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REPORT
OF
THE BOSE INSTITUTE
FOR
1963-64

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

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REPORT OF THE BOSE INSTITUTE
1963—1964

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, 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, and some representative scientists are included.

OBITUARY

The Bose Institute suffered a grievious loss by the passing away on August 13, 1963 of Dr. S. K. Mitra, F.R.S.

As a member of the Governing Body and of the Council since 1951, Dr. Mitra took great interest in the affairs of the Bose Institute. He often presided over the deliberations of these bodies where his wise counsel and guidance were very helpful. From his student days in the Presidency College, Calcutta, Dr. S. K. Mitra had cherished great regard for the personality and the scientific discoveries of Acharya Jagadis Chandra Bose. On two occasions, on November 30, 1946 as Jagadis Chandra Bose Memorial Lecturer and again during the latter's Birth Centenary Celebrations in December, 1958, Dr. Mitra gave lectures on Jagadis Chandra's investigations with short electric waves, using for demonstration the original apparatus employed by the latter. His work as Secretary of the Centenary Celebration Committee contributed largely in making the function a success.

Governing Body

The Governing Body met six times during the year.

Important decisions taken during the year were:

i) Amended some of the Regulations and By-laws dealing specially with the activities of the Governing Body.

ii) Elected Dr. J. C. Sen Gupta as its member in place of late Dr. S. K. Mitra; it also elected him as representative of the Governing Body on the Council of the Bose Institute.

iii) Nominated Prof. B. M. Sen on the Selection Committee for the post of Director.

iv) Nominated six of its members on the new Council which is due to come into effect after April, 1964.

v) Sanctioned the payment of Rs. 2,86,000.00 to the Alipore Treasury as balance of payment due for the acquisition of Rs. 40.99 acres of land at Homaipur, Doharia for the J. C. Bose Centenary Laboratory and Experimental Field Station.

Out of this amount Rs. 1,54,000.00 was an advance payment on behalf of West Bengal Government and the balance represented the contribution by the Central Government, kept in deposit in the Governing Body account.

vi) Authorised the Director to sign jointly with the C.S.I.R. two option agreements with Messrs. Johnson and Johnson, New Brunswick, U.S.A. in respect of an antibiotic from a strain of *Streptomyces bosi* sp. *novum* isolated in the Microbiology Department of the Institute.

Council

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

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

The Council after discussing the report of the Third Reviewing Committee, presided by Prof. D. N. Wadia, F.R.S., forwarded the Committee's Report to the Government of India for implementation of the financial recommendations contained in the Report.

The Council took the opportunity of expressing their deep appreciation of the painstaking, able work undertaken by the Chairman and Members of the Reviewing Committee, in evaluating the research work of the Bose Institute and in making their recommendations for further expansion.

The Council endorsed the view expressed at the meeting of scientists and educationists held in Delhi in August, 1963, for a considerable liberalisation of the amount of foreign exchange made available to research agencies and for a more equitable distribution.

The Council recommended to the Government of India for acceptance that the scale of pay of the future Director of Bose Institute be fixed at Rs. 2000-100-2500/-.

During July 1956 the Council proposed some amendments to the Regulations and By-laws of the Bose Institute, which were then sent to the Government of India. The latter

returned in 1962 the amended Regulations and By-laws with suggestions for minor modifications. At a meeting held in February, 1964 the Council incorporated the modifications suggested by the Government and added a few minor amendments which were considered desirable for efficient working of the Bose Institute.

Similar amendments to the Regulations and By-laws pertaining to the functions of the Governing Body were made by the latter in July, 1963. Both these groups of amendments after incorporation in the comprehensive Regulations and By-laws of the Bose Institute habe been sent to the Government of India for their consideration and approval.

A draft of the project for development of the activities of the Bose Institute during the Fourth Five Year Plan period 1966-67 to 1970-71, has been accepted after scrutiny by the Council, and forwarded to the Government of India. The financial implications of these proposals are:

Recurring Expenditure	..	Rs. 77.92 lakhs.
Non-recurring Expenditure	..	,, 46.07 lakhs.

The Council accepted the decision of the Government of India and sanctioned the payment of ad-hoc D.A. to the members of the staff with effect from July, 1963.

Grants

The following grants were received during the year 1963-64.

(a) *Non-recurring grants:*	(i) Govt. of India, Ministry of Education, Dept. of Science.	Rs.	2,00,000.00
(b) *Recurring grants:*	(ii) Govt. of India, Ministry of Education, Dept. of Science.	,,	8,50,000.00
	(iii) Govt. of India for Research Training Scholarship.	,,	28,686.00
	(iv) Governing Body, Bose Institute. ..	,,	48,645.71

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

Sponsoring Body			
Indian Central Oilseeds Committee.	X-Ray irradiation of oilseeds; radiation mutation and improvement in groundnuts	Rs.	11,000.00
Indian Council of Agriculture Research	Occurence of Rhizobium bacteriophage	,,	8,314.05
-do-	Role of lipoic acid in plant metabolism ..	,,	13,686.35
Council of Scientific and Industrial Research.	Induced mutation in *Aspergillus niger* ..	,,	2,250.00
-do-	Vapour phase Chromatagraphy ..	,,	5,461.86
-do-	Pilot Plant Fermentation	,,	7.560.96

Sponsoring Body—(contd.)			
Council of Scientific and Industrial Research.	Microbial Synthesis of Protein ..	Rs.	7,838.26
-do-	Biosynthesis and Metabolism of Gibberelic acid.	,,	6,032.26
-do-	Enzymatic Synthesis of RNA.	,,	5,000.00
-do-	Indian Medicinal Plants	,,	333.00
-do-	Investigations on Milk Protein ..	,,	919.23
-do-	Gas Chromatography	,,	2,326.63
Council of Scientific and Industrial Research.	Junior Research Fellowship Sm. Malati Mazumdar.	,,	6,176.00
-do-	Jr. Research Fellowship. Sm. Mina Guha	,,	4,600.00
-do-	Pool Officer, Contingent grant. Dr. M. Chakraborty	,,	1,500.00
-do-	Pool Officer, Contingent grant. Dr. A. G. Dutta	,,	1,500.00
-do-	Senior Research Fellowship. B. K. Barman	,,	2,678.33
Department of Atomic Energy, Govt. of India.	(i) Nuclear physics and neutron generator (ii) Induced mutagensis in crop plants etc.	,,	1,10,000.00
Sir J. C. Bose Trust Fund No. 1.	Stipends, and grants for study abroad ..	,,	15,000.00

Acharya Jagadish Chandra Bose Memorial Lecture

The twenty-fifth lecture of the series entitled "The place of science in the defence of a country" was delivered by Dr. S. Bhagavantam, D.Sc., F.N.I., Scientific Adviser to the Ministry of Defence, Govt. of India, on November 30, 1963. The lecture is being published in the Transactions of the Bose Institute.

Acharya Jagadis Chandra Bose Endowment Lectures

The Eighth to Twelvth Endowment Lectures were delivered during the year: (i) Dr. E. L. Nuernbergk, Assistant Director, State Institute for General Botany and Botanical Garden, Hamburg, "On the carbon dioxide metabolism of orchids and succulent plants and its ecological aspects" on October 19, 1963.

(ii) Sir Lawrence Bragg, N. L., F.R.S. Director, Royal Institution, London, "Films of popular science talk; Televised from Royal Institution, London" on January 7, 1964.

(iii) Sir Lawrence Bragg, N. L., F.R.S., Director, Royal Institution, London, "Talk on the Royal Institution, London" on January 7, 1964.

(iv) Dr. T. A. Allibone, F.R.S., Chief Scientist, Central Electricity Generating Board, London, on "New development in electrical power production" on January 23, 1964.

(v) Prof. Richard Abrams, Montifiore Hospital, Institute of Research, Pittsburgh, U.S.A. on "Metabolic changes preceeding DNA synthesis in mammalian cells" on January 27, 1964.

(vi) Prof. Allexander Rich, Department of Biophysics, Massachusetts Institute of Technology, Cambridge, U.S.A. on "Polyribosomes and Protein Synthesis" on February 1, 1964.

Special lectures

1. Prof. A. Sibatani, D.Sc., Professor in the Dept. of Nuclear Medicine and Biology, Hiro-shima University, Hiroshima, Japan, on "Science and Culture in Japan" on February 5, 1964 (under the auspices of the Indian Science News Association).

Degree awarded

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

1. Shri R. K. Mondal, Sr. Research Fellow, CSIR, in the Dept. of Chemistry.
2. Shri B. K. Barman, Dept. of Chemistry.
3. Sm. Parul Chakrabarty, Dept. of Chemistry.
4. Sm. Arati Das, Dept. of Microbiology.
5. Sm. Ashalata Chanda, Dept. of Microbiology.
6. Shri Rabindra K. Sinha, Dept. of Microbiology.
7. Shri Patit Paban Mukherjee, Dept. of Microbiology.
8. Shri Ajit Kumar Mishra, Dept. of Microbiology.

Study abroad and deputation

1. Sm. Kalyani Chatterjee, Jr. Research Fellow, C.S.I.R. has been awarded Research Assistantship in the University of Pittsburgh, U.S.A.
2. Dr. A. K. Mishra, Fellow in the Department of Microbiology, has been awarded a Post Doctorate Fellowship in the University of Rochester.
3. Dr. P. P. Mukherjee, Research Assistant in the Department of Microbiology, has been awarded Post-Doctorate Fellowship in the Vanderbilt University, Tenn. U.S.A.

Grants for study abroad

Grants of Rs. 2000.00 each out of Sir J. C. Bose Trust Fund were made available to Sm. Kalyani Chatterjee, Dr. A. K. Mishra in partial defrayal of their costs of passage abroad.

New appointments and awards

The following appointments were made during the period under review.

A. *Research Fellows*

1. Dr. V. N. Gadgil, M.Sc., D.Phil. Dept. of Botany with effect from 1.4.63.
2. Dr. D. P. Chakraborty, M.Sc., D.Phil. Dept. of Chemistry with effect from 1.4.63.
3. Dr. A. K. Mishra, M.Sc., D.Phil. Dept. of Microbiology with effect from 1.4.63.
4. Dr. M. C. Basu, M.Sc., D.Phil. Dept. of Botany with effect from 9.9.63.

B. *Research Assistants*

1. Shri H. C. Chakrabarty, B.Sc. Dept. of Chemistry with effect from 1.10.63.
2. Shri Sankar Lal Chakrabarty, M.Sc. Dept. of Microbiology with effect from 10.3.64.

C. *Research Scholars*

1. Dr. S. R. Ghoshal, M.B.B.S., D.C.H. Mary & Richard Keating Studentship in the Dept. of Chemistry with effect from 1.5.63.
4. Shri Banidhan Bhattacherjee, M.Sc. Mary & Richard Keating Studentship in Dept. of Botany with effect from 1.1.64.
3. Shri Arun Kumar Chatterjee, M.Sc. in the Dept. of Physics with effect from 18.7.63.
4. Sm. Jharna Mitra, M.Sc. Dept. of Microbiology with effect from 2.9.63.
5. Shri Deb Kumar Mukherjee, M.Sc. Dept. of Chemistry with effect from 24.2.64.

D. *Govt. of India Research Training Scholars*

1. Sm. Protima Roy Chowdhury, M.Sc. Dept. of Botany with effect from 1.4.63.
2. Shri Basudev Prosad Das, M.Sc. Dept. of Chemistry with effect from 1.10.63.

E. *Junior Research Fellows in Research Projects financed by the Council of Scientific and Industrial Research.*

1. Sm. Meena Guha, M.Sc. Dept. of Microbiology with effect from 1.4.63.
2. Sm. Manjushree Bal, M.Sc. Dept. of Chemistry with effect from 22.7.63.
3. Shri Utpalendu Sekhar Maitra, M.Sc. Dept. of Chemistry with effect from 7.10.63.
4. Sm. Anita Roy Chowdhury, M.Sc. Dept. of Chemistry with effect from 7.10.63.

F. *Senior Research Fellow in Research Project financed by the Council of Scientific and Industrial Research.*

1. Sm. Sheela Chowdhury, M.Sc. in the Dept. of Chemistry with effect from 24.1.64.

Termination of schemes

The following C.S.I.R. Grant-in-aid Schemes were terminated during the year.

1. The scheme entitled "Genetical and biochemical studies of induced mutants of *Aspergillus niger*".
2. "Microbial Synthesis of Protein in relation to the Biogenesis of Nucleic acid".
3. "Vapour Phase Chromatography etc."
4. "Pilot plant fermentation studies on antibiotic production from *Streptomyces*".

Seminars

Seminars were held during the period under review when the members of the research staff and invited scientists reported on the results of their respective investigations.

Subjects	*Speaker*
1. "Chemistry of Metachromatic Staining".	1. Dr. M. K. Paul.
2. "Aspects and Prospects of Palynology".	2. Dr. Sunirmal Chanda.
3. "Biochemical Individuality and Nutrition".	3. Dr. Roger J. Williams, Director, Clayton Foundation Biochemical Institute, University of Texas, U.S.A.
4. "Antiviral and Antitumor Effect of Jawaharine".	4. Dr. Durlov K. Roy, D.Sc., Ph.D., Sr. Scientific Officer, Indian Institute for Biochemistry and Experimental Medicine, Calcutta.
5. "Synchronous Culture of Chlorella".	5. Prof. Heroshi Tamiya, Tokugana Institute for Biological Research and Institute of Applied Microbiology, University of Tokyo.
6. "Mechanism of Action of Polynucleotide Phosphorylase".	6. Dr. (Mrs.) Maxine Singer of the National Institute of Health, Bethesda Maryland, U.S.A.

RESEARCH ACTIVITIES

(Non-technical summary)

Nuclear Physics

The series of measurements as described in last year's report on the small angle coherent scattering of gamma rays of different energies has been brought to a conclusion. Attempts have been made to fit in a power law the distribution of the coherent scattering cross sections with atomic number (Z^n); similar attempts have been made to represent its change with energy of the photon.

The nature of the bremsstrahlung produced in scatterers by photo electrons of gamma ray origin has been studied; for heavy elements like lead, the proportion of bremsstrahlung for large angle scattering of gamma rays of energies 1.33 MeV and 2.33 M.e.V. has been found to be of the same order of magnitude as coherent scattering.

A constant sensitivity photon counter consisting of a 2″ NaI/Tl crystal counter with a 8.5 gm/cm² of aluminium filter, whose theory was given in the last report, has been calibrated over a wide spectral range of gamma radiation extending from 0.15 Mev (Co^{58}) to 1.37 and 2.75 Mev (Na^{24}). The results indicate that the performance of the counter both for parallel and wide angled geometry could be satisfactorily accounted for on the theory given in the last Report.

Neutron Physics

A series of measurements for accurate and precision determination of (n, p) cross section for low Z elements for 14 Mev. neutrons has been made. At present effort is being directed to establishing (as secondary standard) the reaction cross section O^{16} $(n\ p)$ N^{16}, which emits β-rays of high energy and short life.

For purpose of absolute measurement of fast neutron flux, investigations are being undertaken to determine a semi empirical relation between the intensity of flourescence of organic phosphor to the energy of protons recoiling from incident neutrons.

Radioactive Fallout

During the period April 17 to June 28, 1962 radioactivity in rain water samples collected on the roof of the Institute building were measured. It was found that unusually heavy radioactivity had been brought down by rain water on April 23 and June 18. From the study of meteorological data for these periods it was concluded that on April 23, the fallout collected mainly in the form of dust, was probably due to nuclear tests conducted in the Siberian region, while on June 18, the unusual radioactivity in collected rain water was probably due to a test carried out in the Pacific Ocean.

During 1963 daily samples of rain water and atmospheric dusts were collected for the period from March 11 to July 14. Attempts are being made to correlate some unusually heavy radioactivity in the samples with the metereological conditions prevalent during these periods.

At the same time attempts were made to determine the nature of the radioactive elements associated with these fall outs. Both chemical as well as gamma ray scintillation counting method failed to reveal the presence of Cs 137 as well as of Sr 90. Using chemical coprecipitation method it was found that the major part of the activity was associated with La (as scavenger). In the solution left after coprecipitation, trace of radioactivity was noticeable—further chemical analysis of this fraction is being carried out.

Radioactive Carbon dating

A convinient method for Radiocarbon dating being developed in the Institute, is to use a scintillation cell containing radioactive toluene in which is disolved diphenyl oxazole, the scintillating liquid and POPOP (a spectrum shifter). The toluene used for this purpose

is synthesized so as to contain (from a sample charcoal) radioactive carbon in the methyl group. The synthesis is a tedious process in which special precaution has to be taken to avoid contamination with atmospheric carbon dioxide. In order to provide sufficient toluene for filling up the scintillation cell several samples have been prepared from the same radioactive carbon source. By the use of two photomultiplier tubes and keeping the whole apparatus in a zero degree room stray scintillation counts will be minimized.

Protein Chemistry

Further physico-chemical studies on crystalline alpha-lactalbumin of goat's milk and estimation of its amino acid composition have shown that this protein has many points of similarity to alpha-lactalbumin of buffalo's and the cow's milk, particularly in respect of molecular weight and amino acid composition. Differences observed in electrophoresis might be ascribed to the number and nature of ionizable groups present in the molecule. A new microcrystalline form of alpha-lactalbumin of cow's and buffalo's milk have been isolated in the course of further purification of these materials.

Studies have been commenced on haemoglobins of Indian zebu cattle and the Indian buffalo. Haemoglobin of about 300 adults zebu cattle have been examined by paper electrophoresis and the occurence of haemoglobin polymorphism has been observed in all the breeds. The *A* and *B* haemoglobins occur singly or in combination in the blood of individual animals. The *A* and *B* varieties have been crystallised and their physico-chemical properties are being studied. Haemoglobin samples from about 50 buffaloes of the same breed show two components in electrophoresis in all the samples, indicating absence of genetic variation.

Proteins of cashew nut have been examined by electrophoresis and sedimentation. Earlier investigations on cashew nut proteins carried out elsewhere reported the presence of a homogeneous globulin. The present studies have shown the presence of five components in electrophoresis and four in the ultracentrifuge.

Organic and Plant Chemistry

In the course of surveying saponin-bearing plants of India a number of new saponins and sapogenins have been isolated. From the fruits of the plant *Barringtonia acutangula*, commonly known as 'Hijal' in West Bengal and used as a powerful fish poison, the active principles have been isolated. The chemical structures of some of these compounds, which were found to be glycosides (saponins), have been elucidated.

The plant *Mollugo spergula* known as 'gima' in W. Bengal is used as a green vegetable and an antiseptic in indigenous system of medicine. It is also a powerful fish poison. The bitter principles have been crystallized and shown to be glycosides.

The seeds of the plant *Albizzia procera* (Beng. 'kori') were known to be fish poisons and insecticides. From these the active principles (glycosides) have been isolated and their chemical structures elucidated. The plant *Lantana camara* has a wide distribution in India and causes severe photosensitization in sheep; from its active principles, isolated as a crude mixture, two new chemical compounds have been prepared.

A new potential antitumor agent has been prepared.

Structural studies of a new group of antifungal antibiotics from higher plants built on simple carbazole and pyrano-carbazole units have been carried out. Some synthetic compounds related to these constituents have been found to have comparable antifungal action.

The position of the family Rutaceae have been analysed from the stand point of chemical taxonomy.

Radiochemical Laboratory

The message in the form of ribonucleic acid that flows from nuclei to the site of protein synthesis carries codes for each amino acid. One of such codes i.e., triple guanylate (GGG) for glycine and tryptophane is experimentally demonstrated.

Differential effect of ultraviolet irradiation on the synthetic messenger RNA lends support to the altered protein synthesis by this treatment. The action of plant growth hormones appears to be concerned with the synthesis of altered messenger RNA. A new enzyme system has been isolated from spinach chloroplasts which can synthesize polynucleotide chains by additions of guanylate and adenylate residues at the end of the pre-existing RNA molecules.

The movement of *Desmodium* leaves has partly been correlated with the augmented phospho-protein synthesis and diminished phosphatase activity in presence of light.

Enzyme and Biochemistry laboratory

Work has been carried out with virulent phage T_2h of *E.Coli* B. and lytic and lysogenic PLT-22 of *S. typhimurin.* Studies on the effect of antibiotics actinomycin *D* and mitomycin *C* on the production of both the virulent and temperate virus have shown that actinomycin *D* has a detrimental effect and motomycin *C* shows enhancement of the rate of production of the virus. Enzyme induction during phage infection by ADP-P^{32} exchange shows that the enzyme level is boosted up by several folds during phage infection.

The effect of the antibiotics and mutagens on the induction of *L*-arabinose isomerase in *Lactobacillus plantarum* have been studied. Actinomycin *D* at a very low concentration completely inhibited the enzyme induction after 15 minutes. Both mitomycin *C* and flourouracil were found to have practically no effect on the enzyme induction. Another inducible enzyme, mannitol-l-phosphate dehydrogenase in *Lactobacillus plantarum* has been purified about 160 fold and its enzymatic properties have been studied.

A new interesting enzyme has been obtained and purified over 60 fold from the extracts of *A. vinelandii* which catalyses the DNA dependent incorporation of radioactive guanylate residues alone. Experiments indicate that only one guanylic acid residue is attached to the terminal position of the DNA chain.

Rat liver nuclei were found to incorporate radioactive P^{32} into nucleic acid. This incorporation was dependent on glucose or adenosine but not on adenine. The incorporation rate decreases after an hour, which might be due to the presence of polynucleotide phosphorylase activity. The presence of this enzyme has been demonstrated. Experiments with sub-nuclear fraction, on the other hand, under the same conditions show the absence of polynucleotide phosphorylase activity.

The reversal of lipoic acid activation reaction has been unequivocally demonstrated for the first time using an assay method based on the formation of ATP^{32} from adenyl lipoate and radioactive pyrophosphate. A new biosynthetic method for the preparation of S^{35} -labelled lipoic acid has also been developed. Recently for the first time the direct formation of adenyl lipoate as an intermediate in the lipoic acid activation reaction has been unequivocally demonstrated.

Analytical chemistry and instrumentation

Gas-chromatography: For high temperature gas-chromatography for the analysis of fats and oils conventional thermal-conductivity cell detector cannot be used. A glass flame-ionization detector has been designed and constructed to replace the thermal-conductivity detector. This flame-ionisation detector is very simple in construction and does not require any airb-lowing from outside or an expensive D.C. amplifier. It can detect organic vapour of as small a volume as one microlitre.

Paper-electrophoresis of maize-szed proteins: From the soluble protein fraction of three different varieties of maize protein constituents were fractionated by ammonium sulphate. Of the three varieties of seeds, the protein fractions from two varieties showed the presence of 3 bands in paper electrophoresis while the protein from the third variety showed 2 bands. Starch gel electrophoresis of the first two varieties also showed only 3 bands.

Clinical method for the diagnosis of different liver diseases: The study of paper electrophoresis of serum proteins along with the serum transaminase activities have been carried out in order to evolve a device for the diagnosis of different liver diseases including liver cancer. Result obtained with 220 cases of different types of liver diseases indicated that typical paper electrophoretic pattern and specific changes in transaminase activities were associated with each clinical entity investigated.

Plant Biochemistry

Plant cancer: Studies were made to convert normal plant tissues into habituated ones: Habituation represents a transitional stage between a normal and cancerous condition of a cell. Work was undertaken to study the relation of auxin with inorganic salts and inositol in the mechanism of transformation of normal plant cell to cancerous cell. Attempts are being made to isolate and cultivate single cells of higher plants in continuous culture.

Botany:

Radiation Mutation

Jute—Some of the effects of radiation (on such characters as—days to flower, average height and pollen sterility) were studied in four varieties of jute (T.M., R. 26, C.G. and D 154) in the X_2, P_2 and S_2 generations; considerable differences were found to exist between the varieties studied.

Paddy:

Aus paddy—Some of the earlier isolated mutants induced by different doses of *X*-rays and P^{32}, were sown together with their controls in replicated plots and their yield and other

characters (such as height, no. of tillers per plant and no. of panicles per plant) were compared with similer characters of their respective controls (viz. Dular and Marichbati). The selection S.1 (black mutant) gave slightly higher yield/plant than its parental (control) type Marichbati; this was followed also by an increase in the number of panicles as well as by an increased panicle length of the mutant.

Boro Paddy—A 3:1 segregtion of green: albino seedlings, was observed in the P_3 generation after P^{32} treatment (30 uc/seed); this indicates that it was caused by the mutation of a single gene. A similar segregation was observed in the next (P_4) generation

Aman Paddy—A mutant having thick grains, isolated from Rupsail variety after P^{32} treatment, in the P_2 generation, was compared to that of its control with respect to straw yield and grain yield in large scale field experiments at Falta and Shyamnagar Experimental Stations. Grain yield and straw yield were found to be lower in the mutant, than in the control.

Chemical Mutation

Physiological and morphological effects of the chemical ethylene-imine on jute in the treated generation were investigated. A striking similarity to *X*-rays at comparable doses (i.e., doses inducing similar number of lethals) was found after ethylene-imine treatment, especially on leaves and stems of the treated plants. Since these types of aberrations may also be induced by auxin treatment; it is suggested that ethylene-imine (like *X*-rays) effects auxin synthesis, resulting in such morphological abnormalities.

Cytology and Cytotaxonomy

(1) Cytological studies have been carried out in detail on four species of Hibiscus and also on one species of Gossypium (G. punctatus). Palynological studies have also been made on five species of the genus Sida and on three species of the genus Abutilon.

Cytological studies on six species of the genus Trigonella as well as on six more species of Acanthaceae has been carried out during the year under report.

The radio-protective action of Methylene Di-isothiourium Bromide was explored and compared to that of *L*-Cysteine on seeds (and chromosomes) of Capsicium annum. *L*-Cysteine was found to give protection against radiation as measured by the reduced number of induced chromosomal aberrations; Di-isothiourium gave no protection at the concentration used (0.1 m/3.0) and had similiar aberration frequency as that of the control (distilled water).

Anatomy

The floral anatomy of eight species of Acanthaceae were studied (belonging to eight different genera). A gradual reduction in the number of stamens were observed. It is suggested that evolution (in Acanthaceae) came through the line of reduction (of stamen numbers) which supports Lindau's classification of Acanthaceae on these lines.

Plant Introduction and breeding—Seeds of about 38 species of fodder grass and fodder legumes were imported from the different parts of the world. These were sown in small plots and their growth pattern and behaviour under local conditions were observed.

Plant Physiology

Preliminary observations were reported last year that electrical impulses appear at the petiole and midrib of the Desmodium leaflet accompanying the movement of the pulvinules. These electrical pulses which produce deflections of a pen recorder are further amplified by attaching to the axis of an optical lever fixed to the tip of a pen recorder and recording the movement of the reflected light spot on a moving film. The electrical impulses given by the midrib and the petiole were of the opposite sign to that produced in the pulvinule. To show that the former are not due to artifacts arising out of the potential fluctuation at the electrodes connected to the pulvinule, records were taken in which only the mechanical movement of the leaflet was recorded simultaneously with the electrical pulsations at the petiole and the midrib. It was found that a positive impulse appears at the midribs and the petiole with the downward movement of the leaflet.

In a previous experiment in which the cut end of a petiole of a Desmodium leaflet is connected to a micropotometer, no movement of the liquid in the capillery tube of the potometer could be detected with the mechanical movement of the leaflet. This may have been due to the considerable viscous resistance to the movement of the small liquid volume in the capilleary tube of the potometer.

This year a modification of J. C. Bose's Spygmograph was used; it enabled the petiole of a pulsating leaflet being placed between the edges of two levers one fixed and the other movable; the movement of the latter could be magnified 10,000 times and recorded by an optical lever. Preliminary indication show that there occurs a change of diameter at the petiole which is correlated to the leaf movement. Detailed study will be undertaken next year by simultaneous recording of the movement of the leaflet and the diametric changes of the petiole.

An investigation has been started to study the biochemical basis of the contractile phenomenon in the pulvinule and leaflet unit of *Desmodium gyrans* to findout whether as in contractile animal tissue, the energy of contraction is supplied by the break down of an energy rich phosphate compound.

Microbiology

Production of antibiotics by Microorganisms

An antibiotic, basic in nature has been isolated in pure form from a *Streptomyces* sp. strain (A_6). Detailed chemical tests of the antibiotic indicate that it is a new member in the streptothricin—streptolin group.

Screening of broad-spectrum antibiotics from Streptomyces spp.

Soil samples collected from different parts of West Bengal were plated; out of these, 640 strains of antibiotic-producing *Streptomyces* spp. were isolated. Ten strains have been found to possess both antibacterial and antifungal antibiotics of which only 5 strains showed activity against human pathogens. A promising strain was finally selected for detailed studies.

Nutritional studies for antibiotic production

Determination of the nutritional requirement of the antibiotic-producing *Streptomyces* strains has been done in several cases and optimum condition for growth and antibiotic production was determined.

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

Attempt was made to compare the active principle extracted from the parent strain AC_{21} 460 as well as the mutant strain by solvent extraction method. The results showed that the antibiotic produced by the mutant strain had two coloured substances of which one is active and the other inactive. The R_f value also differed from each other. The pigment antibiotic was purified by the absorption chromatography using Brockman's alumina as absorbing agent. The details of its chemistry is being worked in collaboratiou with the Dept. of Chemistry. The chemical analysis shows that it could be a new substance so far not reported.

Induced mutation in microorganisms

Experiments were performed to determine the effects of chemicals on ultraviolet radiation on *Aspergillus niger* strain (V_{33}). The following chemicals in different concentrations were used to determine the effects of chemicals on the injurions effect of ultraviolet irradiations : $NaNO_3$, KNO_3, KCl, NH_4NO_3, NaCl, KH_2PO_4, $MgSO_4$, $7H_2O$, and dextrose.

Treatment of Asp. chevalieri with Nitrogen mustard

Spore suspension of *Asp. chevalieri* were treated with 0.1% aqueous solution of *N*-mustard (Bis-chloro ethyl methyl amine HCl) followed by irradiation with *UV* rays after washing, for 2, 4, 6 and 8 minutes respectively. The treated spores were plated out in complete medium and later tested for their biochemical requirements. The frequency of mutation at different treatment levels was compared.

Microbial genetics

Studies with Sordaria bosensis

In *Sordaria bosensis* one *UV* mutant has been isolated which requires glutamic acid and iso-leucine. Physiological studies on growth have indicated that addition of succinic acid, sodium fumarate and fumaric acid to the basal medium encourages vegetative growth of the fungus.

A complete medium having 6 gm. sorbose, malt extraet 5 gm., yeast extract, 2.5 gm., casein hydrolysate 1.5 gm. and glycerine 5 gm. along with the ingredients of the basal medium has been found to be very suitable for compact colony formation in *Sordaria* sp.

Studies on reverse mutations with Neurospora crassa S4894a

Aqueous suspensions of conidiospore were exposed to *UV* rays for 1, 2, 3, 4, 5 and 6 minutes in a wide range of *pH* from 4.2 to 12.0 in order to study the effect of killing and the reverse mutation rate. It was found that the lower *pH* is not too severe on the survival of

spores when irradiated. The maximum killing of spores was observed at *pH* 7.0. Beyond *pH* 12.0 an inhibition of germination of spores has been observed. The maximum number of reverse mutation has been obtained from treatments of 1 and 2 minutes. The highest yield of mutations however, have been scored at *pH* 6.0 with 3 and 4 minutes of treatment.

Studies on Penicillium vermiculatum

UV irradiations have yielded several morphological and biochemical mutants. Maximum number of morphological mutants were obtained at 6 mins. treatment. These are mostly albino and colour mutants. The colour of the mutants so far obtained are white, buff, orange, flesh, pink, grey and intermediate shades.

Studies on the Rhizobium bacteriophage

Different strains of *Rhizobium* were treated with ultraviolet radiation. The probable mutants were divided into several groups, their resistance to the corresponding bacteriophages was tested and a screening was made. Finally cross streak experiments were done to determine the range of sensitivity of the mutants towards unrelated phage strains. A selection of resistant strains was thus made.

Ecology of air microorganisms

A study on the microbial population of air including fungi, bacteria and actinomycetes has been carried out in different localities of West Bengal. It has been found that actinomycetes are of rare occurrence and even when they do so they occur in very small number. On the other hand the bacterial colonies are found to exceed those of the fungi.

A number of fungi reported to be associated with respiratory allergy in human beings have been collected and identified during the course of this study.

Industrial fermentation

Some experiments were conducted with *Asp. niger* mutant strain CGU 163 for the determination of its optimum fermentation conditions. A suitable medium with best fermentative activity with complex nutritional requirement has been recommended and worked out.

Transformation in microorganisms

A strain of *Aspergillus niger* (V_{35}) was used as the donor from which transforming DNA was isolated by the method described by Hotchkiss (1941). Seven strains were selected as receipient strains in the transformation experiment. Transformation was achieved in all cases and the maximum occurrence of transformed colonies was found in the case thymineless mutants.

Studies on the metaphorsis of tadpoles

In previous reports accounts have been given of the retardation of metamorphosis in tadpoles of *Rana tigrina* and *Bufo melanosticlus* by treatment with penicillin, and also by thiourea; and the subsequent restoration of metamorphosis by the action of vitamin B_{12}.

It was also reported that the retarding action of penicillin was due to destruction of vitamin B_{12} secreting gram positive microorganisms in the intestinal flora of the tadpoles. Additional studies made during the past year showed that with the ceasing of treatment with penicillin, some of the retardaled tadpoles started metamorphosing after 30 days due probably to the multiplication from a residual number of vitamin B_{12} producing gram positive microorganisms. It was also observed that after a continuing treatment with penicillin for 100 days, some of retarded tadpoles commenced metamorphosing indicating the multiplication of penicillin resistant vitamin B_{12} producing gram positive microorganism. The effect of these treatments was followed up by histological studies of the trhyroid glands of tadpoles. The glands contain a large number of cells in the vesicles lining the gland : there are colloidal inclusions in them. It was observed that both the total cell area and the colloidal contents was reduced by both by penicillin and thiourea treatment. There was a restoration of these area and its colloidal content after treatment with vitamin B_{12}, but more slowly after thiourea treatment. Recovery was quicker after thiourea treatment with tadpoles belonging to *Bufo melanostictns* than with *Rana tigrina*.

Histological studies on the liver of tadpoles subject to different treatment is being undertaken.

A. PHYSICS

A. 4. Nuclear Physics

A.4.1. (i) *Measurement of the small angle coherent scattering of Gamma rays:*

This series of experiments has been concluded in the period under review. The following are the findings from the experimental results:

(a) Experimental data show better agreement with the form factor calculations of Nelms and Oppenheim than with the calculations of Franz.

(b) It has also been found that a power law distribution of the coherent scattering cross section $\sigma_{coh.}$ with atomic number (Z^n) approximately holds with an exponent n varying with momentum transferred by the photon.

A power law variation of $\sigma_{coh.}$ with energy of the photon has however been found to be valid over a limited range of momentum transfer.

(ii) The effect of bremsstrahlung, produced in the scatterer mainly by the photoelectrons, on the measurement of large angle coherent scattering of gamma rays for heavy elements and high energy has been studied. For energies of 1.33 Mev and 2.62 Mev scattered by lead, the bremsstrahlung contribution has been found to be of the same order of magnitude as the coherent scattering itself.

From the study of the scattered spectrum, in addition to the Compton and Annihilation radiation components, presence of an additional component has been obtained, superimposed on the coherent component.

In view of these results it has been concluded that further extensive work is necessary before any quantitative inference about Delbrück scattering can be made, whose existence has been attempted to be established in the past from the discrepancy between the observed cross sections with the theoretically computed ones.

A.4.II. *Constant Spectral Sensitivity Photon Counter:*

The theory of the wide angle characteristics of the constant spectral sensitivity counter involves corrections due to obliquity effect and to proximity factors. These corrections have been estimated up to $\pm 10\%$ and the effective change of the relative efficiencies of the photon counter can now be calculated to within a few parts per thousand.

Experimental verification of the results obtained in the above manner has been made by using a 2″ NaI/Tl counter with 8.5gm/cm^2 of Aluminium filter. Gamma sources of Co^{58} (0.14 Mev) Hg^{203} (0.28 Mev), Cs^{137} (0.66 Mev), Mn^{54} (0.84 Mev), Co^{60}(1.17 and 1.33 Mev) and Na^{24} (1.37 and 2.75 Mev) have been used for calibration.

The results indicate that the performance of the counter for both paraxial and wide-beam geometries can be accounted for satisfactorily on the basis of the theory developed in the laboratory.

Neutron Physics

A.5. (i) *Utilizing the Institute's Cockcroft Walton neutron generator, the following experiments have been performed:*

Measurement of the (n, p) reaction cross section of low Z elements for 14 Mev neutrons:

Under these series of investigations neutron irradiation of various samples and subsequent activity measurements have been completed. Different thickness of samples have been used and also different neutron flux.

The aim in these measurements were to obtain (i) precise and accurate data of (n, p) reaction cross section in this energy range of neutrons. Previously measurements have been reported with an accuracy of $\pm 20\%$. Thereafter the aim had been to push down this error limit to $\pm 10\%$.

For accuracy, all systematic errors (details of which have been presented in the previous report), have been considered.

For precision, self consistency of data from different sets of measurements have been achieved.

Precise and accurate determination of O^{16} $(n, p)N^{16}$ reaction cross section has been aimed at, so that this value might be used as standard in cases of other (low Z elements) cross section measurements; because in these cases, the (n, p) reaction is responsible for short time activities of very high β-ray energies from the product nuclei N^{16} has a maximum energy of 10.4 Mev.

Periodic check was made on neutron spectra by exposing photographic emulsion plates to the neutron beam.

For extrapolation procedure for determination of self absorption etc. very thin samples of different elements had to be made. Different methods of producing thin films have been used. A method of electrostatic deposition of thin films, following the works of Carswell and Milstead (J. Necl., Energy **4**, 1957, 51) and that of Brunix and Rudstam (Nucl. Inst. and Methods, **13,** 1961, 131) is being perfected.

A.5. (ii) *Investigations on the response of organic scintillators to fast neutrons*

We are investigating the response of organic scintillators to fast neutrons. This study is intended to develop an easy, reliable, absolute method for the measurement of flux of neutrons of known energy. The main difficulty in the use of organic phosphor arise from the fact that the recoil proten pulses in them generate non-linear output light pulse. The specific fluorescence $\frac{dS}{dx}$ of an organic scintillator is related to the specific energy loss $\frac{dE}{dx}$ through the relation

$$\frac{dS}{dx} = \frac{A\frac{dE}{dx}}{1+KB\frac{dE}{dx}}$$

Integrating the above equation numerically, we have compared the experimental pulse height distribution with their predicted values for various values of the constant KB

and we have shown previously that the most appropriate value of this constant is $KB=0.010\pm0.002$ g cm^{-2} Mev^{-1} This method is not very accurate and we have now developed the following procedure for the evaluation of KB. The residual range x of protons of energy E in scintillator is given by a relation of the type for wide variations of E

$$x=aE^n$$

where n varies with the energy interval studied, and with the chemical composition of the phosphor. We have determined the value of S using different rational values of n.

For small values of E we can approximate the above equation by relations of the form $S=D\alpha E^m$.

The values of α and m can be determined by the least square method extended over a continuous range. If we now assume that the $(n-p)$ scattering cross section is isotropic in the centre of mass, then the observed pulse heights should follow the pattern.

We are now experimenting with various values of m and n and other simple relations between S and E to arrive at an accurate estimation of KB from the observed spectrum. Once the value of KB is known the absolute value of flux incident on the phosphor can be calculated in a straightforward manner.

Fallout & Radiocarbon dating

A.6.1. *Radioactivity in rain water and in atmospheric dust over Calcutta*

In 1962 measurement of the radioactivity of rain water samples during the spring was taken up and activities of rain water samples collected between April 17 and June 29 of that year were measured. The results have been published in *Science & Culture* (August 1962). In order to extend this study it was proposed to measure, in addition to the activity of the rain water samples, the activity of the atmospheric dust in the year 1963.

(a) *Radioactivity in rain water, and atmospheric dust* (1963)—Samples of rain water were collected in the Institute from March 11 to July 14 in 1963. The rain water samples were treated similarly as it was done in 1962. The volume of each sample was noted and then filtered through Whatman No. 42 filter paper. The insoluble portion left was then ashed along with the filter paper. The filtrate was evaporated to dryness in order to obtain the soluble part. The relative β-activity of both the soluble and insoluble fractions of each sample were counted by means of an end-window. G. M. Counter of window thickness 4 mg./cm.^{2}. This counter was almost insensitive to α-particles and γ-rays.

To measure the radioactivity in the atmospheric dust a sample collector was designed and constructed. Essentially this was a device to hold a disc of filter paper of 27 cm. diameter horizontally in between two brass rings. The lower rings carries a handle in the form of an U and this can be attached to the upper end of a twenty feet high pole fixed on the roof of our Institute. In order to catch the dust effectively the filter paper disc was coated uniformly with liquid paraffin. For this the disc was immersed in 30% solution of liquid paraffin in petroleum ether (B.P. 40°C—60°C), the excess liquid was drained out and the disc was allowed to stand horizontally for some time so that most of the solvent was removed

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by evaporation. The disc was then fitted to the dust sample collector. As far as possible the duration of each collection of dust sample was 24 hours but in some cases this collection time had to be shortened due to the outbreak of rain. The discs with dust collected, were ashed and the relative β-activity of each sample measured by the same counting system as mentioned above. Unexposed filter paper discs similarly coated acted as controls. The results indicated that the radioactivity of both rain-water and atmospheric dust remain appreciable only in spring in 1963 but for the rest of the year it was very low.

Chemical analysis of the rain water samples—In order to identify the nature of radioactive isotopes present in the above mentioned rain water samples chemical analysis of both the soluble and insoluble portion of those samples that exhibited rather high counts was taken up. Initially the presence of Sr & Cs isotopes were sought for, as they are known to be most abundant radio-isotopes in fallout. But peculiarly enough, though tested repeatedly by well established methods both the two isotopes were found to be absent in the rain water samples. The absence of Cs isotope was confirmed by the fact that none of the sample gave any γ-count when counted by means of a NaI/Tl scintillation γ-counter. Detailed chemical analysis of the samples was then taken up. The samples were repeatedly extracted with boiling conc. HCl until the residues were free from activity. The solution contained a large amount of iron which when precipitated with ammonia as $Fe(OH)_3$ was found to absorb all the activity from the solution. It is well known that $Fe(OH)_3$ absorb a large number of ions from solution. The $Fe(OH)_3$ precipitate was dissolved in 9(N) HCl and was allowed to pass through a column of Dowex-1 anion exchange resin. At this HCl concentration Fe^{+++} formed a complex $FeCl^-_4$ and was retained in the column and the effluent from the column contained all the activity. The effluent solution was then subjected to method of radiochemical analysis based on the principle of coprecipitation with added scavenger ions and frequent use of ion-exchangers. The major activity was found to be associated with the precipitate obtained by using La^{+++} as scavenger. This indicated the presence of radioactive rare earth isotopes. No activity in the precipitate obtained with Ca^{++} excluded the presence of Sr^{90}. The final solution left after all the stages of Co-Precipitation exhibited some activity and further analysis of this fraction has been taken up.

A.6.2. *Radiocarbon dating by scintillation counting*

For radiocarbon dating scintillation counting technique has been taken up in our laboratory. To achieve this toluene has been synthesised from the sample carbon. This is done because, the scintillator, diphenyl oxazole and POPOP (spectrum shifter) are highly soluble in toluene and thereby a homogeneous system is available for counting. The other advantage is that, the toluene contains only carbon and hydrogen as the elements and the quenching of fluorescence is thereby reduced to a minimum and thus a high counting efficiency is obtained.

The toluene, in which the methyl carbon atom is taken from the sample, has been synthesised and obtained in a pure state. The method of synthesis is given below.

The elemental carbon was burnt in a current of pure and dry oxygen in an electrically heated furnace. The carbon dioxide so produced was purified, dried and frozen into several

traps by means of liquid air bath. The residual inert gases if present were pumped out by means of a high vacuum pump. The system was then disconnected from the vacuum line.

While this operation was being carried out, in another system phenyl magnesium bromide (Grignard Reagent) was prepared. Precautions were taken to avoid the contamination with water and atmospheric carbon dioxide. This Grignard reagent was then introduced into a special three-necked flask in an atmosphere of nitrogen avoiding contamination with water and aerial carbon dioxide. This three-necked flask was then connected to the system containing the solid carbon dioxide. The flask containing Grignard reagent was then frozen by liquid air bath. The solid carbon dioxide in the trap was then slowly evaporated and the gaseous carbon dioxide was frozen over the Grignard reagent. This operation was fully controlled by using the open arm mercury manometer as the pressure indicator in the system. The flask was then disconnected from the other part of the system and was stirred by a magnetic stirrer. During stirring the liquid air bath was slowly removed from below. When all the mass was transformed into a liquid state, a cold solution of 6(N) hydrochloric acid was poured into the flask, when solid benzoic acid was precipitated. The carbon atom of the carboxylic group in this benzoic acid thus comes from the sample carbon. This benzoic acid was then purified by recrystallisation from hot water, filtered and dried under suction. The benzoic acid was then esterified to methyl benzoate by reacting it with diazomethane. The methyl benzoate was distilled out and reduced to benzyl alcohol by lithium-aluminium-hydride as the reducing agent and dry ether as the solvent. The liquid mixture was then distilled twice to obtain a pure sample of benzyl alcohol. This benzyl alcohol was then dissolved in dry ethyl alcohol and reduced by hydrogen gas under a pressure of 3-4 atmosphere in presence of palladium-charcoal catalyst at room temperature. Lastly, the toluene formed was separated from ethyl alcohol by adding pure metallic sodium in excess and then distilling the mixture.

The above steps have been repeated several times using charcoal. The yield in each case was found to be high.

B. CHEMISTRY

B.1. Protein Chemistry

B.1.1. *Studies on milk proteins*

(a) *Proteins of goat's milk:* Further studies (cf. Annual Report 1962-63) on the non-casein proteins of goat's milk and on the physico-chemical properties of the crystalline alpha-lactalbumin of this fraction have been continued. Comparative paper electrophoresis experiments with the non-casein fraction of milk and blood serum of goat have indicated that except beta-lactoglobulin and alpha-lactalbumin the rest of the constituents observed on the electropherogram of non-casein proteins are probably derived from the blood serum of the goat. Free electrophoresis experiments at different *pH*'s and variable-solvent solubility tests with the protein have added further information regarding the homogeneity of the material to those obtained from ultracentrifugal studies. The specific refractive increment and the diffusion coefficient of the protein have also been measured and its amino acid composition determined. From the latter, the minimum molecular weight, distribution of side chain groups and partial specific volume of the protein have been calculated. The protein has been found to be remarkably stable towards alkali and heat treatment.

Electrophoresis: In free electrophoresis with 2-3 times recrystallized goat alpha-lactalbumin in acetate and phosphate buffers of 0.1 ionic strength single symmetrical peaks have been obtained in the *pH* range 4.6-6. From the plot of *pH* vs. mobility the isoelectric point has been found to be 5.4.

Variable-solvent solubility test: The variable-solvent solubility curves obtained with goat alpha-lactalbumin at different protein concentrations at *pH* 5.1 show single breaks indicating the presence of one component in the system.

Sedimentation: The radial dilution corrected areas, calculated from the sedimentation velocity patterns of goat alpha-lactalbumin have been found to remain constant. The plot of $D_{20,w}$ (calculated from the sedimentation velocity patterns) vs. time is a straight line parallel to the abscissa.

The results of the above tests show goat alpha-lactalbumin to be a homogeneous protein with respect to these tests.

Specific refractive increment: The specific refractive increment of the protein in acetate-NaCl buffer *pH* 5.3, ionic strength, 0.2 has been found to be 1912×10^{-6} at 546 mμ.

Diffusion coefficient: The value of diffusion coefficient of goat alpha-lactalbumin calculated from the schlieren scanning patterns by the maximum height-area method has been found to be 12.67×10^{-7} cm.^{2}/sec. and the value of $D_{20,w}$ obtained by the method of moments to be 11.18×10^{-7} cm.^{2}/sec. In the calculation of molecular weight and frictional coefficient the $D_{20,w}$ value of 11.18×10^{-7} cm.^{2}/sec. has been used. The frictional coefficient of goat alpha-lactalbumin has been found to be 1.16.

Amino acid analysis: Amino acid analyses of 24 and 72 hours hydrolyzates of goat alpha-lactalbumin have been carried out in a Spinco Model 120 Amino acid analyzer by the courtesy of the Department of Biochemistry, Calcutta University. The minimum molecular

weight of goat alpha-lactalbumin calculated from amino acid composition has been found to be 15,000 g./mole which agrees well with those obtained by other methods (Annual Report 1962-63). From the distribution of side chain groups in goat alpha-lactalbumin, calculated from its amino composition, it has been found that the ratio of polar groups to non-polar groups is 2:1.

Partial specific volume calculated from amino acid composition has been found to be 0.729 c.c./g. This is in good agreement with experimentally determined partial specific volume (0.722 c.c./g.).

Heat stability: Only 1-2% of the protein is denatured when a solution of it is heated at 100°C for 10 minutes.

Detailed comparison of the properties of goat alpha-lactalbumin has been made with those of cow and buffalo alphalactal-bumins. From the close similarity of their properties, the designation "alpha-lactalbumin" given to this protein appears justified.

Determination of Isoionic pH of goat beta-lactoglobulin: Isoionic *pH* of goat beta-lactoglobulin has been determined to be 6.1 by employing the deionisation method through a mixed-bed ion exchange column. The effects of KCl and $CaCl_2$ on the isoionic *pH* have also been studied and the results show potassium ion and calcium ion binding at the isoionic *pH*. Work on the electrometric titration of this protein is in progress.

(b) *Purification of Buffalo and Cow alpha-Lactalbumins by Column Chromatography:* Alpha-lactalbumin obtained from cow's and buffalos' milk is known to contain another protein component, which can not be removed by usual fractionation procedures. Repeated crystallization also failed to remove the impurity. Preliminary experiments have been done with DEAE cellulose equilibrated with phosphate buffer at *pH* 6.8 to remove this component. A fair degree of separation has been achieved and further study of this method is in hand.

A new method of crystallization of Buffalo and Cow alpha-lactalbumins and a new form of crystal of Buffalo alpha-lactalbumin: Buffalo and Cow alpha-lactalbumins have been crystallized by earlier workers in the form of needle and bullet shaped crystals. These crystals were obtained after a series of purification steps.

In the new method, which is a modification of that adopted for goat alpha-lactalbumin in this laboratory, the precipitate of alpha-lactalbumin, freed from beta-lactoglobulin, is dissolved in a minimum volume of water with the help of dilute ammonia and dialysed exhaustively against distilled water at O°C. From the salt free concentrated solution diamond shaped micro-crystals are obtained on keeping it in the cold for 4-5 days.

(c) *Hydrogen-ion dissociation curves of buffalo beta-lactoglobulin:* The acid-base titration curve of buffalo beta-lactoglobulin has been studied in further details (Ann. Rep. 1962-63). The isoionic *pH* of the protein in salt-free solution and at different ionic strengths has been determined. The titration has also been done at different ionic strengths and in the presence of formaldehyde in order to estimate the number of epsilon-amino groups. A count of the tyrosyl groups in the protein has been obtained from spectrophotometric titration. Reversibility of the direct titration curve has been checked by performing back titrations. The number of ionizing groups which contribute to the curve has been estimated and compared with the results of thermal titration of the protein.

The isoionic *pH* was determined by passing a dilute solution of the protein through a mixed-bed ion-exchange column and was found to be 5.48±0.01 at 25°C. Addition of KCl solution lowers the isoionic *pH* indicating potassium ion binding with the protein at this *pH*.

Titration curves covering the acid region at four different ionic strengths in the range 0.02 to 0.25 show that the curves become steeper near the isoelectric point as the ionic strength increases, a fact which is in good agreement with theory.

From the difference between the titration curves in the presence and absence of formaldehyde at *pH* 9.00, 26±0.5 episilon-amino groups per mole of the protein have been estimated. Spectrophotometric titrations at two ionic strengths and at two different temperature indicated the presence of six tyrosyl groups.

In thermal titration, the apparent heat of dissociation (Q′) calculated from the titration data at two different temperatures (25° and 10°C) have been plotted against the number of groups titrated. The plot showed the presence of 49-50 carboxyl and 5-6 immidazole groups per mole of protein.

The results have indicated that buffalo beta-lactoglobulin contains 48 side chain carboxyl groups, 2 alpha-carboxyl groups, 2 alpha-amino, 5 to 6 imidazole, 26 epsilon-amino, 6 phenolic hydroxyl and 6 guanidino groups per mole (35,500 *g*) of protein. The curve has been found to be reversible up to about *pH* 9.00 beyond which the reverse titration curve traces a different path, due probably to irreversible changes in the protein molecule. The thermodynamic analysis of the titration curve in the reversible region is in progress.

B.1.2. *Haemoglobin polymorphism in Indian Zebu cattle and the Indian Buffalo*

In recent years much work has been done on haemoglobin types of domestic livestock, particularly, of cattle and sheep. In cattle two haemoglobins have been identified, one migrating more slowly than the normal adult human haemoglobin in paper electrophoresis and the other migrating slightly faster. The slow and fast moving haemoglobins have been designated as Bovine *A* and *B* respectively. These haemoglobins occur singly or in combination in the blood of individual animals.

Haemoglobin of about 300 adult zebu cattle have been examined by paper electrophoresis at *pH* 8.6. In the following table the results are summarized. These results are being compared with those obtained with the exotic and African breeds by other workers.

TABLE 1

Breed	Total	Hb type		
		A	AB	B
Hariana	102	33	52	17
Sahiwal	44	14	27	3
Tharparkar	25	10	15	—
Red Sindhi	17	11	6	—
Gir	29	9	15	5
Deshi	100	47	47	6

Both *A* and *B* types of haemoglobin have been crystallized and physico-chemical studies on these are in progress.

Indian Buffalo: So far, blood of 48 Indian buffaloes of Murrah breed have been studied by paper electrophoresis. In all of them two haemoglobulin components have been detected, indicating the absence of genetic polymorphism, at least, in this breed.

B.1.3. *Studies on proteins of cashew nut*

In a previous investigation, Damodaran and coworkers (Biochem. J. **30,** 604, (1936) reported the presence of a homogeneous globulin, in cashew-nut.

In the present work paper electrophoresis and ultracentrifugation in the study of cashew-nut proteins have revealed inhomogeneity of the protein.

The protein was extracted by shaking the defatted seed meal with 10% NaCl (buffered to *pH* 7) at room temperature (25°C). The total protein was fractionated to three different fractions by ammonium sulphate fractionation and paper electrophoresis in veronal buffer of fraction-I (55% saturation) gave a broad diffuse pattern. The third Fraction (70%—90% saturation) showed the presence of five distinct components whereas the middle fraction (55%—70% saturation) represented an intermediate zone comprising components of lower and higher fractions.

Ultracentrifuge experiments: Uitracentrifugal patterns of the total protein, in phosphate buffer containing 0.2M NaCl showed the presence of four different components, one being present in large excess of the others. Complete separation of the major component by ammonium sulphate fractionation has not been possible, a small impurity being always present.

B.2. Organic and Plant Chemistry

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

(a) *Barringtonia acutangula* Gaertn. A tentative structure (3β, 16α, 22β, 21 (?), 28-pentahydroxyolean-12-ene) was previously proposed for barringtogenol *C* (vide Ann. Report, 1962-1963). This work has been continued and the complete structure and stereochemistry of barringtogenol *C* have been finally established.

The α-glycol group in barringtogenol *C* was shown to be *trans* by the slow rate of lead tetra-acetate oxidation. Barringtogenol *C* formed a monoacetonide which was obtained in the pure state only as its triacetate, $C_{39}H_{60}O_8$, m.p. 262-264°, $[\alpha]_D^{31°}$ —17.1° ($CHCl_3$). The acetonide formation obviously involved the 22β, 28-diol system, the α-glycol group being *trans* and the acetonide formation between 16α, 28-diol being impossible. 22α, 28-diol does not form acetonide. *Trans* orientation of the α-glycol system in barringtogenol *C* requires the C-21 hydroxyl group to be α as the C-22 hydroxyl group is β. Assuming normal β (equatorial) orientation of the C-3 hydroxyl group, the stereochemistry of barringtogenol *C* may be represented as 3β, 16α, 21α, 22β, 28-pentahydroxyolean-12-ene.

It was only after arriving at the above structure, the striking similarity in the constitution of barringtogenol *C* and barringtogenol *D* became evident and the chemical conversion

of the former to the latter appeared to be quite possible. In fact, on refluxing pure barringtogenol *C* with ethanolic hydrochloric acid (8 per cent), barringtogenol *D* could be obtained in 42 per cent yield. Barringtogenol *D* was previously shown to be 3β, 22β, 28-trihydroxy-16α:21α-oxido olean-12-ene (vide Ann. Report 1960-61). It appears quite probable that barringtogenol *D* is an artifact derived from barringtogenol *C* by dehydration during hydrolysis of the saponin. It was formed only in poor yield (0.05 per cent) during the isolation process most probably because aqueous ethanol (60 per cent) was used for the acid hydrolysis of the saponin.

(b) *Albizzia procera Benth:* The isolation of a number of sapogenins from the seeds of *A.* procera was reported earlier (vide previous reports). Fraction *A* was shown to be 3β-hydroxyolean-12-ene-28→21 lactone. Machaerinic acid furnished a compound m.p. 300-305°, when refluxed with acetic anhydride and fused sodium acetate. This compound was thought to be identical with Fraction *A* acetate (vide Ann. Report 1962-63) as there was no depression in melting point when admixed. But it has now been observed that the above two products are not identical as shown by the difference in their optical rotation and hence the sodium acetate-acetic anhydride treatment product of machaerinic acid should have structure different from that of Fraction *A*.

Fraction C, $C_{30}H_{46}O_4$, m.p. 293-96°, gave purple→violet colour in the Liebermann Burchard test and a pale yellow colour with tetranitromethane. It formed a diacetate, $C_{34}H_{50}O_6$, m.p. 246-248°. The infra-red spectrum of Fraction C showed band at 3550 cm^{-1} for hydroxyl groups and a band at 1765 cm^{-1} which indicated the presence of a γ-lactone ring. Fraction C gave an acid when treated with methanolic alkali followed by acidification. But the acid on refluxing with methanolic or ethanolic hydrochloric acid gave back Fraction C. The above acid on treatment with ethereal diazomethane gave back Fraction C and not the methyl ester. All these observations indicated the presence of a γ-lactone ring in Fraction C. Lithium aluminium hydride reduction product afford a tetrol, $C_{30}H_{50}O_4$, m.p. 298-302°, which formed a triacetate $C_{36}H_{56}O_7$, m.p. 297-99°, indicating the presence of a hindered hydroxyl group in the above tetrol. The infra-red spectrum of the above triacetate showed band at 3500 cm^{-1} showing the presence of free hydroxyl group. The tetrol consumed periodic acid which proved the presence of α-glycol system. Fraction C itself did not consume any periodic acid.

Further work is in progress.

B.2.2. *Chemical investigation of Mollugo spergula*

From the alcoholic extract of *M. spergula* the isolation of some bitter saponins was reported earlier (vide Annual Report, 1962-63). It has now been observed that it also contains some phenolic glycosides. The crude glycoside was hydrolysed with ethanolic hydrochloric acid when a large quantity of aglycone mixture was obtained. The aglycone mixture was extracted successively with ether and chloroform. No detailed work has so far been done on the chloroform soluble part. The ether soluble part could be separated into neutral (Fraction A), acid (Fraction B) and phenolic fractions (Fraction C).

The fraction A was found to be a mixture of sapogenins from which one compound could be obtained in a pure state and its homogeneity was checked by thin layer chromatography. This compound has been found to be a new sapogenin and named as 'spergulagenin A'. It has the molecular formula, $C_{30}H_{50}O_4$, m.p. 278-82° (decomp.), $[\alpha]_D^{32°}$—31.4° ($CHCl_3$). It formed a triacetate, $C_{36}H_{56}O_7$, m.p. 236-238°, $[\alpha[_D^{34°}$ +57.7° ($CHCl_3$) and a tribenzoate, $C_{51}H_{62}O_7$, m.p. 319°, $[\alpha]_D^{32°}$+78.9° ($CHCl_3$) thus indicating the presence of three hydroxyl groups in spergulagenin A. Both spergulagenin A and its triacetate gave pink→violet coloration in the Liebermann Burchard test. But they are saturated to tetranitromethane thus showing the absence of any unsaturation in spergulagenin A. This was further proved by the inertness of the triacetate towards perbenzoic acid and perhydrol-acetic acid oxidation. The absence of unsaturation was further confirmed by the analysis of the NMR spectrum of the triacetate. Both spergulagenin A and its triacetate showed a weak absorption at 280 mμ(logε, 1.17) characteristic of a simple carbonyl group. The infra-red spectrum of spergulagenin A showed a band at 1700 cm^{-1} characteristic of a six membered ring ketone. The presence of a carbonyl group was confirmed by the formation of a triacetyl-mono-2:4-dinitrophenylhydrazone, $C_{42}H_{60}O_{10}N_4$, m.p. 268-70° (decomp.). The carbonyl group appeared to be a keto group as spergulagenin A triacetate could be recovered unchanged after treatment with CrO_3-acetone-sulphuric acid. As spergulagenin A did not react with periodic acid, it can not contain α-glycol or α-ketol system. Pyrolysis of spergulagenin A with Cu-bronze at its melting point, did not give formaldehyde thus showing the absence of any formaldehydogenic group, -CHOH-C̈-CH_2OH, in it. Spergulagenin A did not respond to Zimmermann test for 3-keto group and hence the keto group in it is most probably not located at C-3 position.

Spergulagenin A on oxidation with CrO_3-acetone-sulphuric acid furnished a colourless neutral compound $C_{30}H_{44}O_4$, m.p. 279-282° (decomp). It did not respond to alcoholic ferric chloride test nor did it undergo any change on mild alkali treatment. Thus it cannot be a true α-diketone or an enolizable α or β-diketone. The oxidation product responded to Zimmermann test for 3-keto group thus showing the presence of one secondary hydroxyl group at C-3 position in spergulagenin A. The oxidation product $C_{30}H_{44}O_4$, on reduction by modified Wolff-Kishner method under anhydrous condition, furnished a colourless compound $C_{30}H_{50}$, m.p. 187-91°. No detailed study on the above hydrocarbon has yet been possible. Spergulagenin A is a completely saturated triterpene of the formula, $C_{30}H_{50}O_4$, containing one keto group and hence it should be pentacyclic in nature.

The Fraction B was found to be a mixture from which one compound called spergulagenic acid, could be obtained in a pure state as its dimethyl ester, $C_{32}H_{50}O_5$, m.p. 235-36°. The homogeneity of spergulagenic acid was checked by thin layer chromatography of dimethyl spergulagenate. The above methyl ester formed a monoacetyl derivative, $C_{34}H_{52}O_6$, m.p. 285-87°, $[\alpha]_D^{31.5}$+93.1° ($CHCl_3$). The I.R. spectrum of the methyl ester showed bands at 1715 cm^{-1} (carbomethoxy group) and at 3530 cm^{-1} (hydroxyl group). Dimethyl spergulagenate and its acetate gave pink→violet coloration in the Liebermann-Burchard test. Both gave yellow colour with tetranitromethane thus showing the presence of unsaturation in spergulagenic acid. The double bond was, however, hindered as it could not be hydrogenated over Adam's catalyst under atmospheric pressure. The acetate on refluxing with CrO_3-acetic acid formed an $\alpha\beta$, -unsaturated ketone showing absorption maxima

at 249 mμ. α or β-amyrin group of compounds under similar condition formed 11-keto compounds showing absorption maxima at 245-250 mμ. The dimethyl monoacetyl spergulagenate on refluxing with selenium dioxide in glacial acetic acid furnished a compound which showed triple absorption maxima at 242, 250 and 260 mμ characteristic of $\Delta^{11:12,\ 13:18}$ -dienes obtained from β-amyrin class of compounds.

Dimethyl spergulagenate on oxidation with CrO_3-pyridine complex gave a colourless neutral compound, m.p. 205-207°, $[\alpha]_D^{33°}$+113°($CHCl_3$). It responded to Zimmermann's test for 3-keto group thus proving the presence of a secondary hydroxyl group at C-3 position in spergulagenic acid. On refluxing the above ketone with methanolic caustic potash (20 per cent) for 5 hrs and subsequent esterification of the product with diazomethane gave back the above ketone. The above experiment clearly proved that the keto group is not located at β-position with respect to any one of the carbomethoxyl groups.

Spergulagenic acid is most probably a monohydroxy dicarboxylic acid of oleanane group having a 12:13-double bond. Further work is in progress.

B.2.3. *Chemical investigation of Lantana camara*

The plant *L. camara* causes severe photosensitization in sheep. This plant has been chemically examined by many workers but the active principle has not, so far, been obtained in the pure state. Lantadene B which was isolated from this plant has been found to be inactive. In view of the interesting physiological property of *L. camara* a reinvestigation of this plant was thought to be desirable.

From the petroleum ether extract of *L. camara* two new acids called compound A and B have been isolated as their methyl esters. Besides these two compounds, lantadene B has also been isolated. The homogeneity of compound A was checked by thin layer chromatagraphy of its methyl ester. The methyl ester of compound A, m.p. 196-98°, gave positive Libermann Burchard colour test. It also responded to tetranitromethane colour test for unsaturation. The above ester showed bands at 3480 cm^{-1} (hydroxyl group), 1710 cm^{-1} (carbomethoxyl group) and a series of bands in the region 800 cm^{-1}-1000 cm^{-1} which is probably due to the presence of 1, 2-epoxy ring.

The compound B formed a methyl ester, m.p. 227-229°. The ester gave positive Liebermann Burchard colour test. It also responded to tetranitromethane colour test for unsaturation. Further work is in progress.

B.2.4. *Chemical examination of Ougeinia dalbergioides Benth*

In the previous report the isolation of two compounds from 0. dalbergioides were reported. One of these (Compound A) was characterised as lupeol. Detailed studies on compound B has been made. The presence of primary hydroxyl group in compound B was proved by oxidation of the dihydro derivative with CrO_3-acetic acid when an acid, $C_{30}H_{48}O_3$, m.p. 258-60°, was obtained. The above acid gave positive Zimmermann colour test for 3-keto group. On refluxing compound B with formic acid a compound, $C_{31}H_{50}O_3$, m.p. 310-12°, was obtained which did not respond to tetranitromethane test. The melting point of the above compound was in close agreement with that of allobetulin monoformate. On saponification, the above compound gave a product, $C_{30}H_{50}O_2$, m.p. 273-74°, which did not depress the melting point of allobetulin prepared from betulin. Compound B was shown to be betulin by direct comparison of mixture melting point with an authenitic sample of the latter.

B.2.5. *Chemical investigation of Solanum ferox Linn*

From the berries of *S. ferox* highly bitter glycosides have been isolated. These appear to be glycosidic alkaloids. Further work to characterise the products is in progress.

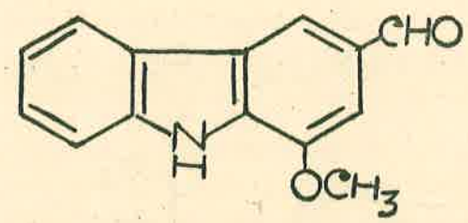
B.2.6. *Preparation of some potential chemical mutagenic agents*

A new compound, 2,' 2-dimethylaziridinyl-p-benzoquinone, has been prepared. Its chemotherapeutic action on tumor-bearing animals is also being tested.

B.2.7. *Structural studies of some constituents of Murraya koenigii* Spreng

(a) *Murrayanine:* The compound $C_{14}H_{11}O_2N$, m.p. 168° (vide previous report p. 26) has been named murrayanine. The molecular formula of murrayanine has been confirmed by mass spectral measurements when molecular ion peak of 225 was obtained.

In the previous report, murrayanine was characterised as 1-methoxy carbazole aldehyde. Further support for the position of the methoxy group in position 1 has been provided by UV data of the borohydride reduction product of murrayanine $C_{14}H_{13}O_2N$, m.p. 127°. The reduction product shows UV spectrum very similar to that of 1-methoxy carbazole. Further studies regarding the position of the aldehyde function has been made. The Wolff-Kishner reduction product, $C_{14}H_{13}ON$ of murrayanine on zinc dust distillation furnished 3-methyl carbazole. It indicated that the aldehyde function in murrayanine is in position 3 assuming that no migration of methyl group has taken place during the drastic cleavage. The UV spectrum of the parent compound was very similar to those of 3-formyl carbazole and 2:4-dimethyl-3-formyl carbazole. From these data the structure of murrayanine as 3-formyl-1-methoxy carbazole or 6-formyl-1-methoxy carbazole is conceivable. From the consideration of the NMR data of murrayanine the following structure is considered more probable.



(b) *Girinimbine:* The compound $C_{18}H_{17}ON$, m.p. 176° has been named girinimbine. In the previous report it was suggested that the structure of girinimbine is built on a methyl carbazole nucleus and a 2′:2′-dimethyl pyran ring is fused to it.

Quantitative hydrogenation of girinimbine has shown that it takes up one mole of hydrogen yielding the dihydro product $C_{18}H_{19}ON$, m.p. 176°.

The isolation of α-hydroxy *iso*-butyric acid by permanganate oxidation of girinimbine further supports the presence 2:2-dimethyl pyran ring in the compound. The ozonolysis

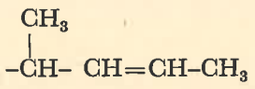
of the compound furnished acetone and a phenolic aldehyde, $C_{14}H_{11}ON$, m.p. 193°. The compound on methylation with diazomethane yielded a methoxy-carbazole-aldehyde. The aldehyde thus obtained gave the corresponding alcohol on borohydride reduction. The UV spectra of these two compounds were similar to those of 1-formyl-carbazole and 2-methoxy-carbazole respectively. From this it has been concluded that the pyran ring is probably fused to the 1:2-position of the carbazole nucleus.

(c) *Mahanimbine:* The compound $C_{23}H_{25}ON$, m.p. 94-95° $[\alpha]_D^{25°}$ $(CHCl_3)+45.4°$ has been named mahanimbine.

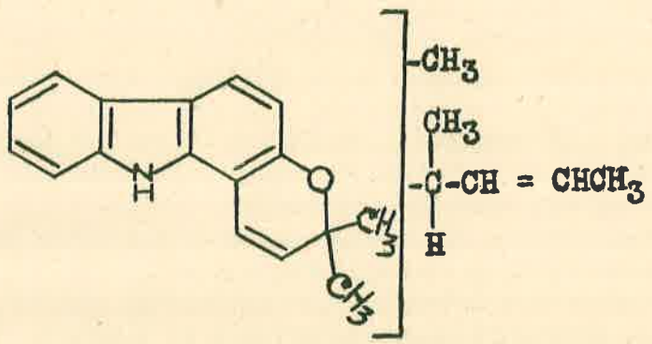
The mass spectral measurements of the compound have confirmed its molecular formula. A molecular ion peak of 331 was obtained. The next important high intensity peak was at 248 as found in girinimbine. This is suggestive of the presence of the same 2:2-dimethyl-pyrano-methyl carbazole unit in mahanimbine. It has been concluded from the mass spectral data that the compound has a side-chain of five carbon atoms besides the pyrano-carbazole fragment.

On ozonolysis in ethylacetate mahanimbine furnished acetone, acetaldehyde, a hydroxy-carbazole-aldehyde and an optically active neutral product, m.p. 213-14°. Acetone and acetaldehyde indicate the nature of unsaturation in mahanimbine. The formation of the α-hydroxy aldehyde shows the presence of 2:2-dimethyl-Δ^3-pyran system in it. The hydroxy aldehyde on methylation afforded a methoxy derivative $C_{18}H_{17}O_3N$, m.p. 176-77° which on borohydride reduction afforded the corresponding alcohol. The UV spectra of the methoxy aldehyde and the corresponding alcohol were very similar to those of 1-formyl carbazole and 2-methoxy carbazole respectively. The evidences show that the pyran ring is fused to the carbazole nucleus in position 1 and 2. The inferences are consistant with results of mass spectral measurements.

The side-chain of 5-carbon atoms has been shown to have the following structure from the structural study of the neutral product obtained by ozonolysis of mahanimbine and from other degradative reactions.



From the results so far obtained the following structure of mahanimbine has been suggested



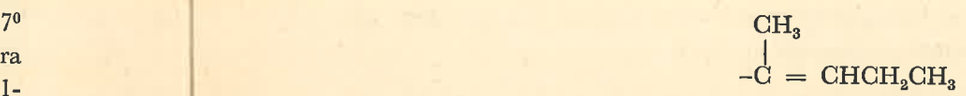
(d) *Isomahanimbine:* From the analytical and mass spectral data, the molecular formula $C_{23}H_{25}ON$ has been arrived at. It is optically inactive.

Like mahanimbine the oxygen function is inert and nitrogen forms a part of an –NH function. On hydrogenation, it yields a tetrahydro derivative, $C_{23}H_{29}ON$, m.p. 90°.

It has been concluded from the results of periodic acid oxidation and chromic acid oxidation of isomahanimbine that one of the two ethylenic bonds belongs to a propylidene group ($=CHCH_2CH_3$) while the other forms a part of 2′:2′ dimethyl Δ^3-pyran system.

On zinc dust distillation the compound yields 3-methyl carbazole which shows that it has a 3-methyl carbazole skeleton as in mahanimbine.

By ozonolysis, acetone, propionaldehyde and a phenolic aldehyde characterised as its methoxy derivative, m.p. 130-31° were obtained. The UV data of the methoxy aldehyde is suggestive of the presence of a 2′:2′ -dimethyl Δ^3-pyran ring fused to the carbazole system in the position 1 and 2. This has also been supported by the mass spectral data of isomahanimbine. The mass spectral data showed it has the same 2′:2′ -dimethyl-pyrano-methyl carbazole skeleton and a side chain of five carbon atoms. Mahanimbine and isomahanimbine differ only in the nature of the five carbon side chain which is perhaps as follows for isomahanimbine.



It has therefore been concluded that isomahanimbine is probably the double bond isomer of mahanimbine. Further work is in progress.

B.2.8. *Structure of mahoganin*

Three crystalline constituents, one bitter and two non-bitter have been isolated from the seeds of *Swietenia mahogani*. One of the non-bitter constituents named mahoganin, m.p. 225-28° has been assigned a molecular formula $C_{27}H_{34}O_8$ from analytical data and mass spectral measurements.

IR spectrum of the compound showed bands at 3580 (hydroxyl), 1725 (carbonyl function) 1505 and 875 cm^{-1} (β-substituted furan). The UV spectrum of the compound showed absorption max at 215. From the NMR spectrum the β-substituted furan, one carbomethoxy group. hydroxyl function and four C-Me groups can readily be recognised.

The compound on hydrolysis yields an acid, $C_{26}H_{32}O_8$, m.p. 223-24 which on methylation with diazomethane yields mahoganin. This confirms the presence of one carbomethoxy group in mahoganin. The methoxyl determination of the compound also support this.

The compound yielded a crystalline DNPH derivative, m.p. 261° showing the presence of the ketonic function. On reduction with sodium borohydride, the compound afforded a product m.p. 192-93° showing the presence readily reducible ketonic function.

On lithium aluminium hydride reduction the compound afforded an amorphous product. This on zinc dust distillation yielded an oily product. The oily product could be

sublimed. The fraction subliming at 180° showed UV absorption max. at 228 and 257.5 mμ.

Work is in progress.

B.2.9. From the leaves of *Ravenia spectabilis*, γ-fagarine and arborinine have been isolated.

B.2.10. *Synthetic studies on carbazole derivatives*

Attempts to synthesise some carbazole derivatives in connection with the constitution of natural products and their biochemical properties are in progress. Some starting materials and intermediate compounds have been prepared.

B.2.11. Paper chromatographic separation of some carbazole derivatives have been made. The most suitable solvent for separation is a mixture of pyridine:water.

B.2.12. *Biochemical properties of natural products*

(a) Some constituents of *M. koenigii* have been found to have pronounced inhibitory action against *Microsporum audouini*, *Nocardia asteroids* and *Trichophyton rubrum*. Some synthetic compounds related to these natural products are more active than the natural products.

(b) The effect of psoralene on hypophysectomised toads syggests that psoralene can induce melanin formation in toads even in absence of MSH.

B.2.13. *Studies in relation to biochemical systematics*

The position of the family Rutaceae has been analysed in consideration of the secondary products like terpenoids, coumarins, flavonoids, amides and alkaloids. The results of the analysis support the placement of *Rutaceae* in the order Rutales of Hutchinson's lignosae.

B.3. Radiochemistry

B.3.1. *The effect of colchicine on nucleic acid and protein synthesis*

Colchicine can induce tumour in *Allium* root tips by interfering with the spindle formation within the cell and thereby resulting in abnormality. Antagonistic activity in inducing tumour formation of the compounds which influence protein and nucleic acid synthesis has been studied. Even 0.01% ATP inhibits tumour formation. The strong inhibition of growth in length which is characteristic of colchicine was overcome slightly due to the presence of ATP. Actinomycin D which specifically inhibits RNA-polymerase had an inhibitory action on tumour formation. In presence of actinomycin D growth of the root tips was also arrested and deformities were observed in less than 5% of the total number of root tips. Chloramphenicol had an inhibitory effect on growth though no effect on tumour formation has been observed. The experiments with colchicine treated tissue showed some differences in nucleic acids and protein syntheses. At least 2-3 fold increase in the synthesis of RNA, DNA and protein has been observed. But just reverse was the case with ATP. The increased C^{14} -incorporation from thymine-C^{14} and uracil-C^{14} in DNA and RNA respectively in the treated ones might be correlated with the decrease in

the level of ATP. The effect of colchicine was found not to be on the production of ATP through adenylate kinase but on its utilization for the synthesis of DNA and RNA. Fractionation of root tip proeins resulted in the isolation of a fraction supposed to comprise the spindle protein which is claimed to be the point of attack by colchicine. As there was very little increase in C^{14}-amino acid incorporation in that protein fraction of treated tissue, it was apparent that colchicine had practically no effect on primary synthesis of protein. Further analysis showed that a decrease in SH content in that protein fraction of treated ones was associated with a concomitant increase in S-S content, suggesting thereby that the regulation of mitotic activity may be achieved by complexing or alteration of an already formed protein molecule.

B.3.2. *Enzymatic synthesis of polyribonucleotides*

This is the continuation of last year's work on enzymatic synthesis of polyguanylic acid by nuclear extract. When crude extract of whole chloroplasts was incubated with four ribonucleoside triphosphates individually or together, it has been found that all the four triphosphates were incorporated. But in presence of other nucleoside triphosphates ATP incorporation was not increased but instead decreased. This inhibition of ATP incorporation was further followed up and the extract from the grana after partial purification showed the maximum incorporation of ATP and GTP but CTP and UTP incorporation could be reduced to nil. Another consistent observation was the inhibition of ATP by GTP and vice versa. ATP or GTP incorporation was not inhibited by other triphosphates. The actual substrates were the triphosphates but not the di or monophosphates. The optimal *pH* was found to be broad in the case of ATP and around 8.5 and in the lower *pH* the incorporation of GTP was found to be more susceptible than that of ATP which might suggest that these two reactions were independent and catalyzed by two different enzyme systems but the other kinetics of the reactions are not in favour of this supposition. Optimal incorporation was observed within 15 min. and the incorporation was linear upto 6 mg. of protein in the incubation mixture. For maximum incorporation of ATP or GTP manganese ions were found to be most effective whereas magensium ions were 50% efficient as compared to the former. Without any divalent cations there was partial activity and that activity could not further be reduced by EDTA. GTP incorporation was inhibited in presence of ATP which was found to be almost competitive and vice versa with a purified preparation from the grana. The Ks value for GTP and ATP were found to be same and approximates to 3.7×10^{-4} M. When preformed poly A or poly G type of substance was added the affinity of the enzyme towards substrate was increased and Ks value in that case approximates to 1.1×10^{-4} M. In order to clarify the nature of the product when both ATP and GTP were present in the system relative nearest neighbour frequencies were determined after incubating with AMP^{32}PP. It has been found that about 90% of the AMP units were incorporated next to another AMP unit and thus favouring the synthesis of poly-adenylate chains when ATP was present alone but in presence of GTP there was little change in the ApA sequence and at least 80% of the AMP units were attached to synthesize poly A. GpA sequence showed a tendency of higher value but that does not prove that any copolymer of poly AG

is formed but instead substantiate that separate chains of poly A or poly G synthesis attached at the end of the preexisting RNA has been favoured. That the product is of poly A or poly G type has been proved by their characteristic influence on the amino acid incorporation by RNP-particles and *pH* 5 fraction isolated from chloroplasts and also from other sources. The physiological role of this poly G type chain has been elucidated as has been surmised last year by the fact that only glycine incorporation was increased among all other amino acids so far tested. Thus far, triplet code GUG, GAG and GCG have been assigned for glycine and that GGG might represent another code for the same has been evident from our experiment inspite of its high interaction. What we have reported here suggests that there are several systems present in the chloroplasts for synthesizing polyribonucleotides. But in what way these polynucleotides are responsible for RNA synthesis in chloroplasts is yet to be answered.

B.3.3. *Biosynthesis of histones*

When intact liver nuclei were incubated with a mixture of C^{14} -labelled amino acids, histones isolated thereafter were found to imbibe radioactivity. When this histone was further fractionated into three principal groups such as (*i*) Arginine rich (*ii*) very lysine rich and (*iii*) slightly lysine rich histones it was observed that in the last fraction more incorporation of C^{14}-amino acids was detected. In presence of chloramphenicol the degree of inhibition of histone synthesis was reflected on these three fractions. Inhibition of the incorporation of C^{14}-amino acids into the first and second fraction was found to be 72 and 75% respectively whereas in the last fraction 86–90% inhibition was recorded. Isolated nuclear ribosomes were also capable of synthesizing histones. This together with differential inhibition of different histones under varied physiological conditions coupled with genetic expression is now in progress.

B.3.4. *Synthesis of protein in chloroplasts*

Some of our observations were reported last year. Ribosomal particles from chloroplasts consisting of various sizes from 50-130S were isolated and found to be active in amino-acid incorporation into polypeptide chains in presence of *pH* 5 fraction, and GTP. Absolute requirement for ATP and generating system could not be demonstrated and later it was observed that adenylate kinase activity was associated with RNP and *pH* 5 fractions. Chloramphenicol was found to be inhibitory in phenylalanine incorporation. Since further addition of poly U in this system did not stimulate the incorporation of phenylalanine it was thought possible that the messenger RNA in the chloroplasts might be more stable and poly U could not affect the incorporation. The effect of poly U in this case was noted after treating the RNP particles with pancreatic RNase for 15 min. Half of the incorporation was diminished after the short treatment with RNase but with the addition of poly U the incorporation could be restored fully. Without this pretreatment there was no effect of poly U. 40% inhibition in phenylalanine incorporation was observed when poly A was used indicating possibly a limiting association with poly U. Poly U which is attached with the RNP-particles may not combine with poly A due to hindrance in hydrogen bond formation. RNA isolated from RNP-particles could restore 85% phenylalanine incorporation possibly

suggesting the stable nature of that RNA. It was observed that with the addition of grana phenylalanine incorporation was inhibited. As a matter of fact a RNase activity was found to be associated with the grana but very little activity was detected with intact chloroplast and stroma fraction.

B.3.5. *Phytin and synthesis of nucleic acids*

In continuation of our work on the relationship of phytin and nucleic acid synthesis an enzyme has been detected from pea seedlings which can specifically transfer phosphate from phytin to ADP synthesizing ATP. This can act as generating system for nucleic acid and protein synthesis. Further purification of this enzyme is now in progress.

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

In the previous report it was mentioned that the plant growth hormone indole acetic acid remarkably affected the synthesis of nuclear RNA and DNA as revealed from studies with coconut milk nuclei. Such studies have since then been extended with kinetin and gibberellic acid and similar effects were observed.

The cell wall components as also nuclei, mitochondria and 12,000×g supernate which includes microsome and soluble RNA, all incorporated P^{32} into their RNA. IAA favoured the incorporation in all the fractions but the stimulation was most remarkable in the case of nuclei which exhibited an 83% increase over the control within 30 minutes of auxin application. Kinetin stimulated the formation of nucleotides and their subsequent incorporation into DNA and RNA. Kinetin-induced stimulation of nucleotide synthesis was apparently unrelated to an activation of the action of adenylate kinase. The possibility of an increased synthesis of adenylate kinase is not ruled out. Kinetin-induced RNA synthesis was associated with comparable increments of growth and kinetin-H^3 was found to bind with DNA.

The action of all these growth substances described above appeared to be concerned with a process mediated by DNA dependent RNA synthesis. Thus, treatment of nuclei with DNase was found to inhibit the incorporation of P^{32} into nuclear RNA. When coconut milk nuclei were pre-treated with actionomycin D, no significant stimulation in RNA synthesis could be detected even in presence of any growth substance, lending further support to the contention that the growth substance action involves DNA in a process concerned with DNA dependent RNA synthesis. The growth substance induced stimulation of nuclear RNA synthesis was discernible within 15 minutes of incubation. The specific activity of the cytoplasmic RNA however, was practically unaffected during this period but increased later and finally exceeded that of the nuclear RNA. In absence of nuclei the cytoplasmic RNA fraction is little affected irrespective of the presence of growth substances. The RNA fraction which is most remarkably affected by growth substance application has a base sequence complementary to that of DNA indicating that it is messenger-type RNA. On the basis of these observations it is proposed that the growth substance action is exerted by a regulation of DNA dependent RNA synthesis which controls the metabolic chain of events leading to the expression of growth, development and morphogenesis. A modification of the DNA dependent RNA synthesis by growth substances will be reflected in a regulation

of protein synthesis which in turn will affect the various physiological processes characteristic of growth substance responses.

B.3.7. *Nucleases from liver nuclei*

Nuclei were extracted in cold by homogenization with Tris *pH* 7.2 and supernatant was made to *pH* 5.5. The precipitate was centrifuged down and from the supernatant a preparation was obtained by ammonium sulphate fractionation (0-30% saturation) which exhibits considerable RNase and DNase activity. This preparation seemed to be completely free from any phosphodiesterase or phosphomonoesterase activity. RNase and DNase activity was found to be in the same fraction. Other nucleic acid-degrading activities studied include those associated with the nuclear histones. Histones have been shown to possess RNA-depolymerising activity recently. In our histone preparations besides RNase activity detectable phosphodiesterase activity has been found as well. Purification of these various fractions and their specificity is now being investigated.

B.3.8. *Mechanism of flowering and studies on photosynthesis*

Within the year under review there had been little progress on mechanism of flowering. The nature of the iron containing compound which was implicated to play a role in photosynthesis was found to be ferredoxin.

B.3.9. *Mechanism of Movement of Desmodium leaves*

It was observed previously that in the dark *Desomodium* leaflets, inspite of supply of glucose, showed marked inhibition of movement. ATPase and phosphatase activity has been found in the *Desmodium* leaves and fractionated further by ammonium sulphate and DEAE cellulose. Light induced an inhibition of ATPase but darkness enhanced this activity. Actinomycin D has also been found to be inhibitory to movement suggesting possibly an association with the messenger RNA synthesis. In presence of light even at O°C phosphorylation of a protein moiety has been detected. This together with the inhibition of ATPase in presence of light also indicates the involvement of light in the movement of *Desmodium* leaflets.

B.4. Enzyme chemistry and Biochemistry

B.4.1. *Host-virus Relationship*

Work has been initiated at the biochemical level to study the relationship between the bacterial host *Salmonella typhimurium* and its lysogenising virus PLT22. For comparative studies parallel experiments were carried out with the virulent virus T2h on its host *Escherichia coli B*. Considerable informations are available in the literature in the latter case. To determine the extent of lysis and lysogency *S. typhimurium* cells were infected at various multiplicities and the optical density of the suspension as well as the plaque forming units (PFU) of the lysate were measured, the latter by the agar plate technique. On increasing the multiplicity of infection from 0.6 to 13.0 there was decreasing amount of lysis. Multiplicity of infection had, however, very little effect on the production of the virulent phage

T2h. Enzyme induction studies have also been carried out in *S. typhimurium* infected with the temperate phage. Method has been developed to study an ADP-P^{32} exchange reaction in the toluenised cells and the level of this enzyme was boosted up several fold in the infected cells. This exchange activity has been partially purified and its characteristics have been studied. It catalyses both ADP-P^{32} as well as ATP-P^{32} exchange reactions but the sole product of phosphorolysis of poly A with P^{32} has been established as ADP^{32} indicating clearly that the fraction must have polynucleotide phosphorylase activity. Further purification is in progress.

The effect of the two well-known antibiotics, actinomycin D and mitomycin C on the production of both the virulent and temperate viruses are being studied in detail. The effects of these antibiotics on the growth of the hosts were first determined. The Gram-negative microorganisms are known to be insensitive to the action of actinomycin D. This was confirmed in both the cases but interestingly enough, slight growth promoting activity was also observed. This was substantiated by the cell-counting method. As expected, actinomycin D had detrimental effect on the production of viruses. Similar studies have been carried out with mitomycin C which was found to have considerable growth-inhibiting activity. This, however, at low concentrations enhanced the production of T2h almost two-fold. The mechanism of this stimulation is being investigated in detail. Some of these results were presented at the Symposium on 'Frontiers of plant science', organised by the Botanical Society of West Bengal and held at Calcutta in February, 1964.

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

In the last year's report (1962-63) the preliminary results obtained in connection with the studies of the induction of L-arabinose isomerase in *Lactobacillus plantarum* were presented. The details have been published in Biochim. Biophys. Acta. (**85,** 152, 1964). In the present year the effect of the antibiotics and the mutagens which selectively affect either the DNA or the messenger RNA synthesis was studied. The influence of those on the growth of the microorganism was first investigated. The final concentrations chosen were in the ranges which were not detrimental to the growth of the organism. At a concentration of 0.1μg/ml of actinomycin D the enzyme induction was completely inhibited though it took about 15 minutes for the exertion of the effect. These results indicate that the messenger RNA already synthesised can carry out its function for about 15 minutes. The induction of L-arabinose isomerase, however, was insensitive to mitomycin C which was expected from the fact that though mitomycin C inhibits the production of DNA it does not interfere with the production of messenger RNA. Similarly, fluorouracil was found to have practically no effect on the enzyme induction. The effect was discernible only after long hours of incubation. The inhibitory effect of fluorouracil as an analogue is due to its wrong base pairing property which is displayed rather infrequently. Further, the wrong bases introduced into a particular messenger RNA may not be harmful to the function of the messenger RNA. Thus, in contrast to the action of actinomycin D the effect of fluorouracil is not "all or none" phenomenon. The effect of fluorouracil observed after long periods was in parallel with the growth inhibitory action of the analogue.

To investigate whether the lag period required for the induction of isomerase had any thing to do with the induction of a specific permease, C^{14}-arabinose was used as inducer. The study was handicapped due to the fact that the inducer itself is actively metabolised by the organism. Attempt is being made to prepare analogues which will act as inducers but not as substrates. Preliminary results obtained with C^{14}-arabinose in short term experiments clearly indicate that the induction of L-arabinose isomerase is preceded by the induction of L-arabinose permease. The induction of the permease was repressible by glucose.

Induction of another enzyme, mannitol-l-phosphate dehydrogenase was also reported earlier. A preliminary communication (J. Bact., **87,** 1247, 1964) containing these results has been published. The details of the induction of this enzyme as well were investigated. Though the action of actionomycin D was more or less the same as that in the case of isomerase yet there was significant difference in the times involved for the exertion of the effect. It was about 2 hours in contrast to only 15 minutes in the latter case. It is quite tempting to speculate that the messenger RNA for the synthesis of mannitol-l-phosphate dehydrogenase was much more long-lived. The dehydrogenase was also found to be less prone to the catabolite repression. The details are being analysed to throw light on the nature of the actual repressors and their modes of action. Since the different sugars and alcohols exert differential effects on the syntheses of the two enzymes it may be possible to pin down the differences in their modes of repression. These results were presented at the International Symposium on Nucleic Acids held at Hyderabad in January, 1964.

B.4.3. *Purification and properties of mannitol-l-phosphate dehydrogenase*

The enzyme mannitol-l-phosphate dehydrogenase which is induced in *Lactobacillus plantarum* by mannitol has been purified about 160 fold starting from the sonic extracts of the microorganism. The reaction catalysed by the enzyme as followed by the measurement of DPNH oxidation using fructose-6-phosphate as substrate had optimum *pH* around 5.0 and was stimulated by phosphate. At *pH* 6.0 the equilibrium of the reaction was in favour of the formation of fructose-6-phosphate: The Km of the enzyme for fructose-6-phosphate has been calculated as 1.36×10^{-3}M. The product of the reduction of fructose-6-phosphate has been tentatively identified as mannitol-l-phosphate. Since the enzyme mannitol dehydrogenase which is involved in the metabolism of mannitol is absent in the mannitol-grown *L. plantarum* it is suspected that the enzyme mannitol-l-phosphate dehydrogenase must be involved in the metabolism of mannitol by the microorganism. It is natural to expect that a kinase phosphorylating mannitol must be the first enzyme involved the pathway of mannitol metabolism by *Lactobacillus plantarum.* It has also been observed that glucose-grown cells have to be adapted to fructose. Mannitol-grown cells, however, grow without adaptation to fructose. But on the contrary fructose-grown cells need adaptation to mannitol. Interrelationship between fructose and mannitol metabolism is being studied. Thise results were presented at the Annual General Meeting of the Society of Biological Chemists held at Bangalore in May, 1964.

B.4.4. *Metabolism of lipoic acid*

Last year (1962-63) an assay method of lipoic acid activating enzyme based on the formation of ATP^{32} from adenyl lipoate and $P^{32}P^{32}$ was reported. The preliminary results

have already been published (Biochim. Biophys. Acta., **85,** 333, 1964). Acetate activating enzyme was found to be absent in the partially purified fraction. Further, from the existing knowledge regarding the reversal of amino acid activation reaction it appears unlikely that any amino acid activating enzyme will carry out the reversal of the lipoic acid activation reaction. Attempt is being made to purify an amidase from the extracts of *A. vinelandii* which will split lipoic acid from the holoenzyme. This will facilitate the study of the process of attachment of the activated lipoic acid to the apoenzyme.

A dihydrolipoic dehydrogenase has been purified 40-45 fold starting from the extracts of mung mean seedlings. This enzyme is diphosphopyridinenucleotide-linked. The rate of reaction with dihydrolipoic acid is slightly faster than that with dihydrolipoamide. Attempts to demonstrate the reversibility of the reaction with either lipoic acid or lipoamide as substrate failed. The enzyme displays maximum activity at *pH* 8.5 in TRIS buffer with dihydrolipoic acid as substrate. With dihydrolipoamide the *pH* optimum is 8.0 in TRIS buffer. The dehydrogenase is inhibited by PCMB but not by arsenite.

Studies on lipoic acid metabolism necessitated the preparation of S^{35}-labelled lipoic acid. The chemical synthesis of S^{35}-labelled lipoic acid being rather complicated, attempt was made with success to prepare it biosynthetically. *Azotobacter vinelandii* cells were allowed to grow on a synthetic medium containing radioactive sulphate. After 18 hours of growth, the cells were harvested by centrifugation and were treated with trichloroacetic acid. The precipitated protein was thoroughly washed and then hydrolysed with 6 N HCl in sealed tube at 110°C for 16 hours. The protein hydrolyzate thus obtained was extracted with benzene with the addition of small quantities of cold lipoic acid as carrier. The benzene extract was evaporated to dryness at room temperature in a flash evaporator. The residue was the crude preparation of S^{35}-lipoic acid which was purified further by extraction with sodium bicarbonate and by paper chromatography. The product thus obtained was identified as S^{35}-lipoic acid by the autoradiographic procedure. The sample was chromatographically pure. The metabolism of S^{35}-labelled lipoic acid will be studied in microbial systems.

B.4.5. *Glutamine activating enzyme from Azotobacter vinelandii*

The first step in protein synthesis is catalysed by the amino acid activating enzymes, each amino acid having its specific activating enzyme. The question whether separate activating enzymes exist for glutamate and glutamine however, remained obscure. Attempt was therefore made to purify glutamine activating enzyme and to test its capacity for activating glutamate. The sensitive ATP-$P^{32}P^{32}$ exchange reaction was used as the assay method for the activating enzymes. Dialysed extracts of sonically ruptured *A. vinelandii* cells showed both glutamine and glutamate-dependent ATP-$P^{32}P^{32}$ exchange activities, the former being much stronger. By ammonium sulphate and acetone fractionations, the glutamine activating enzyme was purified about 12 fold. This partially purified preparation had very little glutamate-dependent exchange activity. Furthermore, the relative rates of glutamine and glutamate-dependent exchange activities changed with the purification steps. These results indicated the presence of separate activating enzymes for

glutamine and glutamate. Further studies involving S-RNA-binding of C^{14}-glutamine and C^{14}-glutamate are envisaged.

B.4.6. *Glutamotransferase and glutamine synthetase in Azotobacter vinelandii*

During the investigation of glutamine and glutamate activating enzymes in *A. vinelandii*, the feasibility of the use of the hydroxamate method for the assay of the enzymes was tested. *A. vinelandii* extract was found to catalyse the formation of hydroxamate in presence of both glutamate and glutamine in an assay system for activating enzymes. By a follow up., two enzymes were found responsible for the formation of hydroxamate, the glutamotransferse and the other, glutamine synthetase. The first enzyme required no nucleotide or metal ion for its activity, while the latter required ATP and Mg^{++}. The latter enzyme, namely glutamine synthetase has been purified 25 fold from *A. vinelandii* extracts by ammonium sulphate fractionation, gel adsorption and heat denaturation precedures. This purified enzyme has very little, if any, glutamotransferase activity. Its properties have been studied in detail.

B.4.7. *Coversion of shikimic acid to aromatic amino acids in mung been seedling extracts*

It has been demonstrated with the quantitative analytical methods that the extracts of mung been seedling are capable of catalysing the conversion of shikimic acid to the aromatic amino acids, phenylalanine and tyrosine. Tyrosine was estimated by a colorimetric method based on the reaction with 1-nitroso-2-naphthol. A crude acetone powder prepared from the cells of *Streptococcus faecalis* served as the source of decarboxylase for phenylalanine. and glutamate were required for the maximum conversion of shikimic acid to aromatic amino acids. No search was made for the presence of the individual enzymes involved in this process but from the requirements of the system it may be concluded that the conventional steps as demonstrated in microorganisms must also be operative in these extracts. Though this conversion is a multistep process and some of the later steps are rather obscure, it can be concluded that all the enzymes catalysing the different steps are quite active in the extracts as the formation of aromatic amino acids can be readily demonstrated. The characteristics of the system were studied in detail.

B.4.8. *Isolation of nucleic acids and their separation on the methylated serum albumin column*

In sharp contrast to other microorganisms DNA from *Azotobacter vinelandii* can be readily extracted by the phenol method. The simple method consists of shaking the cells with aqueous phenol and subsequent addition of alcohol. Native DNA which precipitates in a fibrous form can be lifted out with the help of a glass rod. This was, however, found to be contaminated with considerable amount of RNA. So it had to be subjected to fractionation by CETAVLON and final purification through the methylated serum albumin column. The fractionation of nucleic acids on methylated serum albumin, commonly referred to as Hershey column has been found to be extremely useful in the separation of DNA and different classes of RNA. As mentioned below, it has been successfully used in the identification of the radioactive polyribonucleotides newly synthesised under *in vitro* conditions.

B.4.9. *Polyribonucleotide metabolism in microorganisms*

The purification and properties of an enzyme fraction prepared from the sonic extracts of *Azotobacter vinelandii* which catalyses the soluble RNA-dependent nonterminal incorporation of ribonucleotides into polyribonucleotides were reported last year (1962-63). Preliminary results have been published (Biochim. Biophys. Acta., **76,** 480, 1963) and the details have been presented at the International Symposium on Nucleic Acids held at Hyderabad in January, 1964. Recently Weiss and his coworkers (J. Biol. Chem., **239,** 186, 1964) have reported detailed results regarding the polyribonucleotide-primed synthesis of polyribonucleotides as catalysed by RNA polymerase isolated from the extracts of *M. lysodeikticus*. Similar observations were reported by Krakow and Ochoa (Proc. Natl. Acad. Sci., **49,** 88, 1963). In the present investigations, however, it appears that the RNA polymerase activity and the soluble RNA-dependent activity tend to separate from each other during fractionation procedure. It is not clear whether the fractionation leads to the alteration of RNA polymerase which preferentially catalyses the polyribonucleotide-dependent incorporation of ribonucleotides. Detailed studies to throw light on this are in progress.

Another enzyme fraction has been obtained from the extracts of *A. vinelandii* which catalyses the incorporation of radioactive guanylate residues alone into polyribonucleotide material. The incorporation was absolutely dependent on the presence of DNA, increasing amount of DNA leading to increased amount of incorporation. Addition of other nucleoside triphosphates did not stimulate the incorporation. The incorporation was insensitive to the action of actinomycin D which makes it unlikely that DNA is acting as a template for the synthesis of the homopolymer. The other possibility that the polyribonucleotide chain is built up at the end of DNA chain has to be established by degradation studies. Since actinomycin D combines with the guanylate residuces of the DNA and the cytidine residues must be involved in the priming of the guanylate homopolymer template function of DNA can not be completely ruled out. The mechanism of 'template' action could be as that suggested by Kornberg who visualises the slipping of the template during the priming.

B.4.10. *Polyribonucleotide metabolism in mammalian systems*

Due to the claim that the nucleoli of the mammalian cells may be the seat of ribosomal as well as soluble RNA, the programme of work involving the studies of the syntheses of polyribonucleotides in rat liver nuclear and subnuclear fractions has been initiated. This work has been undertaken according to the suggestion of Dr. Sirlin of the Institute of Animal Genetics, Edinburgh, who is carrying out similar work at the cytological and cytochemical level. The nucleii prepared from livers of rat perfused with saline were found to incorporate P^{32} in presence of an energy source like glucose. Glucose could be replaced with adenosine but not with adenine. The incorporation continues linearly with time up to about 60 minutes and then the rate starts to fall off. Indications have been obtained that this could be due to the presence of polynucleotide phosphorylase activity present in the rat liver nuclear preparations. The radioactive polyribonucleotide isolated by the methylated serum albumin column was found to be of low molecular weight in nature which could be due to phosphorolysis. That the nuclear preparations contained polynucleotide phosphorylase

activity was demonstrated by the ADP-P^{32} exchange which was stimulated by the addition of poly A. The product of phosphorolysis of Poly A with P^{32} was isolated and identified as ADP^{32}. Attempt to purify this activity failed as major portion of the activity was found to be associated with the particulate fraction obtained by the disruption of the nucleii. An aggregate enzyme prepared according to the method of Weiss (Proc. Natl. Acad. Sci., **46,** 1020, 1960) was found to contain polynucleotide phosphorylase activity.

That the P^{32} was incorporated nonterminally into polyribonucleotide of the nucleii was established by the degragation of the isolated material by alkali. All the four bases were found to be radioactive. This incorporation was sensitive to actinomycin D. At a concentration of 50μ g/ml of the antibiotic about 90 per cent of the incorporation was inhibited. Residual polyribonucleotide synthesised under these conditions is being analysed so as to establish whether the incorporation under these conditions is only terminal or partly nonterminal as well. The interesting observation has been made that actinomycin D interfers with the formation of radioactive mucleotide pool as well. A subnuclear fraction has been prepared by brief sonication of the nucleii and following the method of isolation of the nucleoli. This preparation was also found to be capable of incorporating P^{32} though at a much diminished rate in comparison with that of the nucleii. In the former case, however, the incorporation attains saturation level and does not fall off. This was most probably due to the fact that no polynucleotide phosphorylase activity could be detected in these preparations. All the four nucleotides obtained by the degradation of the newly synthesised polyribonucleotide were found to be radioactive but the incorporation of P^{32} was insensitive to actinomycin D. The disruption of the subnuclear preparations and the study of the polyribonucleotide synthesised with radioactive nucleoside triphosphates in these disrupted preparations are in progress. Some of the above results were presented at the Annual General Meeting of the Society of Biological Chemists held at Bangalore in May, 1964.

B.5. Analytical Chemistry and Instrumentation

B.5.1. *Gas-chromatography:*

(a) *Construction of all-glass flame-ionisation detector:* An all-glass flame-ionisation detector has been constructed to detect and estimate very minute amount of effluent from the gas-chromatographic column. This consists of a capillary tubing, one end of which can be attached to the chromatographic column and carries a side tube for the introduction of hydrogen. To the other end is attached a hypodermic needle cut to suitable length. This needle acts as the micro-burner. The burner is surrounded by a glass-chimney. Two platinum wire rings 1.5 cm. in diameter acts as the two electrodes. These are fused to the chimney parallel to one another. The lower ring forming the negative electrode surrounds the tip of the burner the other ring remaining 1.5 cm. above the lower one constitutes the positive electrode. The effluents for the chromatographic column are mixed with hydrogen and lighted to form a micro flame. A potential difference of 150 volts is applied across the electrodes from a battery of dry cells. The change in the current produced by thermal ionisation of effluent gases is recorded by means of a pen recorder. The detector has been found to be highly sensitive. Tests with methyl alcohol, *iso*-propyl alcohol, hexane

etc. indicated that 1 microliter amount of each of these can be easily detected. Good linearity between the amount of the sample and detecter response extends upto 5 microlitres of the sample. Very small dead volume between the column-end and the burner-tip has minimised band spreading. Another advantage of the detector is that it has a very low noise level.

(b) *Influence of column packing parameters on the efficiency in Gas Chromatography:* The efficiency of any particular column in gas chromatography is directly related to the number of theoretical plates per unit length of the column. As such, this number gives directly the efficiency of the column. This quantity depends upon a large number of parameters, such as, temperature, column material, rate of flow of the carrier gas, degree of column packing etc. This value can be calculated from the geometry of the recorded peaks obtained in a gas chromatogram. Experiments have been conducted in our laboratory to determine the effect of the degree of packing on this value. In order to do this other variables are kept constant. Three different liquid phases have been used in this set of experiments. They are dinonyl phthalate, didecyl phthalate, tricresyl phosphate. The same U-shaped 200 cm long glass-column was used in all the cases. The column materials were prepared by intimately mixing the individual liquid phases with the solid support in the ratio of 1:10. The solid support used throughout was 'Chromsorb-P' (special grade celite). For each material chromatograms were taken using columns with different degrees of packing. The degree of packing was varied by packing the column upto the same volume using different amounts of the packing material. The same volume of *n*-Hexane was used in all the cases as the sample. For each packing 12 observations were made and the mean value of the number of theoretical plates per unit length was calculated. The *height* of the theoretical plate values against gms of packing material per cm. of the column exhibit in all the cases a maxima. Works on other liquid phases and solid support is on progress.

B.5.2. *Paper electrophoresis of globulin fraction of Maize Protein*

The protein fraction from three different varieties of maize namely Giant-white, Golden-bantam and Sugar-corn has been extracted by shaking the finely ground seed powder with buffer. Initially the extraction of the proteins from the powdered seed was tried with different buffers containing different amounts of sodium chloride. In this set of experiments it was found that the maximum amount of protein could be extracted with 0.1M phosphate buffer of *pH* 7 without any sodium chloride. The powdered seed was mixed with requisite amount of buffer (4 ml/gm) and stirred for 12 hours at about 4°C. The mixture was centrifuged, filtered through filter-pulp, and the globulin fraction from the clear solution was precipitated by saturating the solution to 70% with ammonium sulphate. The precipitate was centrifuged and dissolved in veronal buffer *pH*—8.6, ionic st. 0.05. Paper electrophoresis was carried out for 6 hours at 180 volts in veronal buffer of 0.05 ionic st. at *pH* 8.6. The globulin fractions from Giant-white and Golden Bantam were separated into three distinct bands whereas, the globulin from Sugar-corn showed only two bands. In order to find out the nature of these three bands the globulin fraction obtained from the Giant-white variety 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 hours. A precipitate was found to form in the bag which was recovered by centrifugation, dissolved in veronal buffer and subjected to paper electrophoresis under similar conditions as above. Only two bands were found in this case, the slowest moving band having disappeared. The supernatant obtained after centrifuging the precipitate from the dialysis bag was also subjected to paper electrophoresis and all three bands were obtained. These results show that one of the fraction of the globulin obtained from the seeds of Giant white variety of maize can be partially separated from the other two by dialysis. Starch-gel electrophoresis of the globulin fraction has been tried. A preliminary run with the globulin fraction from Golden-bantam showed three bands. This work is on progress.

B.5.3. *Paper electrophoresis of serum proteins and serum transaminase activities in Hepatopatheis*

The study of paper electrophoresis of serum proteins along with activities of such transaminases of serum as glutarate—oxalacetate transaminase, glutarate-pyruvate transaminase is being carried out in order to evolve a device for the diagnosis of different hepatopathies (liver-diseases) usually occurring in this country.

The serum samples from 220 patients suffering from different hepatopathies has been tested uptil now. The samples were obtained from Calcutta Medical College Hospital, and Chittaranjan Cancer Hospital. The case history of each patient has also been taken. The different types of hepatopathies that have been studied can be grouped as follows:

(1) Inflammatory-of bacterial, viral or parasitic origin
(2) Degenerative—mainly cirrhosis and post necrotic scarring
(3) Malignancy
(4) Indirect—either mechanical or from any distant organic lession.

Paper electrophoresis has been carried out using veronal buffer *pH* 8.6, ionic strength 0.05 for 4 hours under a potential difference of 240 V across a 24 cm. × 9cm. strip of Whatman no. 1 filter paper. Three samples of serum 0.005 ml each were applied on the strip. The strips were dried and stained with bromophenol blue and washed with acetic acid solution. Of the three different electrophoretic patterns thus obtained from each strip one was kept for visual examination for any change in the pattern from the normal and from the other two the individual bands were cut and eluted in 0.01 N NaOH solution and the colours were measured at 575 mu in a spectrophotometer and from the optical density the content of each band was evaluated using proper corrections.

For the estimation of serum glutarate-oxalacetate transaminase, glutarate-pyruvate transaminase, the method outlined by Frankel & Reitman was used. The principle of the method was to incubate a known volume of the serum with specific substrtate for the two transaminases and to estimate the amount of keto-acid formed from the amino acids in the substrate for a specific time. The estimation of the keto-acids was accomplished by converting them to their respective 2:4 dinitrophenyl hydrazone and measuring colorimetrically the hydrazone colours produced by alkali. The activity of transaminase was expressed in arbitray unit used by Frankel and others. For each samples the total protein

per ml. of the serum was estimated by Biuret method in order to express all the results in terms of gms. of total proteins if required. The results obtained with about 200 cases showed that typical electrophoretic patterns along with departure of activities of transaminase from normal were obtained for each clinical entity investigated.

In some cases, particularly those of malignancy, liver biopsy was done and histopathological studies of the specimen were carried out. In each case, anticipated results were corroborated.

B.6. Plant Biochemistry

B.6.1. *Habituated tissue:*

The strain of habituated tissue isolated previously from normal Hollyhock (*Althaea rosea*) tissue was maintained under cultural conditions. During this period of maintainance it was observed that the rate of growth further declined. It was found that addition of potassium chloride and sodium nitrate in Gautheret's medium acelerated the stimulatory effect of auxin on habituated tissue. These two salts were therefore incorporated in the Gautheret's basic medium for routine maintainance of the tissue.

There are contradictory reports regarding capacity of habituated tissues to form tumours on grafting to healthy hosts. To determine the nature of the isolated strain, the normal, gall and habituated tissue pieces were grafted on healthy hosts using both single and double slit method. Graft tumours developed only in case of gall tissue pieces while both normal and habituated fragments fused with the host tissue but did not develop into tumourous overgrowth. In another experiment, at the time of grafting, lanolin paste containing auxin was applied at the grafting sites. In one set the plants were decapitated and auxillary buds, if any, were removed in order to eliminate, as far as possible, the major sources of naturally occurring growth substances. While in the second set this was not done. Typical graft tumours developed from gall tissue fragments. Where lanolin paste was applied the graft tumours appeared to grow at a slower rate in the beginning. In case of both normal and habituated tissues there was pronounced swelling of the grafted fragments but they failed to grow further and no tumour-like structure was formed. The swelling of grafted fragments was more pronounced where the plants were not decapitated. These experiments support the view that habituated tissues are incapable of tumour formation when grafted on healthy hosts.

Attempts to isolate habituated tissues from *Coreopsis lanceolata* and *Vicia faba* were not successful. The tissues could be grown only for a period of 2 to 3 months without exogenous auxin in the culture medium.

B.6.2. *Normal and Crown Gall tumour tissue*

Experiments were carried out to verify, the report of Braun and 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 four salts, viz., KCl, $NaNO_3$, NaH_2PO_4 and $(NH_4)_2SO_4$ and inositol. For these experiments Hollyhock normal tissue grown in modified tobacco medium containing 2-4-D and coconut milk was used. When these substances

were added to the basic medium a marked increase in the rate of growth of normal tissues resulted. When individual salt was omitted, one at a time, absence of KCl and $(NH_4)_2\ SO_4$ resulted in drastic reduction in growth increment. In the absence of 2-4-D and coconut milk the fortified medium could support the growth of tissues at slow rate only through three successive transfers of twenty eight days interval. In the above medium the growth rate of gall tissue slightly increased but the increase was not significant.

B.6.3. *Single Cell Culture*

Single cell cultures of carrot phloem have been successfully cultivated by Steward and others. Though this technique has been used for a number of years no report is yet available in case of successful cultivation of single cell cultures of gall tumour tissue. With this view, an apparatus on the same principle of 'Auxophyton' have recently been constructed and experiments are being undertaken to obtain single cell cultures of *Vicia faba* gall tissue.

C. BOTANY

C.2. Plant Physiology

C.2.1. *Electrical impulse in ihe petiole and mid-rib of the leaflet of Desmodium and its correlation with the movement of the pulvinule*

It was reported last year that an electrical impulse appears at the petiole and the midrib of the leaflet accompanying the movement of the pulvinule. It was possible to detect this by the attachment of an improvised optical system with the arm of the pen recorder which further magnified the record of the electrical impulse through the amplifier, by about twenty times. Preliminary observations were made by following the spot of light from the optical lever on a moving drum. Later on, arrangements were made to record the electrical changes more accurately by photographic method.

Study was first made by recording simultaneously the electrical changes of the pulvinule and of the midrib or of the petiole by two electrode connections. The electrical impulses given by the midrib and the petiole were of opposite sign to that produced in the pulvinule. In place of negative impulse produced in the pulvinule there was a positive one in those regions. This positive impulse was more pronounced in the midrib than in the petiole. Magnified recording showed that besides the electrical change correlated with the movement of the pulvinule, there are other smaller but sharp impulses occurring quite frequently, which may be attributed to be due to the pulsating activity of the living cells.

Simultaneous appearance of impulses of opposite sign at the pulvinule and at other regions indicated occurrence of diverse reactions at the two points. If the negative sign in the pulvinus be correlated with a process of contraction, the positive sign in the petiole or the midrib may be taken as related to an expansive process. Such counter reaction is possible if sap pressure is increased there due to contraction of the pulvinule.

Following the above observations the question arose whether the simultaneous appearance of impulses of opposite sign at regions other than the pulvinule, some artifacts are involved due to the close proximity of electrode connections. Previously it was observed that when two electrodes are placed very close to each other, an impulse initiated at one electrode induces a fake impulse of opposite sign at the other, the real impulse appearing later in the electrode. This additional impulse appearing simultaneously with the impulse at the first electrode, has been found to be due to the artifact of the instrument.

In order to be definite about the real value and sign of the impulses at the midrib and the petiole, studies were made independent of the influence of the electrode connection at the pulvinule. In such observations only a single electrode connection was made at a point in the petiole or in the midrib and the leaf movement was simultaneously recorded in a mechanical recorder. It was found that in such experiment too a positive impulse appears at the midrib and the petiole with the downward movement of the leaf. So far,

a few photographic records have been obtained. For further detailed studies more records have to be taken in the coming monsoon when healthier plants will be available.

C.2.2. *Influence of Pulvinar movement on the Sap pressure of the petiole of Desmodium*

In a previous investigation attempts were made to detect with the help of a micropotometer the variations of sap pressure in the petiole during pulvinar movements, but no such change could be observed. Revelation of electrical changes in the petiole and its correlation with the movement of the leaf, has indicated that there is possibility of a change of pressure in the petiole. The failure to detect it by potometric method might be due to its magnitude being of very small order. It was therefore proposed to employ a more sensitive method to obtain a direct evidence of the change of pressure in the petiole with the movement of the leaf. For this purpose Bose's Plant—sphygmograph was brought into commission with suitable modification.

The feeler of the sphygmograph was placed at a point in the petiole beneath the pulvinule and diametric changes at that point of the petiole were observed by the movement of the spot of light of the optical lever of the instrument. It has been arranged to record the movement of the light spot photographically with a magnification of about 10,000 times.

Indication has been obtained in the preliminary experiment that there occurs a change of pressure in the petiole with the movement of the leaf. Detailed study will be undertaken this year by simultaneously recording the movements of the leaf and diametric changes of the petiole.

C.2.3. *Multiple electrical response in Biophytum*

A paper on the subject has recently been submitted for publication in Trans. Bose Res. Inst. Some important observations in relation to the investigation were completed during the current year.

It has been previously reported that each of the responses is constituted of two impulses, of which the second one appeared to be reflected from the pulvinus, similar to the biphasic impulse appearing simultaneously at different points in the petiole of Mimosa.

That the second impulse is initiated in the pulvinus was more definitely proved by inducing excitatory transmission through the pulvinus in acropetal direction in the petiole. The simultaneously occurring second impulse in the petiolar responses in this condition preceded the action wave propagation through the petiole.

Study of the repeated electrical responses at the point of stimulation gave clue to the gradual increase of time interval in the appearance of the successive responses at the particular point. It was definitely known that the increase of time interval in the successive responses is mainly due to progressive delay in the execution of excitatory discharges from the point of stimulation, and the slight fall of velocity in the transmission through the conducting tissue, plays only a minor role.

A paper on 'Vascular Anatomy of Mimosa leaf' has recently been communicated for publication in the Trans. Bose Res. Inst.

Studies on the diurnal movement of the pinnule of Mimosa reported last year is being continued. The study has revealed the presence of a peculiar morning rhythm. It was proposed to make a detailed study on the rhythm and requisite arrangements are being made.

An investigation in collaboration with the Plant Biochemistry unit has been started on the biochemical basis of contractility in a pulvinule-leaflet unit of Desmodium gyrans. The object of the investigation is to find out whether as in contractile animal muscles, a protein is involved in which the energy of contraction may be due to the break down of an energy rich phosphate compound.

C.3. Plant Breeding

During the year under review, 33 tomato varieties as left by Sri V. S. Sarma, 6 barley varieties from the Institute collection, some imported wheat varieties were kept under cultivation. Special attention is being given to the introduction of indigenous and cultivated forage crops. For this purpose 20 species of grass and 19 species of fodder legumes have been introduced.

From preliminary field-studies still in progress with 20 species of grasses and 19 species of fodder legumes, there are indications that some of these species of grasses and fodder legumes may show promise in the development of new strains suitable to West Bengal conditions. Among these the following introductions are worth mentioning:

C.3.1. *Species of Desmodium* (*D. uncinatum, D. intortum*) made excellent growth. These perennial legumes may be of great importance as pasture plants in developing the fodder position of West Bengal. In our introduction they await critical comparison with other perennial legumes.

C.3.2. *Phaseolus species* (*P. atropurens, P. lathyroides*) thrives well under our condition and provides good summer feed.

C.3.3. *Annual species of Medicago* (*M. sativa*; *M. seutellata*; *M. tribuloides*; *M. hispida*; *M. littoralis*). There is close relationship, between their normal period of growth and the period over which moisture is available in the surface soil. The stand, in some cases, are completely lost through failure to produce seed owing to high temperature and lack of insect pollinator before growth and seed production are complete. Harbinger-clover (*M. littorilis*) was particularly found very promising with respect to fodder-yield for spring months. It is spreading with excellent growth and gives abundant flowering with good seed set.

C.3.4. *Species of Stylosanthes* (*S. gracillis*; *S. humilis*) From field-performances indications are that these may be developed into valuable pasture species.

They will be subjected to thorough testing in the following years. Their performances will be judged in pasture-combination with certain grasses.

S. gracilis somewhat resembles lucern in appearance, but becomes very woody if allowed to grow larger. If kept short it develops into a bushy plant.

C.3.5. *Species of Trifolium* (*T. pratense*; *T. repens*; *T. sub-terranium* etc.) These are mostly native to the humid-temperate zones of north hemisphere/mostly adapted to cool climates. In regions with hot and dry summer like the climate of West Bengal their growth is confined to winter and spring months in absence in irrigation.

C.3.6. Some of the grasses introduced (such as *Melinis minutiflora*, *Paspalum dilatatum*, *P. commersonii*, *Panicum maximum*, *Chloris gayana*, *Cenchous oeliaris*, *Sorghum almum* and sudan grass) were found very promising with respect to fodder yield etc. They provided abundant feed for spring and summer months. Detailed studies on these are in progress.

C.4. Radiation mutations in Jute

C.4.1. *Radiation mutations in Jute*

C.4.1.1. *Treatment:* The dry seeds of the varieties T.M., R.26, C. G. from *Corchorus olitorius* and D.154 from *C. capsularis* were treated with 1.5 mc, 3.0 mc and 6.0 mc and 12.0 mc radioactive P^{32}. The treated seeds along with controls were sown at the Falta Experimental Station of the Bose Institute. The P_1 generation was raised in the year 1962. The seeds were collected separately and sown again in the year 1963 in the month of April to raise the P_2 generation. In the same year X_2 generation was also raised from the seeds collected from X_1 treated plants.

C.4.1.2. *Flowering:* The flowering behaviour of the P_2 populations along with the controls were noted in the field. The first flowering date of 100 plants were noted at random from each treatment. The mean flowering time with S. E. are shown in the table 1(a) and for a comparative study the mean flowering time with S.E. of the previous year is also included in the table. It is seen from table la that the treated populations in variety T.M. were late in flowering in 1962 but this lateness was not maintained in 1963. In variety R.26 there is no difference in flowering behaviour of the plants in both the years. In variety C.G. the treated populations were late in flowering in the year 1962 and this behavouur was maintained in P_2 also. In the last column of table 1a it is seen that in the variety D.154, the treated populations flowered late in the P_1 generation while in P_2 generation some earliness is observed. From table lb it is seen that the treated populations in varieties T.M., R.26 and D.154, flowered late in the X_2 generation, but in X_1 they started flowering early in comparison to their respective controls. Only in case of C.G. the treated populations flowered late in the X_2 generation.

The height of the individual plant in the control as well as in the treated populations in P_2 and X_2 generations were measured. Altogether 100 plants were measured at random in each treatment. The average of measurements in each treatment is shown in tables 2a and 2b. It can be seen from table 2a that the treated populations in all the varieties do not show any significant differences in height with respect to their respective controls. From table 2b it is seen that the treated populations of variety T.M. show an increase in height whereas those of variety C.G. show a decrease an height when compared to their respective controls. In variety R.26, an increase in height is observed in population after 20,000 r treatment and a decrease after 40,000 r treatment.

TABLE 1a

FLOWERING IN DAYS OF P_2 POPULATION (1963) IN COMPARISON TO P_1 (1962), 1a

Variety	T.M.		R. 26		C.G.		D. 154	
Treatment	Mean time±S.E.		Mean time±S.E.		Mean time±S.E.		Mean time±S.E.	
	1962	1963	1962	1963	1962	1963	1962	1963
Control	99.76±0.52	121.40±0.67	90.15±0.85	109.95±0.85	79.60±0.30	104.05±0.78	101.71±0.34	118.55±0.60
1.5 mc	102.08±0.53	120.01±0.88	91.15±0.72	110.40±0.76	84.45±0.30	106.70±0.96	—	—
3.0 mc	106.69±0.62	121.70±0.79	91.95±0.92	108.05±0.77	86.25±0.83	107.95±0.96	—	—
6.0 mc	—	—	—	—	—	—	105.46±0.25	117.95±0.64
12.0 mc	—	—	—	—	—	—	105.92±0.34	116.10±0.79

TABLE 1b

FLOWERING IN DAYS OF X_2 POPULATION (1963) IN COMPARISON TO X_1 (1962) 1b

Variety	T.M.		R. 26		C.G.		D. 154	
Treatment	Mean time±S.E.		Mean time±S.E.		Mean time±S.E.		Mean time±S.E.	
	1962	1963	1962	1963	1962	1963	1962	1963
Control	106.85±1.16	129.70±0.79	122.58±0.87	119.45±1.06	101.30±1.62	117.80±1.09	114.55±0.66	98.70±0.98
20,000 r	133.40±1.30	127.20±0.92	124.30±0.74	115.10±0.89	98.90±1.64	129.40±0.78	118.67±0.63	92.40±0.76
40,000 r	136.04±0.94	123.70±0.83	123.14±1.03	115.25±0.73	106.00±3.03	125.55±0.91	117.80±0.76	94.60±0.86

TABLE 2a

AVERAGE HEIGHT IN CM. OF P_2 POPULATION IN 1963

Treatment J / Variety	T.M.	R. 26	C.G.	D. 154
Control	77.20	104.70	87.20	78.30
1.5 mc	81.70	104.10	82.50	
3.0 mc	77.90	103.40	88.02	
6.0 mc				76.20
12.0 mc				83.50

TABLE 2b

AVERAGE HEIGHT IN CM. OF X_2 POPULATION IN 1963

Treatment / Variety	T.M.	R. 26	C. G.
Control	100.50	129.60	118.90
20,000 r	126.50	136.70	65.60
40,000 r	140.40	126.90	80.90

TABLE 3a

PERCENTAGE OF POLLEN STERILITY OF P_2 POPULATION IN 1963

Treatment / Variety	T.M.	R. 26	C. G.	D. 154
Control	1.74	0.34	0.18	0.67
1.5 mc	0.54	0.21	1.53	
3.0 mc	0.76	0.29	0.34	
6.0 mc				1.31
12.0 mc				0.77

TABLE 3b

PERCENTAGE OF POLLEN STERILITY OF X_2 POPULATION IN 1963

Treatment / Variety	T.M.	R.26	C.G.	D.154
Control	0.66	0.40	0.77	1.77
20,000 r	1.15	2.04	3.10	2.20
40,000 r	0.96	1.67	1.25	2.70

C.4.1.3. *Pollen sterility:* The pollen grains from the controls and P_2 and X_2 populations were collected and mounted in iodine solution; 100 observations were made in each treatment. The fertile or sterile pollens were counted and the percentage of sterility was calculated. It is seen from table 3a that in the varieties C.G. and D. 154, sterility percentage is higher among the treated populations. In the previous year it was observed that in all the varieties the populations after treatment showed higher percentage of pollen sterility. Again in table 3b it is observed that the treated populations in X_2 generation has higher percentage of sterility in comparison to their respective controls as in the previous year.

C.4.2. *Investigations on induced mutagenesis in paddy using X-rays and radioactive isotopes*

This scheme was financed by the A.E.C., Govt. of India

C.4.2.1. *Studies on Aus Paddy*

C.4.2.1.1. In the year 1958 seeds of Marichbati and Dular varieties of Aus paddy were irradiated with different doses of X-rays and P^{32}. From the treated population, plants were selected with respect to changes in morphological characters. The selected plants were S. 1, (Black mutant), S. 236 (Awned Marichbati) both from Marichbati variety and Selection S. 240 (White Dular) from Dular variety.

These mutant lines were sown along with their parent plants in a randomised block design in the year 1962 in the P_6 and X_6 generation. There were three blocks in this experiments and each block had 3 replications.

TABLE 4

COMPARISON OF THE AGRONOMICAL BEHAVIOUR OF THE MUTANTS AND THEIR RESPECTIVE CONTROL

Types	Mean Height (cm)	Mean number of Tiller	Mean number of Panicles	Mean Panicle length (cm)	Mean yield/ plant (gm)
Marichbati (control)	144.51	6.41	6.01	22.57	12.50
Dular (control)	150.84	6.34	7.62	24.68	9.10
Black Mutant (S. 1)	149.19	11.65	10.96	23.64	15.17
Marichbati awned (S. 236)	142.61	7.20	6.88	24.45	10.80
White Dular (S. 240)	150.46	4.95	4.60	24.20	8.20
±S. E.	3.31	0.70	0.83	0.45	0.45
C. D. at 5%	—	1.98	2.35	1.27	3.00
C. D. at 1%	—	2.64	3.15	1.70	4.00

C.4.2.1.2. *Height of plants, number of Panicles, Length of Panicles:* The mean plant height of selection S. 1 is taller, and the selection S. 236 is shorter than their parent type Marichbati. The mean plant height of Selection S. 240 and its parent Dular Variety shows very little differences.

The mean tiller number of Selection S. 1 and S. 236 is higher than the parent Marichbati variety. The mean tiller number of Selection S. 240 is lower than its parental type. There is significant difference in tiller number of S. 1 over its parental type.

The mean number of panicles of Selection S. 1 is higher than its parent Marichbati variety as well as of the other types tested. Selection S. 236 is similar to its parent Marichbati Variety. Selection S. 240 gives significant lower panicle number than its parent Dular Variety.

The mean panicle length of the Marichbati variety is lower than the two isolated mutant lines, Selection S. 1 and S. 236.

Selection S. 240 has shorter panicle than its parent Dular variety and also shorter than the other types compared in this experiments. The type Marichbati awned (S. 236) gives lower yield than the parental Marichbati variety (control). The mutant line White Dular (S. 240) also gives lower yield than its parent Dular variety (control).

C.4.2.1.3. *Cytological studies of the mutant line:* The growing root tips of the both control and selections were pretreated with Aesculin for 15 minutes and then washed with water (in some cases no pretreatment was given) and fixed in (1:3) propionic alcohol. The fixed root tips were hydrolysed and stained with (9:1) 2.5% Propionic Orcein and Hcl (N). The stained root tips were squashed in 1% Propionic-Orcein and examined under microscope.

TABLE 5

STUDIES ON THE MITOTIC DIVISION OF CONTROL AND MUTANT LINES

Name of Types	Chromosome Number (2*n*)	% early separate at metaphase	% of lagging chromosomal anaphase
Marichbati	24	0.00	0.00
Dular	24	0.73	0.00
Black Mutant (S. 1)	24	0.00	0.00
Marichbati awned (S. 236)	24	0.00	0.00
White Dular (S. 240)	24	2.76	1.76

From table 5 it is noticed that in the Marichbati variety (Control), there is no chromosomal abnormality except that 0.73% of early seperation of chromosomes was noticed in the metaphase division. Similarly the selection S.1 and 2.236 did not exhibit any chromosomal abnormality.

In the Selection S.240, 2.76% early seperation was noticed. Also in the anaphase it was noticed that a single chromosome showed delayed seperation. The frequency of such delayed seperation was 2.75%.

C.4.2.2. *Studies on Boro Paddy*

C.4.2.2.1. After P^{32} treatment (30 uc/seed) one striped plant was isolated (selection S. 385) from the P_3 generation, whose progeny was maintained for further studies.

In the P_3 generation the progeny segregated for lethal albinos and normal green seedlings. The segregation was tested statistically. (Table 6).

TABLE 6

TABLE CALCULATION OF χ^2 FOR ALBINO SEEDLINGS IN P_3 GENERATION

Frequency	Green	Albino	Total	χ^2
Observed	211.0	71.00	282.00	.009
Expected	211.5	70.5	—	—
Deviation	0.5	0.5	—	—

$0.95 > P > 0.90$

Thus in the P_3 generation the ratio of green to albino seedlings were 3: 1.

In the next generation (P_4 generation) further segreggation of albinos were observed.

C.4.2.3. *Studies in Aman Paddy*

C.4.2.3.1. The mutant (thick grained mutant) was isolated in the P_2 generation (1954) after treatment of seeds of Rupsail variety with P^{32}. The previous year's experiments indicated that the grain yield was higher in this mutant, when compared to its control, Rupsail (vide Ann. Rep. 1962-63.)

Encouraged by the above finding two large scale experiments were conducted at Falta and Shyamnagar Experimental Stations in 1963. Randomised block design with sixteen replications at Shyamnagar and with fifteen replications at Falta was used. Each replication consisted of two sub-plots in which the control (Rupsail) and the mutant were allocated at random. The gross area of each sub-plot was $12' \times 9'$. In both the places, seedlings were transplanted with a spacing of $9'' \times 9''$ and at the time of harvest, a row of border plants around each sub-plot was discarded and dry weights of grain and straw yields of the net area ($7.5' \times 10.5'$) of each sub-plot were taken in kilogram.

Analysis of variances of results of the second year's experiments on grain and straw yields at both the experimental stations are shown in Tables 7 & 8. With respect to grain yield, the mutant could be compared favourably with its mother variety at Falta but it showed a significantly poor performance at Shyamnagar. In the case of straw yield, the mutant had significantly lower yield at both the places.

Obviously more tests, over a number of years, are needed for a proper evaluation of the above mutant.

TABLE 7

ANALYSIS OF VARIANCE (1963)

Unit : Kg/plot

		Falta					Shyamnagar			
		Grain yield		Straw yield			Grain yield		Straw yield	
Source	d.f.	m.s.	F.	m.s.	F.	d.f.	m.s.	F.	m.s.	F.
Replication	14	0.1620	3.27*	0.3719	4.46*	15	0.6821	14.93*	0.6624	1.26
Control vs. mutant	1	0.0010	—	1.9253	23.11**	1	0.4027	8.81**	1.9552	3.73**
Error	14	0.0495	—	0.0833	—	15	0.0457	—	0.5245	—

TABLE 8

MEAN YIELDS OF GRAIN AND STRAW (IN Kg/plot)

	Falta		Shyamnagar	
	Grain	Straw	Grain	Straw
Rupsail (Cont.)	2.24	3.58	1.81	3.99
Mutant	2.26	3.08	1.58	3.50
S. E.	0.058	0.075	0.053	0.181

C.5. Chemical Mutagenesis

C.5.1. Investigations were continued on the mutagenic activity of some more chemicals.

C.5.2. The physiological and morphological effects of EI (Ethylene-imine) treatment on Jute (C. olitorius) were studied and compared to those produced by X-rays. The doses given were 12,000 r and 8,000 r X-rays and 0.03% and 0.05% Ethylene-imine. These doses gave approximately the same germination percentage (i.e. from 80% to 95%—Table 1).

EI treatment was found to induce morphological changes similar to X-rays (Tables 9 and 10). The maximum numbers of abnormalities occurred in the 1st few nodes and thereafter their frequency rapidly declined, till normal or near normal leaves started appearing by the 8th or 9th node. This was found to be true in case of both X-ray and EI treatments and indicate that the sensitive stage in jute seeds extend upto the 8th or 9th leaf.

TABLE 9

OCCURRENCE OF (TOTAL) LEAF ABNORMALITIES AFTER DIFFERENT TREATMENTS

Treatment	Leaf in which abnormality occurred												Total No. of abnormal leaves per treatment	No. of plants studied per treatment
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th		
1. 18,000 r	70	35	15	9	4	6	2	–	–	–	–	–	141	87
2. 12,000 r	17	12	7	8	8	4	1	–	–	–	–	–	57	70
3. 0.05% EIM	42	26	2	9	4	8	4	6	2	–	–	–	103	70
4. 0.03% EIM	7	12	15	14	10	8	4	–	–	–	–	–	70	79
Total	136	85	39	40	26	26	11	6	2	–	–	–	371	306

Variety—T. M.
No. of seeds per treatment = 100
Germination percentage = 18,000 r=94%; 12,000 r=80%; 0.05% EI=95%; 0.03%=80%

In table 10 the different types of leaf abnormalities that were found after treatment such as epiascidium or cup shaped leaves, leaf forking and bifurcation, as well as other leaf abnormalities are presented. These "other abnormalities" include leaves, with changes

in shapes of lamina, apex and margin, and also leaves with chlorophyll defects. Stem abnormalities like fasciation, bifurcation and dichotomous branching have also been observed. At the doses given, X-rays are probably slightly more effective than EI in inducing morphological aberrations at approximately the same level of germination. It is also clear that most of the aberrations found after X-ray treatment are also found after EI treatment. Since such effects have been reported also after auxin treatment by different authors on various plants, (and after X-rays as well), it is suggested that EI (like X-rays) affects auxin synthesis, resulting in morphological abnormalities.

TABLE 10

TABLE SHOWING THE FREQUENCY OF OCCURRENCE (IN PERCENTAGE) OF DIFFERENT TYPES OF LEAF ABNORMALITIES, AFTER DIFFERENT TREATMENTS

Treatment	Nature of abnormality		
	Epiascidium	Bifurcation of leaf	Others
X-rays 18,000 r	7.1% (10/141)*	12.1% (17/141)	81% (114/141)
X-rays 12,000 r	12.3% (7/57)	29.8% (17/57)	58% (33/57)
Ethylene-imine 0.05%	2.9% (3/103)	12.6% (13/103)	84.5% (87/103)
Ethylene-imine 0.03%	—	11.3% (9/70)	87.1% (61/70)

*Figures in brackets give the ratios of the actual number of leaf abnormalities found for a particular type of leaf abnormality to the total number of leaf abnormalities scored in the treatment.

C.6. Cytological and Cytotaxonomical studies

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

C.6.1.1. During the year under report (1963-64) some species in the genus *Hibiscus*. Medik, and one species in the genus *Gossypium*, Linn., under the family *Malvaceae*, were under cytological investigations, as continuation of cytological and cytotaxonomical studies in some genera of the family Malvaceae, reported previously. Palynological studies in some species, in the genera *Sida* Linn., and *Abutilon*. Linn., were also carried out.

As reported earlier the genus *Hibiscus* is one of the largest genera of *Malvaceae*, of which 150 species have so far been identified. Many of these species have not yet been cytologically and cytotaxonomically studied because of difficulties in ascertaining the exact chromosome morphology owing to their large numbers and also due to the extreme shortness of the chromosomes in these species.

Some modifications in the schedule of pre-fixing and staining procedure were necessary in order to get properly stained material for the study.

Four species of the genus *Hibiscus* and one species of the genus *Gossypium* have been studied in details.

These are: I.A. Genus. *Hibiscus*. Medik.,

(1) *H. micranthus*. Linn.
(2) *H. Pungens*. Roxb.
(3) *H. Sabdariffa*. Linn.
(4) *H. Ficulneus*. Linn.

I.B. *Gossypium*. Linn.

(1) *G. Punctatum*. Sch. et. Thon.

Palynological studies have also been carried in five species in the genus *Sida*. Linn., and in three species in the genus *Abutilon*. Linn.

There are: II. A. *Genus. Sida*. Linn.

(1) *S. Rhombifolia*. Linn.
(2) *S. Rhomboidae*. Linn.
(3) *S. acuta*. Linn.
(4) *S. spinosa*. Linn.
(5) *S. veronicaefolia*. Lam.

II. B. *Abutilon*. Linn.

(1) *A. indicum*. Linn.
(2) *A. hirtum*. Don.
(3) *A. avicenae*. Gaevtn.

C.6.1.2. A. *Hibiscus*. Medik

C.6.1.2.1. *H. micranthus*. Linn. Plants are shrubs with slender rod-like spreading branches, flower pink, sepals lanceolate, corolla reflexed, anthers whorled, capsule globose, seeds cottony. Generally found in the hotter parts of India; distributed throughout Tropical Africa, Arabia. Garden plants; green fruits are sometimes eaten.

Karyotype analysis of this species reveals that it has a $2n$ complement of 72 chromosomes, which are fairly large. Foreshortening and anastomosis of the chromosomes at the metaphase stage are the common obstructions in getting a well spread metaphase plate for detailed studies. With some modifications in the pre-fixing schedule these difficulties could be overcome. Chromosomes have median to sub-median primary constrictions. There are four pairs of satellited chromosomes. Four parts of nucleoli at telephase as well as eight chromosomes attached to a nucleolus at the early prophase stage were observed using the Feulgen Fast green technique, showing their relationship with the satellited chromosomes.

Earlier worker (Skovsted, 1941), has reported 64 as the $2n$ number in this species. This difference from the present investigation may be attributed to the presence of ecological type.

C.6.1.2.2. *H. Pungens*. Roxb. Annual shrubs, stems erect: leaves cordate, roundish, deeply palmately—7 lobed: calyx 5-toothed, corolla yellow with purple centre. capsule

oblong, pointed, hispid, 5-seeded. Distributed in tropical Himalayan region, from Kumaon to Sikkim, and Khasia hills.

The $2n$ complement of this species consists of 72 very short chromosomes having primary constrictions varying between median to sub-median. There are four pairs of satellited chromosomes of which two pairs have long stalks. Eight chromosomes are attached to a nucleolus at prohpase and the occurrence of eight nucleoli at telophase observed by Feulgen Fast green technique, showed their relationship with the secondarily constricted chromosomes.

Somatic bridges, and laggards were observed as occasionally occurring abnormalities at anaphase.

The chromosome number of this species is being reported for the first time.

C.6.1.2.3. *H. Sabdariffa*. Linn. Annual shrubs, stem erect. stem and leaf-veins bronze coloured, penduncles solitary, axillary, shorter than petiole: sepal deltoid, connate below the middle, transforming into a purplish fleshy cup: corolla yellow, capsule ovoid, pointed, seeds reniform. Generally cultivated in the hotter parts of India and in Ceylon; distributed in Tropics.

This type although taxonomically classified as one with the type reported last year, without assigning any varietal name to it, differs in many respects in their morphological characters, the most important of which is the colour of the fleshy calyx which is edible as pickles and in curries. It is purple when mature in this variety, while it is whitish in the type reported earlier. The plants are bushy in the purple variety but it is long and slender with few branches in the other one. The purple type is cultivated more for the purpose of the kitchen, while the white variety yields very good bast fibre.

This type has a $2n$ complement of 72 chromosomes. Lengths of the chromosomes vary from long to short types; short ones being greater in number. Four pairs of secondarily constricted chromosomes have been recorded. Eight small nucleoli found at the telephase and eight chromosomes attached to a nucleolus at prophase confirm the origin of the nucleoli from the satellited chromosomes.

Abnormalities like somatic bridges and laggards at anaphase and occurrence of hyperploid number were recorded in a few cases.

C.6.1.2.4. *H. Ficulneus*. Linn. This is the same species as reported last year. Further studies in details were necessary in order to establish normally occurring $2n$ numbers because of the difference of the reports of the earlier worker (Skovsted, 1941). With some modification in both pre-fixing and staining technique detailed studies could be possible. $2n$ complement shows 112 very short chromosomes having median or sub-median primary constrictions. Secondary constrictions exist in six pairs. Feulgen Fast green staining reveals the presence of six pairs of small nucleoli at the telophase stage and twelve chromosomes attached to a nucleolus at the prophase stage. This also confirms the origin of nucleoli from the satellited chromosomes.

Abnormalities like the presence of cells with 116 and 122 chromosomes at metaphase and occurrence of laggards, extrusions and bridges at anaphase were found occasionally.

C.6.1.3. *B. Gossypium.* Linn.

C.6.1.3.1. *G. Punctatum* Sch. et. Thon.

The centre of origin of this species is probably in South Mexico. Both cultivated and wild forms are known in that country. It has small bolls, flowers and leaves. Plants are shrubby, annual or perennial.

The species has a $2n$ complement of 52 long and medium size chromosomes. Primary constriction varies between median to nearly sub-median and sub-median. Three pairs of secondarily constricted chromosomes are found. Six small nucleoli at telophase and six chromosomes attached to a nucleolus at prophase were recorded, and establish their relationship with the secondarily constricted chromosomes.

Somatic bridges and laggards at anaphase and hypo- and hyper-ploid numbers at metaphase were found to occur occasionally as cases of abnormalities.

C.6.1.3.2. *Palynological study*

C.6.1.3.2.1. Genus *Sida.* Linn.

(1) *S. Rhombifolia.* Linn.
(2) *S. Rhomboidae.* Linn.
(3) *S. acuta.* Linn.
(4) *S. spinosa.* Linn.
(5) *S. veronicaefolia.* Linn.

Study on the pollen morphology in the five species mentioned above show the following characters.

Polyporate, spheroidal with undulated surface; spinous, spines with broad globular base and sharp points; bacula not uniform, with broad apical region and gradually tapering down and vice versa; sexine tectate, crassi sexinous, scabrate.

C.6.1.3.2.2. Genus. *Abutilon.* Linn.

(1) *A. indicum.* Linn.
(2) *A. hirtum.* Don.
(3) *A. aveicenae.* Gaevtn.

Pollen grains of these species are: triporate, spherodal with undulated surface: spinous; spine with broad globular base and tapering into sharp point; crassi sexinous, sexine scabrate, bacula not uniform, having broad apical region and conical shaped.

C.6.2. *Cytology of Trigonella*

C.6.2.1. During the year 1963-1964 studies the on cytology of the genus *Trigonella* have been taken up. Earlier reports on the cytology of the genus show that the number of somatic chromosomes is 16 for most of the species with few exceptions. The basic number for different species have been variously reported as 8,9, 11 and 14. The present work deals with a detailed study of five species and three varieties of the genus. They are, (1) *T. foenum-graecum*, var. E.C. 18290 (2) *T. foenum-graecum* var. I.C. 74 (3) *T. foenum-graecum*, var. I.C. 1978 (4) *T. corniculata*, (5) *T. calliceras* (6) *T. coerulea*, (7) *T. polycerata.*

For the study of mitotic chromosomes, seeds of different species and varieties were germinated in Petri-dishes and young healthy root-tips were collected and pre-treated with saturated solutions of para-dichlorobenzene for 1½ hours at 16°C. Of the other pre-treatment chemicals tried, 0.2% colchicine for ½ hour gave satisfactory result. After pre-treatment the root tips were fixed in acetic-alcohol (1:2) at room temperature for 1 to 2 hours. They were then stained in 2% propiono-orcein and NHCl in the proportion of 9:1 for 2½ hours. Small portions from root-tips were cut and squashed in 1% propiono-orcein.

Excepting *T. polycerata* where there are 44 chromosomes (reported earlier as 28, 30 and 32), all other species and varieties have a somatic complement of 16 chromosomes.

The three varieties of *T. foenum-graecum* have comparatively larger chromosomes. The total chromatin length of a haploid set are 44.03 μ in E.C. 18290, 47.63 μ in I.C. 74 and 36.61 μ in I.C. 1978. In all individual species and varieties, the size difference of chromosomes though present is not very marked. They form a graded series from longer to shorter ones.

Primary constriction is mostly nearly submedian to nearly median in position in all the materials studied. Subterminal and median primary constrictions are also noted in some members.

Secondary constricted chromosomes of the materials studied, could be divided into three types. Type A,-chromosomes varying from 2.38 to 3.81 μ in length, each provided with two contrictions, primary and secondary; one nearly sub-median and the other subterminal at the end of the shorter arm. Type B,-chromosomes varying from 3.81 to 5.47 μ in length, with two constrictions, one at nearly sub-median position and the other at subterminal position at the end of one arm. Type C,-length of the chromosome 2.74 μ. It is provided with two constrictions, one at the nearly sub-terminal position and the other at the sub-terminal position at the end of the longer arm. An analysis of the chromosome morphology is shown in Table 12.

C.7. Plant Anatomy and Morphology

C.7.1. *Floral Morphology and Cytotaxonomy of some members of Acanthaceae:* There is a growing appreciation of the systematic values of anatomy, cytology, palynology and embryology as tools for classification where systematic botany fails to reach any definite conclusion.

In the previous year (1962-1963) a study of the family Acanthaceae was made in the different branches of Botany viz. anatomy, cytology, palynology and embryology with special reference to the systematic background. During the current year (1963-1964) two branches, namely, anatomy and cytology of some species of the family Acanthaceae have been studied in detail.

C.7.1.1. *Floral anatomy:* The behaviour and the number of vascular bundles have long been recognised as a useful criteria in studying floral morphology which seems to be more important from the taxonomical point of view.

For the study of floral anatomy, eight different species including (1) *Strobilanthes glomeratus* (2) *Barleria prionitis*, (3) *Asystasia gangetica*, (4) *Odontonema nitidum*, (5) *Cardanthera triflora*,

TABLE 11

Species and variety	Number ($2n$)	Length of Largest chromosome in μ	Length of Smallest chromosome in μ	Total chromatine length in μ	Number of secondary constricted chromosome	Position of primary constriction	Nature of secondary constriction
1. *T. foenum-graecum* var, E.C. 18290	16	6.19	4.76	44.03	2	Mostly nearly sub-median	Type 'B'
2. *T. foenum-graecum* var. I.C. 74	16	5.95	4.28	41.63	2	Mostly nearly sub-median	Type 'B'
3. *T. foenum-graecum* var. I.C. 1978	16	5.24	3.81	36.61	4	Mostly nearly sub-median	Type 'A' & 'B'
4. *T. corniculata*	16	3.93	2.38	26.67	2	Mostly nearly median	Type 'A'
5. *T. calliceras*	16	4.05	1.67	24.51	2	Mostly nearly sub-median	Type 'A'
6. *T. coerulea*	16	3.81	2.62	26.76	4	Mostly nearly median	Type 'A' & 'B'
*7. *T. polycerata*	44	3.09	1.67	51.77	4	Mostly nearly median	Type 'A' & 'B'

Investigation on the effect of ra iation on the chromosomes of the different species and varieties is in progress.
*The chromosome number 44 is a new report since it was reported earlier as 28, 30, 32.

(6) *Rhinacanthus nasuta*, (7) *Justicea gendarussa*, (8) *Beloperone guttata* under the different tribes of Lindau's classification have been taken up.

Young flower buds were fixed in Formal: Acetic:Alcohol (F.A.A.), for 24 hours. Dehydration and embedding were done as per normal schedule. Serial sections, 12 to 15 μ in thickness, were made and stained with safranin and light green.

Before considering the taxonomic affinities in light of internal anatomy, it seems better to present in some details the basic plan of the flowers with their variations.

Except for a few instances the flowers of these eight species more or less, follow the same basic plan. The fused calyx at the base receive ten traces of which the alternate five show dichotomy. Though externally the corolla lobes in different species show much variation in forms (sub-equal to bilabiate), the anatomy shows that in all cases, the corolla tube receives five traces for five fused petals. The stamens get one supply each. The external morphology represents the reduction of stamen number from four to two. Internal anatomy reveals the vestige of a morphologically lost stamen in *Barleria prionitis* as well as in *Strobilanthes glomeratus* and both of them possess four fertile stamens. The genera *Asystasia* and *Cardanthera* have four fertile stamens each without having any vestige. In *Odontonema*, out of four staminal traces, two produce fertile stamens while the other two end blindly. *Rhinacanthus*, *Justicia* and *Beloperone* show the presence of two fertile stamens only.

Disc is present in most of the genera studied and gets its supply from the remnant of stamen traces. Sometimes it gets supplies from the residues of both petal and stamen traces. Systematists have described the placentation, in all the members of this family as axile. The behaviour of vascular bundles shows that placentation is more towards parietal rather than the true axile.

The gradual reduction in stamen number as well as the external morphology play great role in the differentiation of the tribes.

The study of floral anatomy also suggests that *Strobilanthes glomeratus* and *Barleria prionitis* are more primitive, having five staminal traces and five sub-equal petals free at the upper end. In *Asystasia*, *Cardanthera* and *Odontonema* there are four staminal traces while in *Odontonema* two remain as vestigial. *Asystasia* having five sub-equal corolla lobes above the corolla tube, *Cardanthera* with corolla lobes of bilabiate form and *Odontonema* having corolla lobe of two-lipped form seem to be more evolved than *Barleria prionitis* and *Strobilanthes glomeratus*. *Rhinacanthus*, *Justicia* and *Beloperone* show extreme reduction in stamen number along with the two-lipped form to bilabiate form of flowers.

The different species studied here suggest that the evolution came through the line of reduction and this finding supports Lindau's classification on Acanthaceae on the basis of palynology.

C.6.3. *Cytology of some Acanthaceae species*

C.6.3.1. Previously the chromosome number of some of the members of the family Acanthaceae were reported some of which were studied for the first time. The present

report also deals with the chromosome number of a few more members of this family. Some of them (1) are newly reported.

*(1)	*Barleria strigosa*	$2n=42$
*(2)	*Barleria cristata*	$2n=42$
*(3)	*Jacobinia tinctoria*	$2n=28$
(4)	*Ruellia formosa*	$2n=34$
*(5)	*Dipteracanthus prostratus*	$2n=44$
(6)	*Cardanthera triflora*	$n=15$, $2n=30$

A detailed karyotypic analysis of the chromosome complements of the different species under investigation has also been made. Such karyotype studies reveal the gross similarity in chromosome morphology of the different species under the same genera with slight variation in chromosome structure as well as in chromosome number. Cytological observations indicate that structural alteration of chromosomes, and polyploidy, especially aneuploidy have played some role in the evolution of most of the materials investigated here.

Further studies in anatomy and on other aspects are in progress.

C.6.4. *Effect of some radioprotective agents in chromosomes of Capsicum annum*

C.6.4.1. During the period under report, an experiment was undertaken to assess radiation protection of a few chemicals on *Capsicum annum* L. In this report results with two chemicals namely, L.—Cysteine and Methylene Di-isothiourium Bromide have been mentioned.

Seeds of the variety Chilli-yellow were used in this experiment. The seeds were soaked for 2 hours prior to irradiation in solutions of the above mentioned chemicals. The strengths of the solutions used were 0.2 M/L-Cysteine and 0.1 M/L-Methylene Di-isothiourium Bromide. The seeds after soaking in each chemical were transferred to sterile Petri dishes, in the bottom of which was a thin film of the solution; the seeds were then irradiated in that condition at 20,000 r. For control seeds were also soaked for 2 hours in Distilled water, 0.2 M L- Cysteine and 0.1 M Methylene Di-isothiourium Bromide, but no radiation was given.

The seeds of all the treatments were allowed to germinate on Petri-dishes and after four days, the root-tips of each treatment were fixed in a solution of 6 parts ethyl alcohol, 3 parts glacial acetic acid and 1 part chloroform for one to three hours. Afterwards the root-tips were stained in propionci-orcein following the usual procedure.

Cytologically, radiation damage is generally assessed from the study of fragments, bridges etc. in the root tip cells or pollen mother cells or both of the irradiated material. In the present experiment, both metaphase and anaphase stages of the dividing root tip cells were studied in detail and the normal cells and the abnormal cells with fragments, bridges etc. were scored for analyses. In each treatment, ten root-tips were examined for this purpose. The result obtained is presented in Table 12.

From the table it is seen that L-Cysteine afforded protection against the production of aberrations after irradiation, whereas Methylene Di-isothiourium Bromide has shown

TABLE 12

EFFECT OF PRE-TREATMENT OF CHEMICALS ON RADIATION INDUCED CHROMOSOME ABERRATIONS IN *Capsicum annum* L. VAR—CHILLI YELLOW

Treatment	METAPHASE				ANAPHASE			
	Number of cells	Normal	Fragments	% of abnormality	Number of cells	Normal	Dicentric Bridges	Dicentric Bridges & fragments	Fragments	% of abnormality
Distilled water number radiation (control)	289	289	—	—	270	270	—	—	—	—
L—Cysteine number radiation (control)	475	473	2	0.42	383	379	—	—	4	1.04
MDB number radiation (control)	322	322	—	—	281	281	—	—	—	—
Distilled water + X-ray	270	73	197	72.96	299	47	58	117	77	84.28
L—Cysteine + X-ray	386	243	143	37.04	342	168	64	69	41	50.87
MDB + X-ray	127	31	96	75.59	159	25	20	81	33	84.27

no protective power at the concentration used. The percentages of abnormalities at metaphase stage were 72.96 and 75.59 in Distilled water and in Methylene Di-isothiourium Bromide, whereas in L-Cysteine it was only 37.04. Similarly at anaphase, the percentages of abnormalities were found to be 84.28 and 84.27 in Distilled water and in Methylene Di-isothourium Bromide treatments, but in L-Cysteine it was only 50.87.

Out of all the control treatments i.e. in non-irradiated treatments, only in L-Cysteine, abnormal cells were found both in metaphase and anaphase stages which were 0.42% and 1.04% respectively. The presence of abnormal cells may be due to the high concentration of the solution used. No abnormality was recorded in Distilled water and in Methylene Di-isothiourium treatment.

The percentages of germinations in the non-irradiated and irradiated populations were high and there were no marked differences in the different treatments. In the control sets of experiments, 98% germination was noticed in Distilled water and in L-Cysteine soaked seeds, whereas 92% germination was recorded in Methylene-Di-isothiourium Bromide soaked seeds. In the irradiated treatment, percentages of germinations were 96, 88 and 84 in L-Cysteine, Methylene Di-isothiourim Bromide and Distilled water respectively.

C.6.5. *Cytology of some Solanum species*

C.6.5.1. Plants of some Solanum species growing around Calcutta were collected and their chromosome numbers determined. It was found that S. *xanehocarpum*, *S. indicum*, and *S. verbascifolium* all had $2n=24$ as their chromosome numbers, thus confirming earlier reports. Observations on their floral biology are being made.

D. MICROBIOLOGY

D.1. Production of antibiotics by microorganisms

D.1.1. *A physico-chemical study on a broad-spectrum antibiotic from a new Streptomyces sp.* (A_6)

An antibiotic, basic in nature, was isolated in pure form in this laboratory from a strain (A_6) of *Streptomyces* sp. The active material was separated in the form of hydrochloride, sulphate, picrate etc. and designated as A_6-hydrochloride, A_6-sulphate, etc.

Electrometric titration of A_6-hydrochloride in aqueous solution with sodium hydroxide indicated it to be a tetra-acidic base with four pK′ a values 7.5, 8.35, 9.85 and a value little higher than 11. A higher pK′ a value of the order of 12.5 due to guanidine group in arginine as also 14 for guanidine have been reported. It is quite likely that the higher pK′ a value might be due to the presence of at least one guanidino group which was later corroborated by strongly positive Sakaguchi test.

Analytical results of some of the derivatives fit reasonably well with the molecular formula of hydrochloride, $C_{23}H_{43}N_9O_8$ 4HCl.

Owing to the basic nature of A_6-hydrochloride as also some of its characteristic positive Sakaguchi, Elson-Morgan, ninhydrine, anthrone, Molisch tests and the nature of UV and IR-spectrum, it was preferred to include this antibiotic as a new member in streptothricin-streptolin group.

In order to obtain a convincing proof, the acid hydrolysed product (6N HCl) was compared with that of roseothricin A. Paper chromatography of the acid hydrolysates were compared in a solvent system of butanol, acetic acid and water (4:1:1). Four ninhydrin positive spots could be seen in both cases with almost identical R_f values. Two of these were found to be identical in R_f values with the known compound reseonine and β-lysine. Thus, it may be inferred that β-lysine and roseonine present in roseothricin A molecule may also be present in A_6-hydrochloride.

Presence of β-lysine as a part of the hydrolysate was proved in another way. The acid hydrolysate was separated in several fractions on a column of Dowex-50 (H^+) and a particular fraction showed its identity in R_f with a known β-lysine also from a mixed m.p. of di-DNP. derivative of this fraction (prepared from 2:4 dinitrofluoro-benzene) with a known di-DNP. β-lysine.

In addition to β-lysine, roseonine was also detected from another fraction of Dowex-50 resin chromatography by comparing its R_f with a known reseonine sample. These two uncommon amino acids, viz., β-lysine and reseonine were only found in streptothricin group of antibiotics thus justifying the inclusion of A_6 -antibiotic under this group. A carbohydrate moiety (Molisch and anthrone positive) probably in the form of amino sugar is present in the molecule because of Elson Morgan positive test. Ammonia as one of the hydrolysed products was detected through as another hydrolysed product was identified through the precipitation of barium carbonate from barium hydroxide.

Finally it was observed that the hydrolysis of A_6 β-hydrochloride produces ammonia, carbon-dioxide, β-lysine and roseonine. In addition, the molecule also contained a carbohydrate moiety as an amino-sugar. All these inferences can only be correlated with if it be

a member of streptothricin group of antibiotics, viz., streptothricin, streptocin, geomycin, roseothricin A, viomycin, racemomycin, pleocidin, myco-thricin etc.

D.1.2. *Screening of broad-spectrum antibiotics from Streptomyces* spp

Soil samples collected from different parts of West Bengal were plated out. Number of soil samples plated out were 45 and altogether 640 strains of antibiotic producing *Streptomyces* spp were isolated. After screening at several steps, 10 strains were found to possess both antibacterial and antifungal antibiotics of which only 5 strains showed activity against human pathogens.

Preliminary screening was done by streak assay method, of which 10 were selected by cup assay method. Number of strains which are antibacterial and also antifungal are 8. The results are given in Table 1.

TABLE 1

ANTIBIOTIC SPECTRA OF ISOLATES

Strains	Antibacterial activity—zone in mm.				
	B. subtilis	E. coli	S. aureus	S. typhosa	V. cholerae
1. 440 Ac_{12}	20	13	23	25	—
2. 444 Ac_2	18	14	20	24	—
3. 444 Ac_4	21	15	21	24	15
4. 444 Ac_8	16	9	17	17	—
5. 444 Ac_9	15	8	13	13	—
6. 444 Ac_{10}	20	17	21	22	17
7. 447 Ac_{15}	16	12	18	17	—
8. 661 Ac_3	18	11	18	17	—
9. 661 Ac_{11}	17	11	13	12	—
10. 665 Ac_{26}	22	—	24	—	—

TABLE 1 (*Contd.*)

ANTIBIOTIC SPECTRA OF ISOLATES

Strains	Antifungal activity—zone in mm.				
	A. niger	A. solani	C. lunata	F.oxysporum	H. sativum
1. 440 Ac_{12}	—	10	13	10	15
2. 444 Ac_2	10	21	15	12	16
3. 444 Ac_4	—	21	12	11	12
4. 444 Ac_8	—	—	—	—	—
5. 444 Ac_9	—	—	—	—	—
6. 444 Ac_{10}	10	27	15	11	18
7. 447 Ac_{15}	—	16	17	10	24
8. 661 Ac_3	15	29	28	14	35
9. 661 Ac_{11}	15	30	26	12	35
10. 665 Ac_{26}	—	19	—	10	10

—indicates no antibiotic activity.

The assay of the antifungal antibiotics active against human pathogens are given in Table 2.

TABLE 2

ASSAY OF ANTIBIOTIC AGAINST HUMAN PATHOGENIC FUNGI

Strains	Trichophyton rubrum	Microsporum gypseum	Epidermophyton floccusum
1. Ac_2444	—	—	12
2. Ac_5444	—	—	10
3. $Ac_{10}444$	10	13	13
4. Ac_3661	17	12	15
5. $Ac_{11}661$	10	10	15
6. $Ac_{28}665$	—	—	14

—indicates no antibiotic activity.

Thermostability of active filtrates: The strains Ac_{12} 440, Ac_2 444, Ac_4 444, Ac_8 444, Ac_{10} 444 Ac_{15} 447 and Ac_3 661 produced antibiotic in broths which were thermostable. Fermented broths kept at 100°C for 15 mins. showed clear zonation against *Staph. aureus* and *Sal. typhosa*. Antibiotic production was recorded everyday in the strains. Ac_{12} 440, Ac_2 444, Ac_2 444 and Ac_{10} 444 which showed positive results from the 2nd day of incubation. Of these strains, Ac_{10} 444 showed highest antibiotic titre. Even after 24 hrs. of inoculation This strain showed good antibiotic titre against *Staph. aureus*.

Comparative study of the four selected strains: The above four strains were grown in different media both solid and liquid and their biochemical properties, colouration, sporulation, growth pigmentation were noted. In biochemical studies liquifaction of gelatin media, nitrate reduction coagulation and peptonization of litmus milk, melanin formation, etc. were noted.

Antibiotic extraction: The fermented broth of those strains were individually extracted with different solvents extracted, solvent layers evaporated and residual materials were dissolved in 0.5 ml. dist. water and assayed against *Staph. aureus*. Butanol, methanol water (4:4:1) and Butanol-ethanol water (4:4:1.5) showed positive result in two cases.

Nutritional study of the orgamisms for antibiotic production: Ac_4 444 and Ac_{10} 444 were selected out finally and grown in 17 different liquid media. Medium A_{13} showed only good result which is selected as the most suitable medium for antibiotic production by the above two strains. In this medium antibiotic titre comes after 24 hours of inoculation. By the variation of the composition of the ingredients optimum production of antibiotic was noted. The most favourable temperature is 28°C for antibiotic production and pH of the medium was suitable at pH 7.2 to 7.4 The cultures were so far grown under stationary condition.

D.1.3. *Study of the nutrition of antibiotic-producing Streptomyces* Ac_5 658

A strain of *Streptomyces* sp was isolated from soils of certain areas in the Dist. of Hooghly, West Bengal. The selected strains showed antibiotic activity against both gram-positive and gram-negative test organisms. As the determination of the nutritional requirement of the antibiotic-producing microorganisms are most important for substantial production of the active substance either in a synthetic or organic medium, it was decided to grow the strains in 12 media having different composition. The organism was grown for 12 days and the assay of the antibiotic was done from the 3rd day of incubation at 28°C. The results are given in Table 3.

TABLE 3

ANTIBIOTIC TITRE OF $Ac_5$658 CULTURE FILTRATE (ZONE IN mm.) AGAIN STAPH. AUREUS

Sl. No.	Medium	Days of incubation at 28°C									
		3	4	5	6	7	8	9	10	11	12
1.	*A_4	—	—	—	—	—	—	+	+	—	—
2.	A_{4h}	—	—	—	—	—	—	+	+	—	—
3.	A_{5a}	10	12	14	18	20	19	+	+	19	16
4.	A_6	20	22	22	24	22	21	+	+	18	17
5.	A_7	—	—	—	—	13	13	+	+	12	10
6.	A_9	10	16	17	22	16	13	+	+	10	9
7.	A_{12}	22	22	22	24	25	25	+	+	20	20
8.	A_{13}	—	—	—	—	10	7	+	+	—	12
9.	A_{16}	—	—	—	—	—	7	+	+	8	—
10.	A_{20a}	—	18	17	17	15	15	+	+	15	14
11.	**CD	—	—	—	—	—	—			—	—
12.	M_{12}	—	—	—	—	—	—	+	+	—	—

*Media A_4–A_{20} were described by Warran et al 1955, *Antibiotics and Chemotherapy*, **5,** 6.
**Synthetic medium;—indicates no antibiotic zone and + indicates results are not recorded.

It will be seen in the above table that medium A_6 would be useful for antibiotic production as the strain Ac_5 658 showed remarkable response from the 4th day of incubation to the 12th day. The utilisation of various carbohydrate in culture medium was considered by Pridham and Gottlieb (1948)† as an important factor in determining the characteristics of an antibiotic producing microorganism specially *Streptomyces* sp. Basal medium was prepared according to the formula of above authors and the strain A_5 658 was grown in 100 ml. flasks each having 50 ml. of the medium. The following carbohydrates were used at 1% concentration w/v. glucose, galactose, mannose, sorbose, maltose, xylose, lactose, sucrose, raffinose, dextrin, starch, glycogen and innulin, The carbohydrates were

† Pridham, T. G. and Gottlieb, D. (1948). J. Bact. **50,** 409.

sterilized separately and added after autoclaving. The flasks were inoculated as usual and incubated for 14 days. The results were however recorded from the 8th day of incubation in Table 4.

TABLE 4

UTILISATION OF CARBOHYDRATE IN GOTTELIEB'S SYNTHETIC MEDIUM AND ANTIBIOTIC PRODUCTION**

Carbohydrate source	Days of incubation												Mycelial growth*
	3	4	5	6	7	8	9	10	11	12	13	14	
L(+) Arabinose	—	—	—	—	—	—	—	—	—	—	—	—	Scanty
D(+) Xylose	—	—	—	—	—	10	11	14	10	10	+	—	Moderate
D—Dextrose	—	—	—	—	—	—	—	—	—	—	—	—	No growth
D—Galactose	—	—	—	—	—	12	13	20	16	16	10	+	Moderate
D—Fructose	—	—	—	—	—	—	—	—	—	—	—	—	No growth
D—Mannose	—	—	—	—	—	—	—	—	—	—	—	—	,,
Lactose	—	—	—	—	—	—	—	—	—	—	—	—	,,
Maltose	—	—	—	—	—	12	12	27	17	17	17	10	Moderate
Sucrose	—	—	—	—	—	—	—	—	—	—	—	—	No growth
1(—) Sorbose	—	—	—	—	—	—	—	—	—	—	—	—	,,
Raffinose	—	—	—	—	—	—	—	—	—	—	—	—	,,
Dextrin	—	—	—	—	—	—	—	—	—	—	—	—	,,
Starch	—	—	—	—	—	—	—	—	—	—	—	—	,,
Glycogen	—	—	—	—	—	—	—	—	—	—	—	—	,,
Innulin	—	—	—	—	—	—	—	—	—	—	—	—	,,

*Growth was recorded after visual observation.
**Antibiotic titre was nil from the 3rd to the 7th days of incubation.
— indicates no antibiotic production.

D.1.4. *Pigment antibiotic of a mutant strain MT-*10 *derived from Streptomyces sp.* (Ac_{21} 460)

Antibiotics compared: Attempt was made to compare the active principle extracted from the parent strain Ac_{21} 460 as well as the mutant strain MT-10. At the preliminary stage paper chromatographic technique with subsequent micro-bioassay was done using the culture filtrate as well as the crude antibiotic mass of both the parent and the mutant strains. Antibiotic principle was extracted by solvent extraction method. The results showed that the antibiotic produced by the mutant strain MT-10 had two coloured substances of which one is active and the other inactive. The active substance produced by the parent was a colourless one and developed only one active spot on the paper. The R_f values also differed from each other. As the antibiotic extracted from MT-10 was coloured and obtained in

appreciable quantity in comparison with that of the colourless substance produced by the parent, further work was done only with the pigment antibiotic of the mutant strain MT-10.

Purifiation of MT-10 pigment antibiotic: The pigment antibiotic was purified by the adsorption chromatography using Brockmann's alumina as an adsorbing agent. The crude antibiotic mass of MT-10 was dissolved in methanol and poured into the alumina column. Elution was carried out with ether when only one fraction could be eluted. This was designated as fraction A and was found to be antibiotically active. The other fraction B could be eluted with methanol but this was antibiotically inactive. The yellow coloured fraction A was further purified by dissolving the solid mass in methanol and then filtering the mixture. The filtrate was allowed to evaporate slowly in vaccum and a solid orange-yellow crystalline substance was isolated.

The physico-chemical properties of the antibiotic indicate that it becomes stable at 30°C; 60% activity was lost at 37°C on the 7th day of observation. It is readily soluble in methanol, ether, chloroform, acetone, ethyl acetate, ethanol, benzene, methyl ethyl ketone, butanol and acetic acid, slightly soluble in carbon tetrachloride and ethylene glycol and insoluble in water and petroleum ether. It is decomposed at 204°C. Ultraviolet absorption spectrum in methanol showed maxima at 442 mμ. The infrared absorption spectrum showed maxima at 3380-3230 Cm^{-1} indicating the presence of bonded hydroxyl. The regions at 1730 Cm^{-1} and 1640 Cm^{-1} are suggestive of the presence of d-lactone and conjugated carbonyl function or unsaturation respectively. The micro-analytical results showed the presence of C-56.37%, H-7.40% and N-10.8%. The molecular weight is 402 (Rast's method) and the probable molecular formula is suggested as $C_{19}H_{31}N_3O_6$. It contains only one active component as found in paper chromatographic studies. The antimicrobial activities of the antibiotic indicate that it is active against a number of plant and human pathogenic fungus. The results showed that among the fungi tested, *Curvularia lunata* was the least resistant to the action of the antibiotic.

D.2. Induced mutation in microorganisms

D.2.1. *Modifying effect of chemicals on Ultraviolet radiation in Aspergillus niger (V_{35})*

Experiments were performed to determine the effects of chemicals on ultraviolet radiation in *A. niger* strain V_{35}. The following chemicals were used at different concentrations. They were dissolved in sterile distilled water and stock solutions were added to the medium to bring the desired concentration: gm/100 ml.

$NaNO_3$	0.3 gm.
KNO_3	0.3 ,,
KCl	0.05 ,,
NH_4NO_3	0.25 ,,
NaCl	0.5 ,,
KH_2PO_4	0.1 ,,
$MgSO_4$	0.05 ,,
Dextrose	4.0 ,,

Four sets of experiments were done with each chemical. (i) preirradiation treatment (ii) treatment during irradiation (iii) post-irradiation treatment (iv) combined effects of all the above. One set of control was set up with irradiated spores suspended in distilled water.

(i) *Pre-irradiation treatment:* In this experiment freshly prepared spore suspension was centrifuged and after rejecting the Ringer's and 'calsolene' solutions from the above, chemical treatment was made. The treated spore suspension was shaken for 6 hours after which the solution was removed by centrifugation. The spores were then resuspended in sterile distilled water. Ultraviolet radiation was given to the spores for 12 minutes and the suspension was plated out in complete medium at different dilutions. After 48 hours of incubation at 28°C the colonies were counted.

(ii) *Treatment during irradiation:* For this purpose the spore suspension was centrifuged and after rejection of the Ringer's fluid and 'calsolene' solution, the sterile chemical solution was added to the spores. The spores were then irradiated for 12 mins. in the presence of the chemical. To stop further reaction the chemical solution was rejected by centrifugation. The spores were resuspended in sterile Ringer's fluid and plated in CM after proper dilution. These were then allowed to incubate at 28°C for 2 days.

(iii) *Post-irradiation treatment*: The spores were irradiated in distilled water for 12 mins and then after centrifugation the chemical solution was added to the spores and kept in a shaking condition for 6 hours. To stop further reaction the chemical solution was rejected by centrifugation. The spores were then suspended in sterile Ringer's fluid and plated in CM after dilution and incubated at 28°C.

(iv) *Combined effect of pre-irradiation, during irradiation and post-irradiation treatments:* The spore suspension was centrifuged and solution of the chemical was added to the spores. These were kept in shaking condition for 3 hours. Then the spores were irradiated for 12 mins. in the presence of the said solution. The spores were allowed to stand in that irradiated chemical solution in a shaker for another 3 hours. And then the reaction was checked by rejection of the chemical solution by centrifugation. The spores were suspended finally in sterile Ringer's fluid and plated in CM after dilution. These were incubated at 28°C for 2 days.

TABLE 5

MODIFYING EFFECTS OF DIFFERENT TREATMENTS IN UV-IRRADIATION
Dose 100 ergs/mm²/sec. Irradhiation with UV for 12 minutes.
Temperature-25°C

Treatment	Survival per cent								
	$NaNO_3$	KNO_3	KCl	NH_4NO_3	NaCl	KH_2PO_4	$MgSO_4$	Dextrose	Control
(i) Pre-irradiation	1.49	2.47	0.98	1.56	2.49	1.77	1.99	2.01	1.43
(ii) During irradiation	0.86	3.13	1.27	1.22	1.75	1.57	0.73	2.64	1.43
(iii) Post-irradiation	1.16	1.32	0.86	1.29	2.01	1.35	1.39	1.43	1.43
(iv) Combined treatment	0.99	7.31	1.16	1.79	3.5	2.61	4.65	2.26	1.43

D.2.2. *Treatment of A. chevalieri with N-mustard*

Spore suspension of *A. chevalieri* was first treated for 50 minutes in aqueous solution of (0.1%) mustard gas (Bis-chloroethylmethyl amine HCl). Preliminary studies showed that the above concentration of nitrogen mustard was without any appreciable mutagenic effect as such. Therefore, an experiment was set up to determine the combined effect N-mustard and UV rays; the spore suspension after 30 minutes of treatment with N-mustard was washed in sterile water and exposed to UV rays for 2, 4, 6 and 8 minutes respectively. The treated spores were plated out in complete medium. The isolates were transferred to MM medium for detecting the morphological and biochemical mutants. Those isolates which failed to grow on minimal medium were classified as biochemical mutants. Morphological mutants were characterized by their growth habits and change of colour of the aerial mycelium.

In the next experiment spore suspension of the *A. chevalieri* was first exposed to UV irradiation for 2, 4, 6 and 8 minutes which was followed by a treatment with nitrogen mustard (0.1%) solution for 30 minutes. After that the spores were washed in sterilized water and plated out in CM. For isolating biochemical and morphological mutants total transfers were made on MM plates. In another experiment *A. chevalieri* spores were irradiated with UV alone. Table 6 shows the survival percentage and frequency of morphological and biochemical mutants.

TABLE 6

THE EFFECT OF NITROGEN MUSTARD, PRE- AND POST-TREATMENT ON THE FREQUENCY OF ULTRAVIOLET INDUCED MORPHOLOGICAL AND BIOCHEMICAL MUTATION IN A. CHEVALIERI

Treatment	Spore concentration ($\times 10^6$/ml.)	Time in minutes	Per cent		
			Survival	Mutant	
				Biochemical	Morphological
(a) N-mustard	5.8	20	20.8	1.2	2.3
		30	12.8	2.3	3.5
		40	11.2	2.8	4.8
		50	10.8	0.1	1.2
(b) N-mustard+UV irradiation	80.0	2	15.5	3.8	4.3
		4	2.1	4.6	4.8
		6	0.66	5.8	6.2
		8	0.4	6.2	5.4
(c) UV irradiation+N-mustard	64.0	2	21.8	2.3	2.8
		4	3.5	2.5	3.1
		6	1.4	2.8	4.2
		8	0.6	1.2	2.8
(d) UV irradiation	24.0	2	11.3	1.4	3.2
		4	2.9	2.5	4.2
		6	0.59	3.8	9.8
		8	0.14	0.4	2.1

D.3 Microbial Genetics

D.3.1. *Experiments on induced mutation with Sordaria sp.*

Following the technique described in the report of the previous year several biochemical mutants had been isolated. These mutants responded to some particular amino acids or vitamins. The nutritional assay of one mutant C/1 isolated from UV-irradiated population revealed interesting biochemical behaviour. It responded to glutamic acid and also isoleucine. No apparent relation between glutamic acid synthesis and that of isoleucine has so far been established. However, it may be that accumulation of some byeproduct on addition of glutamic acid in the medium acts as a breakthrough in some unknown synthetic pathway leading to the formation of essential substances required for growth.

A few mutants of *Neurospora crassa* requiring both isoleucine and valine have been reported. No definite evidence as to the location of genetic blocks causing the requirements could, however, be established. Condensing enzymes, the reducto-isomerase etc. has also been reported to be active in the synthesis of leucine and isoleucine. With regard to the *Sordaria* mutant the known precursors of isoleucine and valine should be tested for growth response in order to establish the possible synthetic steps of the requiring amino acid.

D.3.2. *Experiments on surface sterilization of ascospores*

Attempts have been made to standardize a suitable technique for surface sterilization of ascospores of *Sordaria bosensis* before plating or subjecting to any treatment for induction of mutation. Sodium hypochlorite has been used as an useful sterilizing agent for *Neurospora* ascospores and therefore, freshly prepared sodium hypochlorite had also been used with *Sordaria* ascospores in mutational experiments. In *Sordaria* this technique had to be perfected in view of the fact that excess of chlorine in the sodium hypochlorite during its preparation damage the spores which exhibit a very poor growth in the minimal medium and are likely to be confused with the porbable mutants showing similar growth habit in the minimal medium.

Sodium hypochlorite is an unstable compound and has to be prepared in the laboratory before use. The following is the procedure of the preparation. Concentrated hydrochloric acid is taken in a separating funnel and dripped on sufficient quantity of potassium permanganate in a flask with side tube. The chlorine liberated is collected in a bottle containing 100 ml. 8% sodium hydroxide in water previously cooled and kept in an ice bath. The wt. of the bottle with sodium hydroxide is noted before the experiment and after approximately 8 gm. of chlorine is absorbed in NaOH. The reaction of chlorine with NaOH gives sodium hypochlorite, sodium chloride and water: $2NaOH+Cl_2=NaOCL+NaCl_2H+O$. As sodium hypochlorite is very unstable it is kept in the frezing chamber of the refrigerator. Whenever required the hypochlorite solution is standardized by idometic titration. This is a very critical estimation and has to be done with every care. Different concentrations of sodium hypochlorite in water have been tried ranging from 0.5 to 5%. Suspensions of ascospores were taken in centrifuge tubes and after centrifugation water poured off and treated with different concentration of NaOCl for 2, 3, 4, 5 and 6 mins. Spores were then washed 4 times, diluted to 10^2 and plated 1 ml. in each plate on A_1 minimal medium. A control of untreated and air exposed spores was also plated. It has been found that treatments of 1% solution of NaOCl for 2-3 minutes gives satisfactory results. Higher concentrations of NaOCL and longer duration of treatment give lower colony countings in the plates. At 5 and 6 minutes with 5% concentration spores are totally killed.

D.3.3. *Physiological studies on Sordaria bosensis*

Cultural studies in liquid media with various modifications of the basal medium A_1 have been continued. Dry weights of mycelium growing in 15 ml. of medium in 100 ml. conical flasks have been taken for comparative studies. In each experiment for a particular medium or its modification dry weights of 10 replicates have been taken at an interval of 5 days upto 35 days of growth period. Minimum inoculum was taken for each culture flask from 7 to 10 days old mycelium grown in liquid basal medium A_1. Inoculated culture flasks were incubated at a temperature of 30°C. A detailed comparison of growth rates of cultures in A_1 medium at pH 6.5 and 5.5 revealed that the former gives a better growth of mycelia. As such pH 6.5 has been maintained in all the growth media tried so far. Comparative data showing growth rates is given in table 7.

TABLE 7

TABLE SHOWING THE RATE OF GROWTH OF SORDARIA BOSENSIS IN DIFFERENT LIQUID CULTURE MEDIA

Medium	*p*H	Mean weights of flask culture at different periods of growth						
		5 days	10 days	15 days	20 days	25 days	30 days	35 days
A_1 NH_4NO_3—1 gm. KH_2PO_4—1 gm. $MgSO_4$, $7H_2O$-0.5 gm. NaCl—0.1 gm. Trace element soln.—1 ml. Glucose—25 gm. Water—1 litre	6.5	52.6	158.0	216.3	186.6	155.4	137.8	134.3
,,	5.5	22.5	141.2	199.8	159.9	139.1	131.5	120.5
,, with vitamin (without biotin)	6.5	83.5	228.3	186.3	150.5	112.2	93.2	108.2
,, ,, Sucrose 20 gm. instead of glucose and vitamins (without biotin)	,,	78.6	177.0	137.6	119.0	150.2	105.9	123.2
,, ,, NH_4NO_3—2 gm.	,,	29.3	81.2	208.8	191.3	185.7	153.7	142.9
,, ,, Succinic acid—0.5 gm.	,,	33.7	166.6	202.8	189.1	91.3	87.0	88.9
,, ,, Fumaric acid—1.32 gm.	,,	69.4	238.9	194.7	155.2	165.4	164.6	176.7
,, ,, Sodium fumarate—910 mg.	,,	67.0	245.5	237.5	173.9	193.6	221.1	166.8

Since population is an important factor in mutations studies, search for a suitable medium in which a large number of colonies could be counted was made. Experiments were conducted in order to study the effects of different synthetic media in restricting the growth of colonies of *Sordaria bosensis*. The colonies are usually irregular in size. While some are very minute others are too big and spreading. As such very few colonies could be grown on plates in the normal course. To avoid this difficulty along with the basal medium, various combinations of hydrolysed casein, vitamins, yeast nucleic acids and yeast extracts on the one hand and sugars particularly glucose, sorbose and also glycerine as a substitute for sugar were tried. In all about 60 combinations have been tested. Finally

a combination has been found which has proved to be the most suitable one as a complete medium. This medium not only quickens the germination of spores but also restricts the colonies to such an extent that countings could be done with ease even upto 300 colonies in a 9.5 cm. dia. Petri dish. To make survival counts on treatment of spores with UV and other mutagenic agents this medium has been found to be particularly suitable. The growth of colonies in this medium stops beyond a certain limit and they can be left as such for even two weeks or more before they are counted or otherwise made use of. Thus it has been found that the most suitable medium for compact-colony formation is closely comparable with Westergard's P-complete medium. The composition of the complete medium for *Sordaria* is as follows:

NH_4NO_3	..	..	1.0 g.
KH_2PO_4	..	..	1.0 ,,
$MgSo_4$, $7H_2O$	..	..	0.5
NaCl	..	..	0.1 ,,
$CaCl_2$	..	..	0.1 ,,
Trace elements	..	..	1 ,,
Yeast extract	..	..	2.5 ,,
Casein hydrolysed	..	..	1.5 ,,
Malt extract	..	..	5.0 ,,
Sorbose	..	..	6.0 ,,

This medium may further be simplified by eleminating yeast extract.

D.3.4. *Studies on reverse mutation with Neurospora crassa S4894a methionineless*

Studies on reverse mutation with *Neurospora* have been taken up with the strain S4894a in a more extended scale compared to the experiments done previously. The survival rates of conidiospores on UV radiation for 1,2, 3, 4, 5 and 6 minutes at different pH of the spore suspension have been observed. The Table 8 shows the results of the experiments.

TABLE 8

TABLE SHOWING PERCENTAGE OF SURVIVAL OF CONIDIOSPORES TREATED AT DIFFERENT *p*H WITH DIFFERENT DOSAGES OF UV

*p*H of spore suspension at treatment	Percentage of survival of conidiospores at different treatments					
	Mins.					
	1	2	3	4	5	6
4.2	79.8	56.1	49.8	47.7	5.61	0.62
5.5	67.6	34.7	3.69	0.62	0.27	0.17
6.0	32.0	20.2	1.0	0.15	0.01	0.0049
6.5	61.5	23.27	1.47	0.17	0.08	0.0021
7.0	51.5	27.8	3.8	0.43	0.11	0.086
7.5	52.3	18.4	4.6	0.42	0.035	0.031
8.0	36.9	15.2	3.2	0.70	0.22	0.025
9.0	96.9	6.11	0.77	0.20	0.049	0.0098
10.0	61.7	17.3	2.20	0.08	0.062	0.0106
11.0	61.6	22.2	3.51	0.60	0.121	0.038
11.5	74.2	26.3	3.80	0.37	0.152	0.053
12.0	49.2	24.48	8.41	1.23	0.019	0.034

It will be seen from Table 8 that at lower pH e.g. 4.2 the effect of irradiation is not too severe on the survival of spores. With increasing pH. however, the survival percentage decreases upto 6.5. With increase towards alakalinity at pH 7.0 the survival percentage increase but with further alkalinity it decreases again. At pH 11.0, 11.5 and 12.0 irradiation has been found to affect the germination of spores at a lesser degree whereas, beyond pH 12 an inhibition of germination has been observed.

Studies on the survival of conidiospores on irradiation were mainly done with an object to find out a relationship between the percentage of survival and the mutational response to UV irradiation at different pH. From an analysis of the data obtained so far it is evident that the maximum number of reverse mutations are obtained from treatments of 1 and 2 minutes. The highest yield of mutations, however, have been scored at pH 6.0 with 3 and 4 minutes of treatment. This work is being further continued with different strains and mutagenic agents other than UV.

D.3.5.1. *Studies on P. vermiculatum Dangeard* (77.62)

A strain of *Penicillium vermiculatum* Dangeard (77.62) which was initially a conidia producing one and seldom produced any sexual spores, was received from the Indian Jute Mills Association Research Institute, Calcutta. But because of long preservation or due to some other physiological factors this strain had lost the conidia producing habit. The strain was tried with the following media that would facilitate conidia production and it was found that excepting only one all other media failed to induce production of any asexual conidiospores.

Different media tried were:—

1) Czapek Dox Agar (C.D.A.)
2) C.D.A.+2% Malt extract
3) C.D.A. modified

Glucose	..	..	..	30.0 gm.
$NaNO_3$	..	..	..	2.0 ,,
KCl	..	..	..	0.5 ,,
$MgSO_4$, $7H_2O$		..	..	0.5 ,,
K_2HOP_4	..	..	..	0.75 ,,
KH_2PO_4	..	..	..	0.25 ,,
Water	..	..	..	1000 ml.

4) C.D.A. with 20% Glucose.

It was observed that in the medium No. 1, the vegetative growth was very poor. In medium No. 2, it was some what better but in medium No. 3 vegetative growth was found to be profuse. In all the cases perithecia were found to develop within three to four days and became mature within 8-10 days. In all the three media, however, conidiospores did not develop. In medium No. 4 with 20% Glucose the growth was satisfactory and conidia were formed within three days from inoculation.

It was found that glucose is more suitable than sucrose and when it was supplemented with vitamins (without Biotin) mycelial growth was even better.

D.3.5.2. *Effect of temperature on growth*

To study the effect of temperature the cultures were kept at 25°-30°c and also at room temperature between 25° to 32°C. At room temperature vegetative growth showed at its best; at 25°C growth was better but at 30°C it was very poor.

D.3.5.3. *Experiment on induced mutation by UV radiation*

The conidiospores from 5-6 days old cultures were subjected to UV radiation for 2, 4, 6, 8, 10 and 15 mins. It was found that 95% of the spores were killed at 4 mins. treatment.

Maximum number of morphological mutants were obtained at 6 mins. treatment. Morphological mutants are mostly albino and color mutants. The color of the mutants so far obtained are white, buff, orange, flesh, pink, grey and intermediate shades.

Some of the mutants are identical with the parent strain so far as the mycelial habit is concerned but produce yellow brown or red pigments as metabolic byeproducts. A few bio-chemical mutants have also been isolated. These are under observation.

D.4. Studies on Rhizobium bacteriophage

D.4.1. *Induced mutation in Rhizobium sp. by UV radiation from phage sensitivity to resistance*

In this study the different strains of *Rhizobium* sp. were treated with Ultraviolet radiation and the isolated mutants were tested for resistance to the action of their corresponding bacteriophage.

The following *Rhizobium* sp. were used for UV radiation experiments with a view to develop phage-resistant strains. The corresponding phages used for sensitivity tests are given in the list below.

Rhizobium sp. for	*Symbol of the strain*	*Phage*
Crotolaria juncea	CJ	P_1
Trigonella foenum-graecum	TFG	P_4
Lens esculenta	LE	P_{15}
Cicer arietinum	CA	P_6
Lathyrus sativus	LS	P_8
Cajanus indicus	CI	P_{12}
Phaseolus radiatus	PR	P_{13}
Arachis hypogaea	AH	P_{19}

The UV source used, was a 4-watt General Electric Germicidal lamp of bent-tube construction emitting over 95% of its radiant energy in the region of 2537Å. The distance of the object for irradiation from the lamp was set up in such a manner that the energy incident on the surface was 100 ergs/mm./sec. for all experiments.

Preparation of the bacterial suspension for irradiation: The culture of *Rhizobium* sp. was purified by single colony isolation by dilution method. 50 cc. amount of media was distributed in 250 cc. flask, sterilized and solidified. Bacterial suspension was made with 24 hrs. old slant culture with 9 ml. sterilized distilled water. 1 ml. of the suspension was spread on the flask's media and incubated at 28°-30°C. After 24 hrs the bacterial colonies were scraped off by means of sterilized bent glass rod. The suspension was made in normal saline and filtered through sterilized absorbent cotton wool so as to remove bacterial clumps. The whole of the filtration assembly was sterilized previously. It was seen that by this method a suspension of bacteria were obtained in which all the cells were separated from each other. The whole process was done at 10°C to reduce the possibility of multiplication of the bacterial cell during the operation. 5 ml. quantities of bacterial suspension were pipetted to a number of sterilized Petri dishes each being placed on the shaker plate one after another.

The irradiation experiment was conducted in total darkness in order to avoid photoreactivation. The irradiated suspension was washed off and diluted before plating in agar media and the desired strength was attained by preliminary plating experiments. The plating was done on YWA media. A large number of Petri dishes were used varying from 10-25 for each dilution. One ml. of the diluted suspension was pipetted into a sterilized Petri dish to which 25 ml. of the medium at 45°C was added. The irradiated suspension of *Rhizobium* sp. in each set was plated out in quintuplicates and the plate cultures were incubated at 28-30°C for 48-96 hours.

The survival percentage was calculated and a selection of morphological and colonial mutants was made amongst the survivors. The probable mutants were divided into several groups, their resistance to corresponding bacteriophages was tested and a screening was made. Finally cross-streak experiments were done using 28 phage strains to determine the range of sensitivity of the mutants towards unrelated phage strains. A selection of resistant strains was made at this stage which represented different groups of mutants both from phage induced and UV induced mutant strains.

D.5. Ecology of air microorganisms

D.5.1. *Studies on microbial population of air*

A study on the microbial population of air, including fungi, bacteria, and actinomycetes has been carried out in three different localities—Bose Institute campus (Calcutta), and Experimental Stations at Falta and Shyamnagar in 24-Parganas, West Bengal.

The composition of air microflora and their frequency at monthly intervals, have been observed. Air samples were taken bi-monthly, and the mean data were recorded for the particular month. The method consists in exposing sterilised Petri dishes, containing a moderate layer of malt-agar medium for 5 minutes at different sites in a particular locality. The plates were than incubated at room temperature in the laboratory and colony counts were made after 3 to 4 days of incubation. The colonies of fungi, bacteria and actinomycetes were then isolated separately. After 6 or 7 days of incubation, the fungi were examined for identification.

The most commonly occurring fungi so far identified upto genera are the following representing by more than one species.

(1)	Aspergillus	10 sp.
(2)	Penicillium	3 ,,
(3)	Gladosporium	2 ,,
(4)	Curvularia	2 ,,
(5)	Alternaria	2 ,,
(6)	Chaetomium	6 ,,
(7)	Nigrospora	2 ,,

The forms frequently found and represented by only one species each, are the following:

(1)	Phoma	(8)	Trichoderma
(2)	Fusarium	(9)	Pestalosia
(3)	Mucor	(10)	Cephalosporium
(4)	Rhizopus	(11)	Neurospora
(5)	Stemphyllum	(12)	Monochaetia
(6)	Spicaria	(13)	Humicola
(7)	Helminthosporium	(14)	Sordaria
		(15)	Ceratocystis.

Of these the last 5 types appeared only once or twice during the whole period of the survey.

Along with these there are some unidentified species forming vegetative growths only. These are referred to as the sterile forms.

The fungi identified upto species are the following:—

(a) *Aspergillus niger*, (b) *A. fumigatus*, (c) *A. terreus*, (d) *A. oryzae*, (e) *A. nidulans*, (f) *Penicillium tardum*, (g) *P. implicatum*, (h) *Curvularia tuberculata*, (i) *Sordaria hypocorpoides* and (j) *Ceratocystis paradoxa.*

From a survey of the above study, it has been found in all the three localities, that among the colonies developed on exposed plates, actionomycetes are of rare occurrence, and even when they do so, they occur in very small number. On the other hand, the bacterial colonies are found to exceed the fungal number in almost all cases, as it will be evident from Table 9.

TABLE 9

Locality	Organisms	*May* 1963 (month of warm climate)	*July* 1963 (rainy season)	*December* 1963 (month of cold climate)
Bose Institute (Calcutta)	Fungi	11.7	11.9	27.3
	Bacteria	43.9	17.7	32.5
	Actinomycetes	0.9	0.2	1.2
Shyamnagar (Research Station)	Fungi	5.1	6.6	18.5
	Bacteria	20.8	11.3	23.9
	Actionomycetes	0.5	0.1	0.1
Falta (Research Station)	Fungi	6.2	8.4	35.4
	Bacteria	12.0	18.5	35.0
	Actinomycetes	0.0	0.6	1.0

The mean numbers of the colonies of different organisms, per plate exposed for 5 minutes are given in Table 9.

The fungal colonies are found to decrease in number, especially in the rainy-season in all the three localities. The fungal colonies developed from the air-samples taken at Shyamnagar, were found almost always to exceed that of the samples taken at Falta and Calcutta. The variation may be due to the fact that wild vegetation is very common in Shyamnagar, at Falta some cultivated crops are present and in Calcutta vegetation is very poor. This leads to the idea that the aerial microflora emerges from the debris of the vegetation, on which they grow saprophytically or parasitically and sporulate. The spores are blown and carried by the wind and settle on exposed plates thereby constituting the particular microflora of the air.

Climatic factors e.g. wind current, air temperature, relative humidity, rainfall and storm play most important part in determining the distribution of the aerial microflora of a locality; and because of these factors, the monthly or seasonal variations in the frequency of the fungal units present in air are observed. The average data of monthly temperature, relative humidity and rainfall have been recorded. For the determination of sources of the fungi occurring in air, plant parts, especially leaves and stems of the surrounding vegetation were examined microscopically, after incubating them in humid chambers. For this purpose a number of crop plants, garden plants and wild plants were examined. These samples were collected along with the air samples at different localities. Fungi found in the dead or diseased leaves and stems are as follows—*Aspergillus niger*, *Penicillium sp.*, *Cladosporium sp.*, *Alternaria sp.*, *Chaetomium sp.*, *Helminthosporium sp.*, *Curvularia sp.* and some unidentifiable sterile forms.

Of the fungi, *Aspergillus sp.* was found to predominate in the summer from March to June and the number gradually comes down and ultimately becoming scanty during the winter season. On the other hand, *Cladosporium sp.* occurs in very high number during the winter season (November to February) and suddenly becomes absent during the following months as the temperature rises with a rise in the relative humidity. *Penicillium spp.* remains more or less moderate in number through out the year. *Curvularia spp.* also appears throughout the year in high or low rate somewhat irregularly. Others do not follow any regularity with regard to their occurrence. In almost all cases, the sterile forms constitute a fair portion of the total number of colonies.

The effects of different periods of the day on the occurrence of fungi, bacteria and actinomycetes, have been determined. The variations in the number of these organisms in different periods of a particular day is closely related to the climatic factors—temperature and humidity, which also vary in different parts of the day. Exposures were made at 6 A.M., 10 A.M., 2 P.M. and 6 P.M. and the colony counts of all the organisms and the identification of the fungi, upto their respective genera were carried out. The experiments were conducted from June '63 to February '64; and it was found that in almost all cases, the fungi occurring in the morning are less in number than those occurring in other periods of day.

The genus *Chaetomium* in general is known to act as a cellulose-decomposing agent, and hence 6 species of *Chaetomium* obtained from the air samples, were taken for determining their ability to decompose cellulose. Results of experiments with three species of *Chaetomium* have been so far obtained. In all the three cases it has been found that these fungi, can easily utilise cellulose and thereby decompose filter papers in media (C.D.) completely devoid of any sugar or carbohydrate.

5.1.2. *Allergenic principle from fungal spores*

A number of fungi have been reported to be associated with the respiratory allergy in human beings and animals. Some of these are spp. of *Aspergillus*, *Penicillium*, *Cladosporium*, *Mucor*, *Rhizopus*, *Phoma*, *Trichoderma*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Nigrospora*, *Alternaria* etc. occurring in air. The seasonal variations of these fungi have been investigated in the above study but their allergenic principles have not yet been determined. *Aspergilli* are prominent from March to May, the months of highest temperature, while *Cladosporium* follows closely the air temperature towards the lower range, the highest number being recorded in January, the month of lowest temperature.

D.6. Industrial Fermentation

D.6.1. *Determination of the conditions for optimum fermentation of citric acid by a selected mutant of A. niger strain CGU*-163

It has been mentioned earlier that there are two ways of increasing the yield of a product elaborated by a microorganism. One of these involves the induction of high-yielding mutants from the original parent followed by selection of a superior one (in respect of yield value), while the other is improvement of the fermentation environment. After a highly potent mutant is selected out the next step to get the higher yield from it is to follow the second pathway, for finding out the combination of conditions optimum for maximum production.

In fermentation experiments, several factors play important role in the growth and metabolism of the organism, e.g., aeration, agitation, pH of the medium, temperature of incubation and control of nutrients. Our knowledge of citric acid production by fermentation under controlled conditions was mentioned in the review of earlier works done in the line. It is apparent, that specific differences exist among the fermentation strains with respect to their ability to produce citric acid under optimum fermentation conditions. It was desired, therefore, that the physiology of each strains should be studied thoroughly in order to determine the highest yield of citric acid by a particular strain.

A number of experiments were, therefore, done with CGU 163 for the determination of its optimum fermentation condition. Each experiment was repeated several times to test the reproducibility of the results. Erlenmeyer flasks of 100.0 ml. capacity, each containing 25.0 ml. of the medium were used in all the experiments. To start with, Doelger and Prescott's medium was used as the basal medium. Following each experiment, the medium was modified according to the results observed in respect of a variable.

After an intensive study on the effect of variation of the constituents of the medium and other environmental conditions on citric acid production a suitable culture of defined composition has been accomplished. The media used by many earlier workers with different compositions might have been formulated on the basis of the requirement of their specific fermenting microorganism. It has been well established that nutrient requirements vary with strains and each one might perhaps perform well in fermentative activity with complex nutritional requirement.

The presence of trace elements, specially iron and manganese in combination significantly increased citric acid production. The reasons for divergent results obtained by different investigators are probably due to variation in the requirement of metallic elements among fermenting strains. However, the requirements of the strain CGU 163 has been determined on the basis of the experimental results obtained in the study of detailed fermentation. The composition of the medium has been recommeded as follows:

	Wt./litre
Sucrose ..	150 gm.
NH_4NO_3 ..	2.3 ,,
K_2HPO_4 ..	1.0 ,,
$MgSO_4$, $7H_2O$ ..	0.25 ,,
Fe(as $FeSO_4$, $7H_2O$)	10.0 mg.
Mn(as $MnSO_4$, $4H_2O$)	10.0 ,,
Water to make up 1 litre.	

The medium should be purified and finally the pH should be adjusted to 2.0 by (N) HCl.

The constituents of the above medium were used in fermentation experiments and the pH of the broth was carefully adjusted in each batch. Of the environmental conditions, ratio of the surface to the volume of fermentation liquid was thought to be one of the most important factors concerned in a successful process. The ratio was maintained at 1.2 to 1.4 in the present case. At low pH (2.0) the oxalic acid production was nearly suppressed during fermentation and danger of contamination was also eliminated. The citric acid produced was determined colorimetrically as described before.

D.7. Transformation in microorganisms

D.7.1. *Transformation reaction in Aspergillus niger*

A strain of *Aspergillus niger* (V_{35}) was the donor from which transforming DNA was isolated by the method described by Hotchkiss. A slight modification of the method was considered necessary as in the particular case the material was rather different. A stock of nutritionally deficient mutants were maintained in this laboratory of which 7 strains

were selected as recepient strains in the transformation experiment. Table 10 shows the strains with genetic markers and phenotypes.

TABLE 10

ASPERGILLUS NIGER WITH RESPECTIVE NUTRITIONAL DEFICIENCES

Deficiency in nutrition as genetic marker	Strain Index	Conidial colour
1. Methionine	H_{19}	Black
2. Leucine	H_{25}	,,
3. Adenine	H_{26}	,,
4. Choline	H_{45}	,,
5. Cytosine	H_8	,,
6. Thymine	C_{94}	,,
7. Arginine	H_{14}	,,

As a routine procedure the mutants were grown in liquid complete medium and the spores were harvested after 5-7 days of incubation. The suspension of the spores were filtered through pads of sterile cotton wool under aseptic condition and repeated by washing with normal saline to remove any trace of nutrient material adhering to the spores. The spores were centrifuged, washed, pooled in normal saline and stored at 4°C before use.

The experiments were set up in 100 ml. conical flasks each receiving 9 ml. of the minimal medium inoculated with 1 ml. of the stock spore suspension of the recipient strains. The DNA solution with concentration 20 μg/ml was added to the flasks meant for treatment while those for the controls received equal volumes of sterile water only. The flasks were kept in a waterbath at a constant temperature of 28°C. After 2, 4, 6, 8 and 10 hours of treatment, the spores were collected and washed repeatedly with normal saline. The spores were centrifuged and plated out in minimal medium at proper dilutions. The culture plates were incubated and the growth of the colonies on MM was recorded. The parallel examinations of the control plates were also made and the results are given in Table 11.

TABLE 11

TRANSFORMATION IN ASPERGILLUS NIGER

Strain Index	Nutrition marker	Time in hours contact with DNA	Number (total) Spores treated with DNA X10⁷	Number (total) Transformed colonies	P.C. of transformation X10⁻⁶
H_{14}	Arginine	0	279.00	0	—
		2		120	4.2
		4		30	1.07
		6		70	2.5
		8		11	3.9
		10		10	3.5
H_{19}	Methionine	0	342.00	0	—
		2		100	4.1
		4		110	4.5
		6		130	5.3
		8		140	5.5
		10		120	4.9
H_{25}	Lucine	0	243.00	0	—
		2		170	6.9
		4		530	2.1
		6		670	4.5
		8		500	2.05
		10		486	2.0
H_{45}	Choline chloride	0	495.00	0	—
		2		750	15.00
		4		800	18
		6		810	10
		8		816	17
		10		805	16
H_3	Cytosine	0	1035.00	0	—
		2		300	28
		4		350	33
		6		355	34
		8		340	31
		10		333	32
C_{84}		0	126.00	0	—
		2		468	37
		4		475	37
		6		489	38
		8		490	39
		10		480	37

The colonies arising on the MM agar plates were considered transformed and they were subsequently subcultured in the same medium in which there was no reversion.

E. ANIMAL PHYSIOLOGY

E.I. Studies on the metamorphosis of tadpoles

E.1.1. (a) *Vitamin B_{12} production in the guts of tadpoles*

It has already been reported that penicillin has a marked inhibitory action on the metamorphosis of tadpoles of *Rana tigrina* and *Bufo melanostictus*. Similarly vitamin B_{12} displays a stimulatory action on the metamorphosis. The stimulatory effect of vitamin B_{12} was demonstrated more effectively in the recovery of metamorphosis of tadpoles which had earlier been retarded by treatment with penicillin. The stimulatory action of vitamin B_{12} on the metamorphosis was also supported by the fact that when the penicillin treatment was carried out with simultaneous presence of vitamin B_{12}, no inhibition of metamorphosis was observed.

The examination of the microflora of the alimentary tracts of normal and penicillin treated tadpoles for the presence of vitamin B_{12}-producing micro-organisms suggests that penicillin treatment gives rise to a deficiency of this vitamin in both the species of tadpoles. The bacterial population in the guts of penicillin treated tadpoles is greatly reduced and the Gram positive bacilli are completely eliminated. Only the Gram negative few survive. The bacteria isolated from the guts of normal tadpoles comprise a large number of Gram positive bacilli which produced varying, but significant amounts of vitamin B_{12} in the culture medium. These Gram positive vitamin B_{12}-producing bacilli were absent in the guts of the treated tadpoles. The Gram negative bacilli isolated from the guts of penicillin treated tadpoles did not produce the vitamin under identical conditions excepting a few strains which produced but a negligible amount (similar were the Gram negative bacilli isolated from the normal tadpoles). The elimination of these vitamin B_{12}-producing bacteria on treatment with penicillin may lead to a deficiency of this vitamin in the tadpoles. That such a deficiency exists in these animals, at least in the guts, is supported by the facts that this vitamin was not found in any detectable amount in the guts of penicillin treated tadpoles and that the inhibitory action of penicillin can be prevented by feeding the treated tadpoles with the gut contents of normal tadpoles, but not so by feeding with the same of penicillin treated tadpoles.

Though the vitamin B_{12}-producing bacteria disappeared in penicillin treated tadpoles, such organisms gradually developed after the discontinuation of penicillin treatment. On the 30th day of the treatment the B_{12}-producing bacteria were not detected in the guts. Thereafter the B_{12}-producing organisms began to grow in the guts and within 25 to 30 days of withdrawal of penicillin the nature of the microflora of the guts of the treated tadpoles was more or less the same as in controls. With the growth of the B_{12}-producing bacilli in the guts of tadpoles, the metamorphosis advanced gradually.

Penicillin treatment for a longer period of time, say 100 days, did not show any further inhibition of metamorphosis than that effected by penicillin treatment for 30 days. This was due to development of penicillin resistant vitamin B_{12}-producing bacilli, synthesizing increased amounts of B_{12} in the guts and thereby stimulating the process of metamorphosis.

Thus the penicillin treatment for such longer time first delayed the metamorphosis to a marked degree but later did not affect it very much.

E.1.2. *Effect of vitamin B_{12} on the metamorphosis of thiourea treated tadpoles*

Possible relation of vitamin B_{12} with the thyroid glands of tadpoles were further examined and for this purpose attempts were made to retard the metamorphosis of *Rana tigrina* and *Bufo melanostictus* tadpoles by thiourea, a known anti-thyroid drug, and to reverse this effect by vitamin B_{12}.

When vitamin B_{12} (in doses of 100 μg per litre) was present with thiourea (1 gm. per litre), *Rana tigrina* tadpoles did not show any sign of metamorphosis. Similar was the result with *Bufo melanostictus* tadpoles, although in this species the growth of hind limbs was somewhat more marked in tadpoles treated with vitamin B_{12} and thiourea together than in those treated with thiourea alone. These results show that the depression in the activity of thyroid glands of tadpoles by thiourea could not be overcome by a simultaneous administration of vitamin B_{12}.

After the discontinuation of thiourea treatment the treated tadpoles recovered and gradually metamorphosed. But the recovery was significantly accelerated by vitamin B_{12} and more rapidly so by thyroxine. The recovery of *Bufo melanostictus* tadpoles was comparatively faster and they metamorphosed within 20.8 days of withdrawal of thiourea. This period was shortened by another 5.7 days by vitamin B_{12} and 12 days by thyroxine. The recovery of *Rana tigrina* tadpoles was slower and the thiourea treated ones metamorphosed within 92 days. Treatment of the inhibited tadpoles by vitamin B_{12} and thyroxine helped them to metamorphose within 30.6 days and 12.7 days respectively.

E.2. Histological and histochemical studies on the thyroid and liver of tadpoles (*Rana tigrina* and *Bufo melanostictus*)

E.2.1. *Thyroid activity of tadpoles under different experimental conditions*

The histological demonstration of the activity of thyroid glands of tadpoles by measuring the cell area lining the vesicles and the colloid area in the vesicles showed that with the gradual progress of the tadpoles in their stages of metamorphosis, the activity of thyroid glands gradually increased. The increased activity of the thyroids was demonstrated by increase of the colloid area as well as the cell area.

When the tadpoles (*Rana tigrina* and *Bufo melanostictus*) showed just emergence of fore limbs (stage XX), the colloid area became maximum. The cell area reached a maximum level at XXV stage, i.e. when the metamorphosis was complete with absorption of tail. The colloid area, i.e. the amount of thyroglobulin, was reduced at XXV stage than that observed at XX stage. But it was higher than that of thyroids of tadpoles of stages between XVII and XVIII. The same results were obtained in both the species.

Penicillin treatment of tadpoles (*Rana tigrina* and *Bufo melanostictus*) resulted in considerable reduction in the activity of thyroid glands, as evidenced from the marked decrease in

both the cell area and colloid area. Vitamin B_{12} slightly increased the activity of thyroid glands of tadpoles.

The depression of activity of thyroid glands of tadpoles (*Rana tigrina* and *Bufo melanostictus*), as evidenced from the cell area and colloid area, under the influence of penicillin was successfully counteracted by vitamin B_{12}. The cell area and the colloid area of thyroids of the penicillin plus vitamin B_{12} treated tadpoles were about the same as the untreated controls, but much higher than those of thyroids of penicillin treated tadpoles.

The colloid area, representing the stored hormone of thyroid gland, was above 6000 sq. mm. in *Rana tigrina* tadpoles and above 4500 sq. mm in *Bufo melanostictus* when they showed just emergence of front legs (i.e. stage XX). It is likely that during the larval life of *Rana tigrina* and *Bufo melanostictus* maximum amount of the hormone of thyroid gland is formed and stored and secreted during this time in preparation for the metamorphic changes. But when these morphological changes were completed, as in stage XXV, the colloid area was diminished.

That thiourea exerted a more pronounced inhibitory action on the metamorphosis of tadpoles was also supported by its profound inhibitory action on the activity of thyroid glands. The cell area and specially the colloid area of thyroids were greatly decreased by thiourea treatment in comparison with the controls in both the species of tadpoles. The thyroid glands of thiourea treated tadpoles, as observed from its histological picture, appeared to be atrophied.

In *Bufo melanostictus* tadpoles the same atrophied condition of thyroids developed, but after the discontinuation of thiourea treatment this condition improved more readily than in the *Rana tigrina* tadpoles.

E.2.2. *Histochemical studies on liver of tadpoles*

Histochemical studies on the activity of alkaline and acid phosphatases, RNA and DNA levels, glycogen, protein, melanin, tyrosine, arginine and ascorbic acid contents in liver of tadpoles of *Rana tigrina* and *Bufo melanostictus* at various stages of larval life and also of the treated tadpoles have been undertaken. Preliminary studies indicate that alkaline and acid phosphatase activity, glycogen and protein contents and RNA and DNA levels vary at different stages of development. Treatment with penicillin, vitamin B_{12}, thiourea and thyroxine also affect the levels of these substances in liver to a marked degree. However, the results are not conclusive and the detailed studies are in progress. Attempts are being made to correlate these changes with the stages of development of tadpoles and whether the changes under the treatments are due to the effects of the substances concerned or due to changes of stages caused by the substances.

Another interesting histometric work which has been undertaken is the determination of the cellular and nuclear volumes of liver of tadpoles at various stages of larval life. It has been observed that the average volumes of the cells as well as of the nuclei gradually increase with the gradual advancement of different stages.

F. LIBRARY

The library contains 6519 volumes (3062 books and 3457 bound periodicals). During the year under review 481 volumes (203 books and 278 bound periodicals) were added to the stock as against 258 volumes during the previous year. The total number of periodicals received was 241 out of which 124 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 is 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 normally meant for use of its research staff. But provision has been made to admit, on the basis of reader's card, bonafide non-Institute research workers and scientists for use of its Reading Room only.

The library grant received during the year was Rs. 26,000.00. After meeting the mounting cost of periodicals subscriptions, specially of American origin, not much was left for purchase of books. However, a non-recurring grant of Rs. 10,000.00 was made available to the library for purchase of books and important reference work.

Need is being felt for providing additional floor space both for the display of current journals as well as for additional stack rooms. Plans have been included in the Fourth Five Year Plan programme for raising another storey to the library buildings for this purpose.

II. PUBLICATIONS

During the year under report Vol. 25, No. 2 (June 1962 issue) and Vol. 25, No. 3 (September 1962 issue) 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".

Other publication during the year was "Report of the Bose Institute for 1962-63".

G. WORKSHOP

During the year under report Workshop handled about 500 jobs from the departments of the Institute. These include works which according to the nature may be categorically divided into the following groups.

I. Fabrication of (a) specially designed apparatus and instruments and (b) of small equipments, gadgets, and spare parts for imported instruments:—
   - i) 100 kv X-ray unit have been completed.
   - ii) One Auxophyton machine for single cell Tissue culture work has been constructed.
   - iii) Paper Electrophoresis apparatus of modified design
   - iv) Strach gel Electrophoresis Apparatus Vertical type
   - v) Apparatus for the investigations on the coherent scattering of Gamma-ray
   - vi) Heavy conical shielding arrangement for one curie Gamma-ray source and mechanical arrangement for its suspension
   - vii) Brass double core shield and Aluminium stand for the above
   - viii) Photoelectric cross section apparatus with spherical absorbers
   - ix) New Target assembly for 14 Mev neutron generator incorporating provision for liquid air cooling
   - x) Probes for ion source
   - xi) Vacuum valves for Neutron generator
   - xii) Photomultiplier housing
   - xiii) Laue Camera for X-ray Crystallography and Spectrometer for X-ray identification of elements
   - xiv) Lead Shield with attachment for γ radiaction counting system
   - xv) Centrifuge buckets
   - xvi) Gadgets for transferring irradiated samples from the neutron generator to counting system
   - xvii) Modification of Gas phase chromatography apparatus

II. Besides the maintenance of Refrigerators, Air Conditioners and other refrigerated equipments, the refrigeration section has installed Air conditioning plants for the Microbiology, Chemistry, Botany, Plant Physiology, Protein Chemistry laboratories. The section has also completed the construction and installations of 0° Cold Rooms for the Microbiology and the Chemical Instrumentation Laboratories.

A new 0°/21°C Cold room is under construction for the Organic chemistry laboratory. It will be divided into two compartments (i) 21° degree cold room and (ii) Cold Room whose temperature can be varied from 0° to –10°C. The latter will be used for store of chemicals including inflamable organic liquid.

III. Due to expansion and increase of maintenance work the Refrigeration section has been shifted for more commodious accommodation to the ground floor of the North Building for convenient space.

IV. A new Electronic Instruments section is also under construction to undertake the repairs, maintenance and also the construction of electronic instruments needed for the Institute.

APPENDIX A

I. Governing 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
Prof. Sisir Kumar Mitra, F.R.S.*
Shri Abani Nath Mitter
Shri S. C. Mukherjee, I.C.S. (*Retd.*)
Dr. Basanti Dulal Nag Chowdhuri
Prof. Bhupati Mohan Sen, I.E.S. (*Retd.*)
Dr. J. C. Sengupta

II. The Council

Shri K. N. Chatterjee
Shri S. K. Mandal
Prof. S. K. Mitra, F.R.S.
Shri A. N. Mitter
Dr. B. D. Nag Chowdhuri
Prof. B. M. Sen
} Nominees of the Governing Body

Dr. D. M. Bose, Director, Bose Institute (*Ex-officio*)
Secretary to the Government of India, Ministry of Scientific Research and Cultural Affairs or his nominee.
Director-General, Council of Scientific and Industrial Research or his nominee.
Shri Sachindra Chowdhuri, M.P.
Prof. P. Parija, Vice-Chancellor, Utkal University.
Prof. K. Ahmed Chowdhuri, Professor of Botany, Muslim University, Aligarh.
} Scientists from outside West Bengal, nominated by Govt. of India.
Shri Nandadulal Sreemany (Representative of the Calcutta Corporation).
Registrar, Secretary.

III. Finance Committee

Prof. B. M. Sen, Chairman (Representative of the Council)
Shri P. K. Bardhan, Accountant General, West Bengal.
Dr. D. M. Bose, Director (*Ex-officio*)
Registrar, Secretary.

IV. 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.

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* Deceased since August 13, 1963.

APPENDIX B

Director : Dr. D. M. Bose, M.A., Ph.D., F.N.I.
Registrar : Shri N. Das Gupta, B.Sc., B.L.

Department of Physics

Head of the Dept. : The Director
Research Fellow : Dr. T. C. Bhadra, M.Sc., D.Phil. (on study leave)
Research Scholar : Shri Arun Kumar Chatterjee, M.Sc.
D.A.E. Junior Fellow : Shri Amalendu Nath, M.Sc.
Mary & Richard Keating Scholar : Shri Amitava Ghosh, M.Sc.
Shri Banidhan Bhattacherjee, M.Sc.
Attached workers : Sm. Krishna Paul, M.Sc.
Shri Prabhat Kumar Bhattacherjee, M.Sc.

Department of Chemistry

Head of the Dept : Dr. P. K. Bose, D.Sc., F.N.I.
Reader Dr. A. Sen, M.Sc., Ph.D. (Leeds).
Dr. S. K. Roy, M.Sc., Ph.D. (Lond.), D.I.C. (on leave)
Research fellow : Dr. A. K. Barua, M.Sc., D.Phil.
Dr. B. B. Biswas, M.Sc., D.Phil.
Dr. D. P. Chakravarty, M.Sc., D.Phil.
Dr. V. N. Gadgil, M.Sc., D.Phil.
Shri J. Dutta, M.Sc.,
Research Assistant : Dr. N. K. Sinha, M.Sc., D.Phil.
Shri H. C. Chakravarty, B.Sc.,
Research Scholars : Shri B. K. Burman, M.Sc.
Shri Kalachand Das, M.Sc.,
Sm. Susweta Sen, M.Sc.
Sm. Parul Chakravarty, M.Sc.
Shri Deb Dutta Roy, M.Sc.
Govt. of India Scholar : Sm. Shila Chowdhury, M.Sc.
Shri Deb Kumar Mukherjee, M.Sc.
Shri Ranjit Roy Chowdhury, M.Sc.
Shri Ashoke Chandra Ghosh, M.Sc.
Sm. Sujata Roy, M.Sc.
Shri Basudev P. Das, 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
Shri S. P. Saha, M.Sc.
Sm. Anima Dutta, M.Sc.

Department of Botany

Head of the Dept : Dr. Sunando Bose, M.Sc. (Cal.), Filosofic Doctor (Lund.).
Research Fellow : Dr. R. K. Basu, D.Sc.
Dr. M. C. Basu, M.Sc., D.Phil.
Statistician : Shri A. K. Roy Chowdhury, M.Sc.
Research Assistant : Shri B. K. Dutta
Shri A. T. Guha Thakurta
Shri A. K. Adhikari, M.Sc.
Research Scholar : Shri B. R. Banerjee, M.Sc.
Shri S. S. Sarker, M.Sc.
Sm. Anima De, M.Sc.
Govt. of India Scholar : Sm. Protima Roy Chowdhury, M.Sc.
Sm. Sheela Sen, M.Sc.
C.S.I.R. Pool Officers : Dr. J. G. Bhowal, M.Sc., Ph.D.
Dr. Sunirmal Chanda, D.Sc.
Attached Worker : Shri D. N. Chakravarty, M.Sc.

Department of Microbiology

Head of the Dept : Dr. P. N. Nandi, M.Sc., Ph.D. (Lond.), D.I.C.
Research Fellow : Sri K. L. Chowdhury, M.Sc.
Dr. A. K. Mishra, M.Sc., D.Phil. (on study leave)
Shri R. K. Sinha, M.Sc.
Research Assistant : Shri P. P. Mukherjee, M.Sc.
Shri S. L. Chakravarty, M.Sc.
Sm. Ashalata Paul, M.Sc.
Shri Bijoy Ranjan Maity, M.Sc.
Sm. Jharna Mitra, M.Sc.
Birla Scholar : Sm. Gita Nanda, M.Sc.
Govt. of India Scholars : Sm. Kalyani Bhattacherjee, M.Sc.
Sm. Roma Borat, M.A.
Attached Workers : Dr. A. C. Das, M.Sc., Ph.D.
Shri Narendra Nath Shee, M.Sc.
Shri Biswanath Mukherjee, M.Sc.,
Sm. Ashita Mukherjee, M.Sc.,
Sm. Purabi De, M.Sc.,
Shri S. C. Mishra, M.Sc.

Department of Animal Physiology

Research Assistant : Shri G. C. Bhattacherjee
Research Scholar : Sm. Roma Ghosh, M.Sc.
Attached Worker : Shri A. K. Medda, M.Sc.

Workshop

Superintendent : *General* Shri M. L. Chatterjee
do *Electricals and Refrigeration* Shri S. R. Ghosh.

Library

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

STAFF APPOINTED UNDER GRANT-IN-AID SCHEMES

A. Department of Atomic Energy, Government of India.

Scheme No. 1. Nuclear Physics investigations with Neutron Generator

Investigator-in-Charge : Shri A. M. Ghosh, M.Sc.
Shri B. Mitra, M.Sc.

Research Assistant : Dr. Amalendu Nath, M.Sc. D.Phil.

Scheme No. II. Investigations on the induced mutagenesis in crop plants using X-rays and radioactive isotopes.

Investigator-in-Charge : Dr. Sunando Bose.

Junior Research Assistant : Shri Balaram Majumder, M.Sc.

B. Indian Central Oilseeds Committee

Inducing mutants with desirable economic characters in Til, Rape and Mustard by X-rays.

Investigator-in-Charge : Dr. Sunando Bose.

Botanist : Shri Ashoke Das, M.Sc.

Asstt. Botanist : Sri P. R. Das Gupta, M.Sc.

Chemical Assistant : Shri Amalendu Gayan, M.Sc.

*Discontinued with effect from 30th September, 1963.

C. Indian Council of Agriculture Research

(*i*) Investigation on the role of lipoic acid on plant metabolism with radioactive tracers.

Investigator-in-Charge : Dr. D. P. Burma

Senior Research Asstt. : Shri Samir Kumar Mitra, M.Sc.

Junior Research Asstt. : Shri Chirabrata Majumdar, M.Sc. (upto 1.8.63.)

(*ii*) Studies on the occurrence of Rhizobium bacteriophage and its effect on host legumes grown in Indian soil.

Investigator-in-Charge : Dr. P. N. Nandi

Jr. Research Asstt. : Sm. Itu Sanyal, M.Sc.

D. Council of Scientific and Industrial Research

(*i*) Scheme on Application of Vapour phase Chromatography in the analysis of Indian natural oils and the essential oils present in Indian tea and studies on the possibilities of using this technique as a preparative tool.

Investigator-in-Charge : Dr. D. M. Bose

Jr. Research Asstt. : Md. Maminul Haque, M.Sc.

(*ii*) Scheme on Microbial Synthesis of Proteins in relation to the biognesis on nucleic acids.

Investigator-in-Charge : Dr. D. P. Burma

Sr. Research Fellow : Dr. R. K. Mandal, M.Sc., D.Phil.

(*iii*) Scheme on Enzymatic synthesis of RNA and its role in Protein synthesis.

Investigator-in-Charge : Dr. B. B. Biswas

Jr. Research Fellow : Shri A. K. Chakrabarty, M.Sc.

(*iv*) Scheme on studies on the Pilot Plant fermentation for antibiotics production from streptomyces species

Investigator-in-Charge : Dr. P. N. Nandi

Jr. Research Asstt. : Shri R. K. Sinha, M.Sc.
Shri S. L. Chakrabarty, M.Sc.

(*v*) Scheme on Genetical and Biochemical studies on induced mutants of *Aspergilus niger*

Investigator-in-Charge : Dr. P. N. Nandi

Jr. Research Fellow : Sm. Arati Sarker, M.Sc.

(*vi*) Scheme on studies on the recovery from radiation damage in micro-organisms.

Investigator-in-Charge : Dr. P. N. Nandi

Junior Research Fellow : Sm. Malati Majumdar, M.Sc.

(*vii*) Scheme on Effect on ultraviolet light on DNA transforming factor in Micrococcus Pyogenes var aureus.

Investigator-in-Charge : Dr. P. N. Nandi

Junior Research Fellow : Sm. Meena Guha, M.Sc.

(*viii*) Scheme on Physical Chemical Study on Milk Protein, comparison of proteins of cow's and buffalo's milk.

Investigator-in-Charge : Dr. A. Sen

Senior Research Fellow : Sm. Sheela Chowdhuri, M.Sc.

(*ix*) Scheme on Application of Gas chromatography to (1) analysis of fatty acid and glyceride contents of some fats in oils of economic importance and (*ii*) the separation of the important amino acids from their mixture.

Investigator-in-Charge : Shri J. Dutta.

Junior Research Fellow : Sm. Anita Roychowdhury, M.Sc.

(*x*) Scheme on Biosynthesis of soluble RNA

Investigator-in-Charge : Dr. D. P. Burma.

Junior Research Fellow : Sm. Manjusri Bal, M.Sc.
Shri Utpalendu S. Mitra, M.Sc.

BOSE INSTITUTE, CALCUTTA

List of Published Papers 1963-64

Title of papers	Authors	Where published	Reference : Vol. page and year
1. Incorporation of radioactive phosphate into RNA of cell-free preparations of *Azotobacter vinelandii.*	R. K. Mandal D. P. Burma	*Biochim. Biophys. Acta.*	**72**, 162 (1963)
2. Molecular basis of heredity—A review	M. Chakravorty D. P. Burma	*Science & Culture*	**29**, 167 (1963)
3. Soluble RNA-dependent non-terminal incorporation of ribonucleotides from the nucleoside triphosphates into RNA	C. Majumdar D. P. Burma	*Biochim. Biophys. Acta.*	**76**, 480 (1963)
4. Biosynthesis of nucleic acids A review	D. P. Burma	Advances in Biochemistry (Proceedings of the Summar School of Biochemistry held at Srinagar, 1962)	p. 4 (1963)
5. Genetic code for amino acids in the protein synthesis—A review	D. P. Burma	Advances in Biochemistry (Proceedings of the Summer School of Biochemistry held at Srinagar, 1962)	p. 37 (1963)
6. Effect of some antibiotics and antimetabolites on protein and nucleic acid syntheses in *Azotobacter vinelandii* and *Lactobacillus arabinosus*	R. K. Mandal	*Trans. Bose Res. Inst.*	**24**, No.2 (1963)
7. Incorporation of radioactive nucleotides into polyribonucleotides	D. P. Burma D. Majumdar U. S. Maitra	Proceedings of the International Symposium on Nucleic Acids held at Hyderabad in January 1964.	In press

Title of papers	Authors	Where published	Reference : Vol. page and year
8. Reversal of lipoic acid activation reaction	S. K. Mitra D. P. Burma	*Biochim. Biophys. Acta.*	**85,** 333, (1964)
9. Induction and repression of L-arabinose isomerase in *Lactobacillus plantarum*	M. Chakravorty	*Biochim. Biophys. Acta*	**85,** 152, (1964)
10. Metabolism of mannitol and induction of mannitol-1-phosphate dehydrogenase in *Lactobacillus plantarum*	M. Chakravorty	*J. Bact.*	**87**, 1247, (1964)
11. Induction and repression of enzymes in *Lactobacillus plantarum*	M. Chakravorty	Proceedings of the International Symposium on Nucleic Acids held at Hyderabad in January, 1964	In press
12. Radiation-induced Crinkled Leaves in Jute	R. K. Basu	*Genetica*	**34,** 287-295, (1963)
13. Variable Manifestation of some Morphological Mutations in Jute	R. K. Basu	*Trans. Bose Res. Institute*	**26,** 27-32, (1963)
14. Radiation-induced Genetic and Physiological colour changes in Jute	R. K. Basu	*Indian J. Genet. & Pl. Breeding*	**24,** 42-50 (1964)
15. Cytotaxonomical Studies in some Species of *Sida*	A. Adhikary	*Trans. Bose Res. Inst.*	**26,** (3) (1963)
16. Induced variability of some quantitative characters of aman paddy.	A. K. Roychowdhury	-do-	**25,** (3) 81-87, (1962)
17. Behaviour of early flowering mutants	A. K. Roychowdhury	-do-	
18. Components of variation in *n*th generations of selfing	A. K. Roychowdhury	*Sci. & Cult.*	**29,** (404), (1963)
19. Anatomical interpretation on naturally occurring abnormality in *Corchorus capsularis* Linn.	Anima De	*Trans. Bose Res. Inst.*	**25,** (173-179), (1962)

	Title of papers	Authors	Where published	Reference : Vol. page and year
20.	Electrical reaction of the pulvinus of M. pudica in a state of excitation.	B. K. Dutta A. Guhathakurta	*Trans. Bose Res. Inst.*	**26,** (3), (1963)
21.	The constitution of barringtogenol D	S. K. Chakraborti A. K. Barua	*Tetrahedran*	**19,** 1727, (1963)
22.	Studies on the constitution of barringtogenol C	A. K. Barua S. K. Chakraborti Parul Chakraborti P. C. Maiti	*J. Ind. Chem. Soc.*	**40,** 483, (1963)
23.	Chemical investigation of *Ougeinia dalbergioides* Benth	D. K. Mukherjee A. K. Barua P. K. Bose	*Trans. Bose Res. Inst.*	**26**(2), (1963)
24.	Chemical investigation of Mollugo spergula	Parul Chakraborti A. K. Barua	*Ind. J. Chem.*	In press
25.	The structure and stereochemistry of barringtogenol C	A. K. Barua Parul Chakraborti	Symposium on glycosides and saponins	(1964)
26.	Isolation of the active principles from a strain of *Bacillus subtitio*	Mira Sen P. Nandi	*Ind. Jour. Chemistry*	**1**(3), 135, (1963)
27.	A new antifungal antibiotic produced by Streptomyces sp Ac_2 435	Ashalata Pal P. Nandi	*Experientia*	**20,** 321, (1964)
28.	A broad-spectrum antibiotic from a *Pseudomonas* sp.	P. P. Mukherjee P. Nandi	*Naturwissenschaften*	**51,**(13), 322, (1964)
29.	The effect of colchicine on nucleic acids and protein synthesis.	A. Chakraborty B. B. Biswas	*Expt. Cell. Res.*	**36,** (1964)
30.	Characterization of DNA from spinach chloroplasts	S. Biswas B. B. Biswas	*Sci. & Cutl.*	**29,** 618, (1963)
31.	Nucleic acids and protein synthesis in chloroplasts	S. Biswas B. B. Biswas	Symp. on Frontiers of Plant Sciences	(1964)
32.	The effect of U.V. on amino acid incorporation by chloroplasts	S. Biswas B. B. Biswas	Proc. 4th Intl. Photobiology Cong.	**25,** (1964)

	Title of papers	Authors	Where published	Reference : Vol. page and year
33.	Histones biosynthesis	A. Chakraborty B. B. Biswas	*Ind. J. Biochem.*	In press
34.	Effect of indole acetic acid on RNA and protein synthesis	Anima Dutta B. B. Biswas	*Naturwissens chaften*	In press
35.	The effect of nuclear RNA on on nuclear protein synthesis	B. B. Biswas	*Exptl. Cell. Res.*	**32**: 177, (1963)
36.	The conversion of Thymine-2 -C^{14} and its incorporation into nuclear RNA of endosperm nuclei of *Cocos nucifera*, Linn.	R. Roychowdhury S. P. Sen	*Biochem. Biophys. Res. Com.*	**14**: 7, (1964)
37.	Studies on the mechanism of Auxin action: auxin regulation of nucleic acid metaboliom in pea internodes and coconut milk nuclei	R. Roychowdhury S. P. Sen	*Physiologia Plantarum*	**17**: 352, (1964)
38.	Polynucleotide synthesis of chloroplast extract	Susweta Biswas A. Chakbravorty B. B. Biswas	Symp. Nucleic acids. Pergaman Press, New York.	(1964)
39.	Enzymatic synthesis of RNA and its role in protein synthesis : Part 1—The effect of chloramphenicol and actinomycin D on nuclear RNA and protein synthesis	A. Chakbravorty B. B. Biswas	*Indian J. Biochem.*	**1**: 13, (1964)
40.	Mechanism of action of colchicine	A. Chakbravorty B. B. Biswas	*Bull. Bot. Soc. Bengal*	**18**, (1964)
41.	Hormonal regulation of nucleic acid metabolism and its significance in the mechanism of hormone action.	R. Roychowdhury S. P. Sen	*Bull. Bot. Soc. Bengal*	**18**, (1964)