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REPORT

OF

THE BOSE INSTITUTE

FOR

1960–61

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

CONTENTS

REPORT OF THE BOSE INSTITUTE
1960-61

The Bose Institute was founded by Jagadis Chandra Bose in 1917 for "the fuller investigation of the many ever opening problems of 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 Instititute maintains experimental stations at (i) Mayapuri —Darjeeling (High altitude station), (ii) Falta—24 Parganas, (iii) Shamnagar—24 Parganas and (iv) Nilgunge—Barrackpore.

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 grant-in-aids 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, on which some representatives of the Governing Body, nominees of the Government of India, and some representative scientists are included.

Governing Body

Four meetings of the Governing Body were held during the year. It nominated six of its members to serve on the Council of the Bose Institute for a period of three years. At several of its meetings proposals for acquisition of about 125 bighas of land near Madhyamgram on the Jessore Road, for erection of the Centenary Memorial Laboratory were considered. It authorised the Director to negotiate with the Ministry of Scientific Research and Cultural Affairs and with the Education Directorate, Govt. of West Bengal for the sanction of non-recurring grant on 50 : 50 basis for purchase of the proposed area of land through the department of Land Acquisition, West Bengal. In this connection it sanctioned the payment of Rs. 22,029.00 to the Collector, Land Acquisition, 24-Parganas as deposit for land acquisition establishment, contigent and revenue charges.

Council

Four meetings of the Council were held during the year.

The draft revised Regulations and Bylaws of the Bose Institute which were sent to the Ministry of Scientific Research and Cultural Affairs, Government of India, in July 1956, are still awaiting the approval of the Government of India.

On the recommendation of the Council, the Government of India has approved with effect from 1st April 1960, the enhancement in the Institute contribution to the Provident Fund Account from 6.1/4% to 8.1/3%, provided an equal contribution is made by the employee.

The Council approved the principle of extending to the employees of the Bose Institute, the benefit of H.R.A., city allowance and medical relief as per Government of India scales and has requested the Director to take up the matter with the Government of India.

The Government of India has approved the amalgamation of grades of Readers and Research Fellows, change of designation from Research Fellows to Research Officers and introduction of two grades, Grade I and Grade II in the scale of Rs. 500-25-650-EB-30-800/- and Rs. 300-20-500/- respectively.

The Council has welcomed the view of the Estimates Committee, Government of India, on the appointment of Reviewing Committees at intervals of five years, to assess the activities of the research institutes receiving substantial grant-in-aid from the Government of India, and of the Ministry's proposal for the appointment of a Reviewing Committee at an early date, to assess the research work carried out in the Bose Institute during the last 13 years, to make recommendations on its future programme of work, and on the scientific and technical staff required for this purpose.

The life of the present Council has consequently been extended for a period not exceeding two years with effect from 1st October 1960 to enable this Council to deal with the recommendations of the proposed Reviewing Committee, and the present Director, Dr. D. M. Bose has been requested to continue in his office for another two years, within which period the recommendations of the Reviewing Committee will be available for consideration and implementation by the Council.

The Council has approved the revised leave rules framed by the Heads of the Departments which have been put into effect from 1st April 1960.

Shri S. K. Chatterjee, Registrar, was permitted to resign his post with effect from 28th August 1960. Pending the appointment of a permanent Registrar, Shri Nirmalendu Das Gupta, a retired Deputy Controller, Relief and Rehabilitation, Government of West Bengal, was appointed Offg. Registrar, with effect from 29th August 1960.

The Council has subsequently, on the recommendation of a Selection Committee, appointed Shri Nirmalendu Das Gupta, as Registrar, Bose Institute. He will be on probation for one year.

The Council also took cognisance of the resolutions adopted by the Governing Body for the acquisition of about 125 bighas of land on the Jessore Road near Madhyamgram for the erection of the proposed Centenary Memorial Laboratory.

Grants

The following grants were recieved during the year 1960-61 :

(*a*) *Non-recurring grants*	(*i*) Government of India, Ministry of Scientific Research and Cultural Affairs ..	Rs. 2,58,000.00
	(*ii*) Ministry of Scientific Research and Cultural Affairs, Govt. of India, grants to individual worker for Fundamental research ..	1,936.00
(*b*) *Recurring grants*	(*i*) Government of India, Ministry of Scientific Research and Cultural Affairs ..	Rs. 5,73,000.00
	(*ii*) Government of India for training in Scientific Research	13,141.50
	(*iii*) Governing Body, Bose Institute ..	65,000.00

(*c*) *Grant-in-aid schemes* — The following grants-in-aid were received during the year :

Sponsoring Body	*Schemes*	*Amount received*
Indian Central Jute Committee	Radiation mutation in jute	17,175.62
Indian Central Oilseeds Committee	X-ray irradiation of oilseeds	26,600.00
do	Radiation mutation and improvement of groundnut	19,050.00
Council of Scientific and Industrial Research	Antibiotic production	8,188.00
do	Isolation of macromolecules by counter-current method	6,300.00
do	Induced mutation in *Aspergillus niger* ..	4,500.00
do	Mycology and plant pathology ..	7,211.34
do	Microbial synthesis of proteins ..	1,300.00
do	Chemistry of Indian plant resins ..	7,131.83
do	Biosynthesis and metabolism of gibberellic acid	9,800.00
do	Application of vapour phase chromatography in the analysis of Indian natural oils etc.	10,485.80
Dept. of Atomic Energy Govt. of India	(*i*) Cosmic ray (*ii*) Nuclear Physics (*iii*) Neutron generator (*iv*) Induced mutagenesis in crop palnts	1,10,872.60
	AEC Fellowship	3,778.00

Indian Central Oilseeds Committee & Indian Central Jute Committee	For erection of a 20 curie Cobalt 60 source at Nilgunge	21,879.36
Indian Lac Cess Committee	Constitution of lac resin	2,300.00*
Sir J. C. Bose Trust Fund No. 1	Research scholarship, stipends and foreign scholarship	14,000.00

Centenary Memorial Laboratory

The Government of India has sanctioned Rs. 1,37,500.00 to meet half the cost of land required for the erection of Centenary Memorial Laboratory and the Government of West Bengal, Directorate of Education, has also agreed in principle to share the cost of land purchase on a 50 : 50 basis. The Government of West Bengal has been requested to provide, in addition to the cost of land acquisition on a 50% basis, a grant towards the cost of building construction and equipments. Following the normal procedure, a copy of the application to the Director, Land Acquisition, West Bengal, for the acquisition on behalf of the Bose Institute of about 125 bighas of land near Madhyamgram, Jessore Road, has been sent to the Directorate of Education, West Bengal with the request that the application may be forwarded with their recommendations to the Department of Land and Land Revenue, West Bengal.

The scheme for the erection of the Centenary Memorial Laboratory has been enlarged and merged into a plan which has been submitted to the Government of India, Ministry of Scientific Research and Cultural Affairs, as part of the Bose Institute development programme for the Third Five Year Plan period. On the acquired land it is proposed to errect (*i*) a high energy particle accelerator laboratory and gamma garden (*ii*) an air-conditioned Biochemical and Radio-biological laboratory and (*iii*) on the rest of the area a Plant Experimental Station. After the site has been sufficiently developed for the latter purpose, it is proposed to transfer the existing Experimental Stations at Falta, Shamnagar and Nilgunge to the new site.

Acharya Jagadis Chandra Bose Endowment Lectures

The third and the fourth Endowment Lectures were delivered by (*i*) Prof. P. C. Koller, Professor of Cytogenetics, Chester Beatty Research Institute, London, on 'Origin and growth of Cancer' and by (*ii*) Prof. Michael Polanyi, F.R.S., Professor Emeritus, Manchester University, on 'The unaccountable element in Science' respectively. The lectures are being published.

* Received after 31st March 1961

Acharya Jagadis Chandra Bose Memorial Lecture

The twenty-second lecture of the series entitled 'Some aspects of the cancer problem' was delivered by Dr. V. R. Khanolkar, M.D., Director, Indian Cancer Research Centre, Bombay, on November 30, 1960. The lecture is being published in the Transactions, Bose Research Institute.

New Construction

The construction of the western block with its equipments is nearing completion. The south wing of the ground floor is being equipped as a chemical process unit, it contains at present a 100 litre stainless steel fermentation pilot plant fed by steam from an oil fired steam generating unit housed in a separate shed. When not required for the fermentation plant, the steam from the boiler can be let to a condenser unit for production of distilled water.

The northern half contains the workshop administrative unit and a refrigeration assembly and repair workshop.

On the first floor on the southern side, is the new seminar room with accomodation for about one hundred persons. It is being at present fitted up with furnitures, black-board, projection unit and chairs etc. The northern wing of the first floor is divided into two mezzanine floors; it is being fitted up as an enzyme laboratory. One of the mezzanine floors is being air-conditioned, while the other is being equipped for chemical operations which could be carried out at the room temperature. A store room is also being provided. To the north of this building, in continuation of the western block, a neutron laboratory has been erected and put into working condition. Details of this laboratory is given in A.6 (Neutron generator).

During the last year work in the laboratories was being hampered by increasing difficulty in providing an adequate supply of water with low content in salts and of maintaining the supply of electric energy at constant voltage and progressively increasing in amount. Due to the recent shortage of electric power supply, the Bose Institute, like other large consumers of electric power, is being faced with difficulties. The equipment of the enzyme laboratory will require a substantial increase, by about 20 KW of electricity and of water supply. The difficulties can only be overcome by spending more money in sinking deeper tube-well of larger bore and by arranging for a bulk supply of electricity. During the later part of the year under report work in the different laboratories had been often interrupted by breakdown of electric power supply.

Two air-conditioned sets of rooms with artificial illumination have been fitted up for the study of plant growth under controlled environmental conditions. One of them is a basement room under a glass house of size 18′--0″×13′—0″. This room is divided into two chambers, both air-conditioned for investigations on short day and long day plants. The other air-conditioned set of rooms of which the temperature can be brought down to 55°F are being utilized at present for investigations on some temperate climate plants

with short growth cycle of about eight weeks. Such plants are suitable for investigation with chemical mutagens.

Study abroad and Deputation

1. Dr. T. C. Bhadra, Research Fellow in Physics, was awarded a Post doctoral Research Fellowship by the University of California, U.S.A.

2. Dr. D. P. Chakraborty, Research Assistant in the Department of Chemistry, was awarded a Post doctoral fellowship by the University of Miami, U.S.A.

3. Sm. Anjali Chowdhuri, Research Scholar in the Department of Physics, was awarded a scholarship for higher studies by the Government of Sweden.

4. Shri Rabindra Prashad, Research Scholar in the Department of Chemistry, was awarded a scholarship by the University of Pensylvania, U.S.A.

5. Dr. K. T. Jacob, Head of the Dept. of Botany, was granted deputation leave for one year, to enable him to join as Director, Jute Agricultural Research Institute, Barrackpore.

6. Sm. Maharani Chakraborty, Senior Research Fellow—C.S.I.R. was awarded a scholarship by the University of New York.

7. Dr. S. P. Raman, Senior Research Fellow—C.S.I.R. was awarded a post doctoral fellowship by the University of Wisconsin, U.S.A.

8. Dr. J. G. Bhowal, Cytogeneticist—in a grant-in-aid scheme in the Department of Botany, was awarded a post doctoral fellowship by the National Research Council, Canada.

Grants for study abroad

Grants of Rs. 2000.00 each out of Sir J. C Bose Trust Fund were made available to Dr. D. P. Chakraborty, Dr. S. P. Raman and Sm. Maharani Chakraborty in partial defrayal of their cost of passage abroad.

Degrees awarded

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

1. Shri S. P. Raman—Department of Chemistry.
2. Shri K. Venkateswara Rao—Department of Chemistry.
3. Shri V. N. Gadgil—Department of Chemistry.

Visitors

The following visited the Institute during 1960-61.

(1) Dr. H. R. Ambler, the Scientific Adviser to the U.K. High Commissioner in India. (2) Dr. Ernest C. Watson, Science Attache to the American Embassy in India. (3) The Soviet Scientific delegation to Indian Science Congress 1961. (4) Dr. P. Jacquinot, Professor of Physics in the Faculté des Sciences of Paris. (5) Prof. R. H. Burris, Chairman

of the Department of Biochemistry, University of Wisconsin, (6) The Soviet Scientists engaged in I.G.Y. research programme on board the ship VITYAZ. (7) Dr. F. N. Russanov, Director, Botanical Gardens, USSR. (8) Prof. Sir John Cockcroft, F.R.S., Churchill College, Cambridge. (9) Prof. Harlow Shapley, Harvard University. (10) Prof. Michael Polanyi, F.R.S., Professor Emeritus, Manchester University. (11) Dr. Walter S. Blair, Director, Wheat Loan Educational Exchange Program. (12) Prof. F. G. Young, F.R.S., Department of Biochemistry, University of Cambridge. (13) Scientific Delegation from Indonesia. (14) Prof. P. C. Koller, Chester Beatty Research Institute, London. (15) Shri Harish Chandra, Chairman, U.P. University Commission. (16) Prof. H. Webster, University of Queensland, Australia. (17) Prof. Maier-Leibnitz, Technische Hochschule, München. (18) Prof. N. W. Pirie, F.R.S., Professor of Biochemistry, Rothamstead Experimental Station, U.K.

Special Lectures and Seminars

(1) Under the auspices of Indian Interplanetary Society, Prof. Harlow Shapley, the world-renowned astronomer, delivered a lecture on 'Origin and Evolution of Life.'

(2) Prof. Sir John Cockcroft, F.R.S., Churchill College, Cambridge, gave a talk on 'Some British contributions to Atomic Energy'.

(3) Prof. N. W. Pirie, F.R.S., Professor of Biochemistry, Rothamstead Experimental Station, gave a talk on 'Green leaf proteins' with a film show.

(4) The Soviet Scientists engaged in I.G.Y. research programme on board the ship VITYAZ gave a talk.

New Appointments and Awards

The following appointments were made during the period under review.

A. *Research Fellow*

1. Dr. Ashoke Gopal Datta in the Department of Chemistry with effect from 24.10.60.

B. *Research Assistant*

1. Shri V. N. Gadgil M.Sc. in the Department of Chemistry from 1.5.60.

C. *Research Scholar*

1. Sm. Gita Bhattacharjee, M.Sc. in the Department of Microbiology from 1.7.60.
2. Shri S. S. Sarkar, M.Sc. in the Department of Botany from 11.4.60.
3. Shri Kalachand Das, M.Sc. in the Department of Chemistry from 13.2.61.

D. *Government of India Research Training Scholar*

1. Shri Josy Joseph, M.Sc. in the Department of Botany from 29.6.60.
2. Shri A. K. Chatterjee, M.Sc. in the Department of Physics from 18.7.60.
3. Sm. Shila Chowdhuri, M.Sc. in the Department of Chemistry from 13.2.61.
4. Shri Deb K. Mukherjee, M.Sc. in the Department of Chemistry from 24.2.61.

National Institute of Sciences of India—Senior Fellow.

1. Dr. Smritimoy Bose, in the Department of Botany from 1.8.60.
2. Dr. Mira Sen, in the Department of Chemistry from 3.8.60.

Appointments under Grants-in-aid Schemes

Indian Central Oilseeds Committee

1. Shri C. Banerjee, M.Sc. (Tech.), Chemical Assistant from 1.4.60.
2. Shri P. R. Dasgupta, M.Sc., Assistant Botanist from 1.4.60.

Indian Lac Cess Committee

1. Shri S. K. Chakraborty, M.Sc. Senior Chemist from 6.3.61.
2. Sm. Parul Chakraborty, M.Sc. Junior Chemist from 13.2.61.

Council of Scientific and Industrial Research

1. Sm. Anima Datta, M.Sc. Senior Research Fellow from 1.12.60.
2. Shri K. C. Padmanabhan, M.Sc. Senior Research Fellow from 23.7.60.
3. Sm. Kalyani Chatterjee, M.Sc. Junior Research Fellow from 28.7.60.

Resignations

The undernoted members of the research and administrative staff were relieved from their respective assignments in this Institute from the date noted against each.

1. Shri S. K. Chatterjee, Registrar, from 28.8.60.
2. Shri Rabindra Prashad, M.Sc. Research Scholar from 8.8.60.
3. Sm. Anjali Chowdhuri, M.Sc. Research Scholar from 13.11.60.
4. Sm. Archana Sengupta, M.Sc. Government of India Research Training Scholar from 25.2.61.
5. Sm. Maharani Chakraborty, M.Sc., C.S.I.R. Research Fellow from 1.5.60.
6. Dr. S. P. Raman M.Sc., D.Phil., C.S.I.R. Research Fellow from 23.7.60.
7. Dr. A. N. Lahiri, C.S.I.R. Research Fellow, from 30.9.60.

Seminars

Fortnightly seminars were held during the period under review, when the members of the research staff reported on the results of their respective investigations and reviewed the progress of work done on selected topics.

	Subject	*Speaker*
1.	The effect of visible light on the mitotic spindle of Areca nuclei	Mrs. Mridula Datta
2.	Statistical method in plant breeding	Shri A. K. Roychowdhur
3.	Some aspects of the contractility in *Mimosa pudica*	Dr. D. P. Chakraborty
4.	Effect of gamma radiation on fungi	Shri A. K. Mishra
5.	Milk proteins	Dr. A. Sen
6.	Karyotype evolution in *Lycoris Amarryllidaceae* ..	Dr. Smritimoy Bose
7.	Biochemistry of photoperiodism : Some facts and problems	Dr. S. P. Sen
8.	Plant tissue culture	Shri V. N. Gadgil
9.	Neoplastic growth	—do—

Obituary

During the past year the Bose Institute had lost two of its valued workers :

(*i*) Dr. Jyoti Prakas Sircar and (*ii*) Shri Indralal Chakraborty, both of whom passed away on the same day, June, 13, 1960.

In 1905, while still a medical student Dr. Sircar was assisting Acharya Jagadis Chandra's researches in the Presidency College. He was one of the original band of workers who were initiated by Acharya Jagadis Chandra on the Foundation Day of the Bose Institute, November 30, 1917.

With occasional breaks he was associated with the research work of the Bose Institute till the time of his retirement in May 1954 as Workshop Superintendent. Dr. Sircar was an accomplished experimentalist with a flair for technical craftsmanship; many of the present generation of workers still remember the assistance they received from him in solving their technical problems. During the period 1923-30, Dr. Sircar used to accompany Acharya Jagadis Chandra in his yearly European tours. After his retirement in 1954 he continued to occupy a set of rooms in the Institute residential block. His advice was frequently sought and valued by the administration and workshop staff of the Institute. He was widely admired for his cheerful and affectionate personality, and for his readiness to help people in difficulties.

(*ii*) Indralal Chakraborty, M.Sc. joined the Bose Institute in 1945. From the beginning he was associated with several schemes receiving grants-in-aid from the C.S.I.R. and the Department of Atomic Energy, Government of India. He began in 1949 his main line of investigations on the nuclear active component of the Cosmic Rays, both at Calcutta and at Darjeeling. Under the guidance of Dr. S. D. Chatterjee he constructed a 40 litre pressure ionisation chamber with which during 1949-50 he recorded several instances of sudden changes in Cosmic Ray intensity associated with solar flare and magnetic storms. In 1954 he constructed a fast recording device connected to a small pressure ionisation chamber, with which was associated a bank of Geiger Counter. With this arrangement he commenced measurements on nuclear active cosmic ray particles at Darjeeling. During the period 1958-59 he was entrusted with continuous measurement of cosmic ray intensity at Darjeeling as part of the IGY programme. For this purpose he constructed a cubical

meson telescope and a neutron monitor. Due to his being entrusted with such subsidiary tasks, the writing of his own thesis for the D.Phil (Science) degree of the Calcutta University was delayed. His thesis has been accepted by the Calcutta University for the award of the D.Phil (Science) degree.

Indralal Chakraborty had acquired a sound theoretical knowledge of the problems he was investigating and had planned for their extension when death suddenly snatched him away on June 13, 1960, while he was in Dehra Dun. He was liked and esteemed by every one with whom he came in contact; his cheerfulness, modesty of behaviour and readiness to help others attracted people.

Retirement

The Institute has lost a valuable collaborator, due to the appointment of Dr. M. S. Sinha as Professor of Physics in the Regional Technological Institute at Durgapore. After passing his M.Sc. examination in 1937, M. S. Sinha joined the Bose Institute as Research Scholar. From the beginning he had concentrated his energy to the development of the Wilson Cloud Chamber in its various cylindrical and rectangular forms. For several years he has been using these instruments for the study of the different components of the secondary particles produced in absorbers by the action of penetrating cosmic ray particles both at Darjeeling and recently in the underground tunnel under the Maithon Dam.

His recent studies were directed to the measurement of electron decay asymmetry of mu-mesons with different starting energies. Several research workers under his guidance have obtained the D.Phil. (Science) degree of the Calcutta University and others are completing their theses.

Dr. M. S. Sinha was elected a Fellow of the National Institute of Sciences of India in 1960.

RESEARCH ACTIVITIES—NON-TECHNICAL REPORT

Cosmic Rays

The study of the asymmetry in the direction of electron emersion from stopped mu-meson is being continued by placing a large Wilson chamber $(90\times80\times50)\text{cm}^3$ in a tunnel under the Maithon dam under a depth of 138 metres equivalent of water. The Cloud chamber was placed under such a large thickness of absorbing materials with a view to eliminating the presence of strong interaction particles (II, P) whose presence in the cosmic ray beam can badly distort the picture of mu-meson interaction.

At present the production of penetrating shower by fast mu-meson is being investigated. So far out of 1300 pictures taken since October 1960, shower production by seven penetrating particles have been observed, giving a value for the shower producing cross section $\sigma=4.7\pm2.2\times10^{-30}\text{cm}^2$/nucleon.

Further study of the energy dependence of decay asymmetry of cosmic ray meson has been made at Darjeeling. In about 36000 pictures taken with a cylindrical cloud chamber

fitted with fifteen 1/4″ copper plates, 1500 cases of muon decay have been observed, of which 1021 were due to mesons in the momentum region 0.35 Bev/c and 479 cases in the region 1.3 Bev/c. For these momenta regions the degrees of polarization of the muons were found to be 33% and 34%. These results differ from those of Alikhanyan as they do not show any dependence of polarization on the momentum of muon.

Investigation on the scattering of mu-meson in the momenta region $40\angle.p\beta\angle170$ Mev/c has been found to be in satisfactory agreement with the distribution expected on the purely electromagnetic interaction between mu-mesons and the nucleons. This result indicates that there is no anamalous scattering of mu-meson in the momentum region below 200 Mev/c.

Neutron Generator

A positive ray tube of total length 3 meter and 15 cm diameter, made up in four sections, has been assembled. In it 14.4 Mev neutrons are produced by the impact of deuterons on a target containing absorbed tritium, according to the reaction $H_1^2+H_1^3\rightarrow He_2^4+n_o'$. The upper half of the discharge tube with its deuteron ion source is insulated for 400 kv. The deuteron ions are accelerated by connection of the insulated electrode with the positive terminal of a Cockroft Walton high voltage circuit, which can develop potential difference upto 320 kv. The earthed target end of the discharge tube passes through a hole in the floor to an underground chamber. The target is made of titanium foil on which tritium gas has been adsorbed. At present it is possible to obtain a 400μA deuteron current beam, which can be accelerated to a potential difference of 175 kv. The upper chamber contains the insulated arrangements for producing a deuteron beam and the accelerating voltage. While the underground room contains a fast pumping system by which a vacuum of the order of 10^{-4} mm can be maintained. At present, arrangement is being made to measure from the control room the neutron flux produced by the positive ion tube and of the beta and gamma decay characteristic of the artificial radio activity which develops in the basement room due to the emitted neutrons.

Differential coherent scattering cross section of gamma photons

A gamma beam scattered by a given material contains two kinds of photons :

1. Whose energy and therefore frequency has been reduced by Compton scattering;

2. and a smaller number of coherent scattered photons (Rayleigh, Thomson and Delbruck scattering). The aim of the present investigation is to determine the dependence of the latter on the atomic weight of the scatterer and on the energy of the photon. The substances selected as scatterer are copper, mercury, bismuth, lead etc. The gamma radiations used are from Co^{60} and Cs^{137}. The experimental arrangement used is a Compton gamma ray spectrometer and is based upon a modification of the usual two crystal arrangement of Hofstadter, with a much better energy resolution and increased efficiency. The apparatus is still in the stage of construction.

Vapour phase chromatography

A vapour phase chromatography apparatus constructed in the workshop has been provided with different absorbent columns, with which it is possible now to separate a large number of volatisable organic compounds such as hydrocarbons, alcohols, esters, fatty acids, ketones, aldehydes etc.

Preliminary separation of low boiling fractions of Indian petroleum has been performed. Six different peaks were obtained, corresponding to C_4, C_5, C_6 hydrocarbons. Experiments for further resolution of these fractions with the help of suitable multiple column arrangements, using didecyl phthalate, squalane and diglycerol columns placed in series have been taken up.

Preliminary fractionation of essential oils extracted from a sample of tea has been achieved. Five broad peaks, corresponding to six and seven carbon alcohols, aldehydes, ketones and phenolic bodies have been obtained. These broad peaks are being further analysed with the help multiple column system.

Uranium and thorium uptake in plants

Recently collections have been made of plants growing in monazite areas in South India. Studies on these plants have been undertaken in the Institute from two points of view. The radioactive contents of leaves and fruit pods are being measured after ashing. By suitable extraction method, the radio-active ions present in the ashed plant organs are extracted and passed over ion exchange columns. By this method the relative amounts of uranium and thorium ions present in the ash as well as their total amounts are being determined. This ratio of uranium to thorium is compared to the ratio of the same elements present in the monazite sand soil on which the collected plants were growing. This invesigation will give some information on the percentage solubility of uranium/thorium compounds present in the soil and their respective uptake through the plant roots.

Milk proteins

Detailed investigations on the non-casein proteins of buffalo's milk have been continued. It has been confirmed that β-lactoglobulin and α-lactalbumin of buffalo's milk do not exhibit polymorphism as found in cow's milk. In crystalline shape, in electrophoresis, sedimentation and ultraviolet absorption, buffalo β-lactoglobulin and cow β-lactoglobulin-B show close similarity. It is probable that only minor structural differences exist between the two proteins. The molecular weight of buffalo β-lactoglobulin has been found to be approximately 35,000 g./mole.

Electrophoretic studies of buffalo lactalbumin indicates its properties to be different in some respects from cow α-lactalbumin-B. The observation made here and by other workers that many Indian and African cows show α-lactablumin polymorphism containing α-A and α-B (singly or in combination), of which the former moves in paper electrophoresis to the same position as the buffalo lactalbumin, renders the possibility of detecting adulteration of one milk by the other uncertain.

The non-casein proteins of goat's milk have also been examined. It has been found to contain three distinct components by paper electrophoresis at pH 4.6. No genetic control of occurrence of these proteins has been observed in forty animals so far examined. A simple method of preparation of crystalline β-lactoglobulin has been given.

Seed proteins

The crystalline and amorphous α-globulin of sesame seeds has been shown to be heterogeneous in the ultracentrifuge. Its pH stability range lies between pH's 4.5-9, below and above which it breaks down into smaller units and changes thus brought about are irreversible. The toxicity of different protein fractions of *Abrus precatorius* has been tested on mice. Of the three fractions prepared, two haemogglutinating fractions were fairly toxic while the non-haemogglutinating fractions were less toxic.

Plant chemistry

The survey of saponin bearing plants of India is being continued, as well as of Indian plant resins. A new scheme of research on the constitution of lac has been taken up this year. Palas seed lac was fractionated by temperature phase separation in acetone. A residue left after ether extraction on fractionation at 30° from acetone is a light brown amorphous powder. The study of the characteristic properties of the powder viz. melting point, acid value, saponification value, hydroxyl value etc. is being undertaken.

Radiochemistry

In this Institute for some years the presence of free nuclei and their division have been observed in milk of young coconut, arecanut and palm fruits. By using P^{32} as tracer, investigations are being carried out to find out the interrelationship between the synthesis of RNA (present in the milk) and DNA (present in the nucleus). The investigations indicate the possibility of transfer of tagged phosphate residue of RNA to untagged DNA, and vice versa. Correction has been made for the non-metabolic exchange reaction, by which radio-active and non-radioactive isotopes get interchanged.

Investigations are being undertaken to study the site of action of antibiotic substance in the bacterial cell. For this purpose S^{35} and H^3 labelled penicillins have been prepared.

It is generally believed that one of the major sites of action of penicillin is the cell wall. This contention has been confirmed by incubating Staphylococcus cells in the presence of labelled metabolities and penicillin. Penicillin treated cells absorbed more P^{32} than the untreated ones.

In view of certain structural similarities between penicillin and nucleic acid, the effect of penicillin on nucleic acid metabolism in *Staphylococcus aureus* is being studied using P^{32}.

A new line of investigation on the biosynthesis and metabolism of gibberellic acid has been commenced. The presence of gibberellin type of compounds has been found in the seeds of several plants belonging to Cucurbitacae and Leguminosae. Partially purified extracts were tested by the dwarf maize bioassay method. Preliminary results obtained indicate that extracts of unripe green gram (*Cicer arietinum*) and *Cucumis melo var. momordica* have marked growth stimulation properties, which characterise gibberellin type of compounds.

Enzyme chemistry

It is now generally accepted that the RNA molecule present in the cytoplasmic microsomes are the templates for the synthesis of protein molecules—the specificity of each type of protein molecules is ultimately derived from the DNA molecules of the genes via the intermediary of the giant RNA molecules present in cytoplasmic microsomes. Recent investigations are providing detailed pictures of how the protein molecules are synthesized from individual amino acids. The first step in the process is the activation of the individual amino acids. The second is the linking of the amino acids to a relatively small soluble ribonucleic acid (sRNA). Then this intermediary, according to theory, attaches itself to specific spot on a large RNA template in the cellular particles called microsomes. The biosynthesis of protein in relation to the biogenesis of nucleic acid is being studied in this laboratory using *Azetobacter vinelandii* as the study organism. Kinetic studies on P^{32} incorporation with growing cells of A. *vinelandii* reveal that particulate fractions of RNA are more rapidly saturated with the tracer than the soluble fraction.

Investigations are being carried out on subcellular fractions with respect to their RNA content and their function in protein synthesis. The technique of ion exchange chromatography and starch gel electrophoresis are being used for the separation of soluble and particulate RNA constituents.

Crown Gall tumour

Investigations on the production of crown gall tumour in plants by innoculation with *Agrobacterium tumefaciens* in different host plants like Bryophyllum, Kolonchoe, Nasturtium, Hollyhock and their bacteria free in vitro culture has been going on in the Institute for several years. Successful implantation of in vitro grown bacteria free gall tissues on healthy plants is considered to be a strong proof of the autonomous nature of the implanted gall tissues. Last year in vitro culture of Hollyhock gall tissues were successfully implanted on Hollyhock plants. This year bacteria free gall tissues of some plants with small chromosome numbers and with large single chromosome like *Vicia faba*, *Coreopsis lanceolata* and with *Ixora sp.* which were grown in bacteria free in-vitro culture for two years, have been successfully grafted into healthy tissues of their respective host plants, and have given rise to typical undifferentiated tumours. When these tumours attained a moderate size, tissues isolated from them were grown in synthetic media. They were found to be bacteria free and continued to grow undifferentiated in the synthetic media. Normal callus tissues of Hollyhock, Coreopsis and Nasturtium have been grown successfully in tobacco medium agar slant, supplemented by coconut milk and low concentration of auxins; the latter differ for different plant tissues. It has been maintained that while normal tissues grown in synthetic medium are incapable of continued growth in absence of any externally applied growth substance, Gautheret has, however, claimed that it has been possible in the case of some normal plants tissues, to habituate them to grow in culture solutions to which no exogenous growth substance had been introduced. Similar experiments were tried in the laboratory to induce habituated growth of hollyhock and coreopsis tissues, without positive results.

Glycolytic enzymes of higher plants.

In last year's report it was mentioned that it had been possible to isolate and obtain in pure crystalline form the enzyme aldolase. Microscopic examination of aldolase crystals obtained from mung seedlings had revealed the same shape and size for these crystals as reported by Taylor for rabbit skeletal muscle aldolase. On a careful re-investigation during this year it was found that the crystalline material was not a homogeneous protein. A modification of the method of preparation of this substance was tried this year. The specific activity of the enzyme had, during the process of purification, resulted in a net 60 times increase in the concentration of this enzyme; the latter could not even at this stage be crystallized. Further purification of the enzyme is being attempted.

The aldolase activity in whole mung seedlings and in different regions and at different ages of the plant has been investigated. In addition, the triosephosphate dehydrogenase, triosephosphate isomerase, and α-glycerophosphate dehydrogenase activities in young mung seedlings and in the different organs are under investigation.

Plant Physiology

The electrical correlate of the mechanical pulsations of *Desmodium gyrans* is not a replica of its mechanical pulsation; similar differences have been noticed between electrical and mechanical records of pulsating animal hearts. Attempts have been made during the year to obtain simultaneous record of electrical pulsations from different quadrants of the pulvinule of D. gyrans. After many attempts to find suitable electrodes, it was found that thin tempered steel electrodes could be inserted into the pulvinules without producing much injury. The records so far obtained show that the main seat of the electrical phenomena is located on the lower side of the pulvinule. There is also a difference in pattern of the electric pulsation records obtained from the upper and the lower side; that from the upper side is feeble in intensity.

It has been reported previously that in *Mimosa pudica* during the contraction of the pulvinus a biphasic electric response appears almost simultaneously at different points along the petiole. Further there is a time lag of 1/10 to 1/20th of a second between the commencement of the almost simultaneous appearance of electric response along the petiole and the contraction of the pulvinus.

Some experiments carried out during this year indicate that the biphasic electric response is initiated in the pulvinus by the contraction of the active cell. The resulting hydrostatic

pressure appears to produce by impact, simultaneous electrical changes throughout the petiole. The socalled simultaneous appearence of biphasic response along the petiole length may be due to the small resolving power of the pen recorder. It is proposed to investigate this type of response by a fast recording cathode ray oscillograph.

Radiation genetics

The following kinds of radiations are used for inducing mutagenesis in different types of plants. Gamma radiation (G), X-ray (X), β-radiation from phosphorus 32(P) and from sulphur 35(S). In addition the effect of chemical mutagens have been studied. The economic crops which have been investigated included oilseeds—Til (Sesamum), Rape and Mustard (Brassica), Groundnut, Jute and Paddy.

Sesamum. Two mutants isolated in the X_3 and X_4 generations, the white flowered mutants and the small seeded mutants from Sesamum Va. 16, have continued to breed true upto X_{11} generation and have maintained their increase in oil content by about 4 and 11 percent respectively over their controls. The keeping qualities of the oil from these mutants are better than those from the controls. A new mutant, a small seeded mutant 2 (SSM. 2) has been isolated in the X_3 generation from Sesamum var. T. 10 and gave 8 percent more oil than the control.

Brassica. The two recessive mutants, the appressed pod mutant 'APM' and the thickened pod mutant 'TPM' isolated from Brassica variety Rai. 5 (West Bengal) continued to be non-shattering. The oils from these mutants were not found however to be superior either in quality or in quantity over the controls. Besides these morphological mutants, selections made in the different generations from plants radiated by X-rays, and beta rays from P.32 and S.35, of Sesamum and Brassica, gave significantly higher yield and early flowering types compared to their control.

Groundnut. Most of the previous years selections and mutants were grown in the year under report. The mutant 'Dwarf' isolated last year as a result of P.32 treatment on seeds of the variety Small Japan, continued in general to breed true. The creeping mutants isolated from the variety A.K.10 after X-ray treatment, and which gave rise to plants with bold and small seeds, further segregated into creeping bold, creeping small, erect bold and erect small types.

Some statistical analysis of quantative data (such as flowering time, maturity, pod number, pod weight and spreading habits) were made on P.3 and S.3 selections in the varieties small japan and TMV-3. An increase in genetic variability and mean value, were observed in the third generation with respect to pod weight and pod number.

Two mutants (dwarf and creeping) isolated last year were crossed to their respective parental types (small japan and A.K.10 respectively) but no seeds could be obtained from the crossings.

Gamma radiation

A few selection in the G.1 and G.2 generation of different varieties of Sesamum gave higher oil yield or were earlier in flowering. In Brassica variety Y.10, *Yellow Sarson* nearby all G.1 plants showed early flowering, while selections from other varieties showed increase in yield and early flowering behaviour. In groundnut in the variety TW-3 one abnormal plant, having stout and erect branches with tips of branches pointing upwards, was observed in the G.2 generation.

Jute. In one variety of Jute, D-154, after treatment with P-32 produced some trailing plants; in the P.2 generation, there was segregation. Most of these plants died out during the different stages of growth and only two plants survived and yielded few seeds.

Rice. Rupsail—with P^{32} treatment an easly flowering in this variety, isolated in the second generation, flowered 29 days earlier than the control.' In the third generation, the early flowering mutants showed very poor grain yield : 3.2 gms as against 15.9 gms. in control; this was due to a decrease in fertility.

In the variety Patnai, one off-type plant was isolated in P.3, which had narrow grains. It was found to breed true in the P.4 generation while the grain yield of the control was 7.16 gms, that of the mutant was 13.11. The number of ears and grain yield was more in the mutants.

From three varieties of aus paddy *Marichbati*, *Dular* and *Jhanji*, treated with X-rays and P^{32}, 300 selections were made on basis of morphological, cytological peculiarities and pollen sterility. The morphological behaviour of plants grown in the next generation are being studied.

Chemical mutagenesis

The effect of two chemical mutagens dimethyl sulphate and ethylene imine on jute plants variety D154 have been studied. The effect of the first chemical has been inconclusive, probably due to its decomposition.

Ethylene imine. In the 2nd generation of treatment of D.154 spirally growing plants have been observed. Similar results have been obtained by the action of gamma radiation on D.154. Amongst plants grown from seeds collected, one has retained its spiral character.

Effect of irradiation on the anatomy of plants. Usually attention is concentrated on studies of morphological mutations produced in plants by radiation—like small seeded mutants, white flowered mutants in sesamum, thickened pod mutants and appressed pod mutants in *Brassica* and so on. A series of anatomical investigations on the anatomical changes in the root tips and other parts of these mutants have been commenced.

Cytological and cytotaxonomical studies in *Amaryllidaceae.* Efforts are being made to collect bulbs from different geographic regions with a view to study the phylogeny, karyotype evolution and interrelationship between different taxa—Cytological confirmation of taxonomy of the different genus of *Amaryllidaceae* and their evolutionary relationship is being studied. The role of polyploidy, hybridization and chromosome changes in speciations in the family is also being taken into consideration.

Zoology

It was reported previously that penicillin had an inhibitory effect on the growth of bacteria found in the intestine of normal tadpoles. Some of these bacteria have been found to produce vitamin B_{12} in suitable media; it was also observed that such vitamin producing microorganims were not found in the penicillin treated tadpoles. During the year under report a large scale investigations have been started on the presence of vitamin B_{12} producing microorganisms isolated from the intestinal flora, of control and penicillin treated tadpoles of *Rana tigrina*. Some of the bacteria isolated from the alimentary canal of untreated tadpoles produced Vitamin B_{12}. The rest of the organisms are still under investigation.

Ennucleation of and nuclear exchange between Amoeba

It is proposed to study the transfer of nutrients between nucleus and the cytoplasm in amoeba of the same species and between amoeba of different species. It has been possible by use of micromanipulators to ennucleate some of the organisms and to study how long they could be kept alive in such condition. So far it has been possible to rear clones of *A proteus* and *A lymax*. Attempts are being made to rear clones of *A. descoides*.

Microbiology

The investigations carried out in the Microbiology Department, dealt with

(*a*) detailed study of the bactereostatic properties of the broad-spectrum antibiotic substance isolated from *Streptomyces sp.* A.14(475), and from *Pseudomonas sp.* Homogenity of the purified antibiotics was established by paper electrophoresis and counter current distribution studies. Toxicity test on white mice showed that the first antibiotic was relatively non-toxic.

(*b*) An antifungal substance extracted from an actinomyces Ac_2 (435) has been studied. A pigmented antifungal substance was obtained after chemical purification, which on further counter-current treatment was found to consist of two components of which the active principle was found to be yellow coloured.

(*c*) Investigations on the effect of radiation on *Aspergillus niger* continued. Treatment of irradiated spores with vitamin B_{12}, indicated slight recovery from radiation damage inflicted on them. The mutagenic action of nitrogen mustard compounds on spores on *A. niger* show that with treatments at different dilutions, some morphological mutants are produced, whose number increases with concentration of nitrogen mustard.

(*d*) Similarly the mutagenic properties of certain chemicals on *Neurospora crassa* is being studied. The chemicals employed were dimethyl ethylene immine, para-amino-hippunic acid and acridine orange. So far negative results were obtained with dimethyl ethylene immine; due to non-availability of this coumpond, the investigation has been discontinued for the present. With the second compound some reverse colonies were

observed on the minimal plating; the results have been rather irregular in relation to the concentration employed.

With acridine orange, the experiment had to be carried out in the dark to prevent side reactions; in about a week's time, a fair number of reverse colonies grew up on the minimal plates. At pH 6.1, 2 hours and 4 hours treatment gave the maximum number of colonies on the minimal plate.

(*e*) Biochemical mutants were produced by ultraviolet radiations of spores of *Asp. chevalieri strain No.* 88. Six mutants have been isolated and are being tested.

(*f*) An improved method using auxanographic technique was employed for isolation of biochemical mutants of a strain of Streptomyces sp. Ac_2 (460), produced by irradiation. Out of a total of 2870 colonies picked up from filtered irradiated spore suspension, 22 strains were isolated showing nutritional deficiency, the nature of such dificiencies has been further analysed in detail.

(*g*) The healthy growth of leguminous plants depends upon the presence in the soil of appropriate *Rhizobium* bacteria. It has been found that different strains of bacteriophage, which are specific to the different *Rhizobium, spp.* occur in the soil. A series of investigations have been started (*i*) to determine the presence of *Rhizobium* bacterial strains in the experimental plots at Falta, (*ii*) to study the effect of innoculation of seed with appropriate bacteria; how far the yield of plants grown from innoculated seed are higher than those from the controls, (*iii*) to isolate and purify bacteriophage from leguminous soils and study their rate of killing of different bacterial strains (*iv*) and lastly to develop phage resistant mutants from these group of bacteria. The investigation is proceeding.

(*h*) Yield of paddy from plots subject to different manurial and cultural treatment were continued. The difference in yield between the different treatments tried has not been found to be statistically significant. Pests and diseases of rice plants are being studied. Some amount of work has been done in the isolation and identification of fungi from soils and roots of rice plants. About 210 fungi belonging to *Imperfecti* group, 6 belonging to *Physomycetes* and 6 to the *Ascomycetes* group have been identified.

(*i*) Bacterial transformation investigation which was started last year is being continued. Desoxyribonucleic acid (DNA) collected from the cells of *Micrococcus pyogenes var. aureus* was introduced in a nutrient medium innoculated with freshly grown cells of *Micrococcus pyogenes var. albus.* and cultured. The number of colonies both coloured (transformed) and white (albus) varieties were examined and the frequency of transformation induced calculated. The coloured colonies appearing in culture plates were isolated in pure culture and their biochemical characteristics are being studied. About 400 isolated cultures are being studied in detail.

A. PHYSICS

A.1. New Apparatus

During this year a number of useful equipments have been included in the laboratory instrument list. As reported last year (vide Sec. A.3.2) Tektronix 545A Cathode Ray Oscilloscope has arrived along with its plug-in unit. The construction work of the Electromagnet, capable of producing a field of about 10,000 gauss, is in progress. The energising unit of the Electromagnet, consisting of an A.C. voltage stabiliser, a Variac, a Selenium Rectifier unit with adequate filter arrangements, has been commissioned to work. Two amplifiers, one wide band amplifier type—hp-460 AR and another Fast Pulse Amplifier type—hp-460BR have been imported from the U.S.A.

A.2. Microwaves

Preliminary arrangements are in progress for the much needed equipments of the microwave laboratory. Though a number of microwave components and other allied accessories have been imported within last two years, but still to start working on the installation of microwave spectroscope, a few number of vital components are necessary. Their arrival is being expected.

In the mean time efforts are being made to construct the Electromagnet as reported above, and to improve the magnetic field strength meter using Proton Resonance phenomenon. Improved types of circuits for this purpose, are under investigation. Active consideration has also been given to the problem of getting an uniform magnetic field over a considerable area of the pole-face. The distribution of the magnetic field over the pole-face is being investigated with the help of a scanning-probe constructed here. This probe has got arrangements for smooth vertical and horizontal movements and the proton sample rests inside it. By scanning the pole-face in this manner, and by polishing the pole-face repetitively, the field uniformity in between the pole faces will be increased considerably. With the increased field uniformity, the resolving power of the spectroscope will also increase and that will help us in the investigation of (*i*) paramagnetic resonance due to ions in a crystalline lattice whose energy levels can be influenced, by an external magnetic field, and (*ii*) the free-radicals produced in living tissues due to radiation.

A.4. Cosmic Rays

A.4.1. *Under ground studies of cosmic ray mu-mesons*

It was expressed in our last report that in order to get a more accurate picture of mu-meson interactions we should extend our experimental studies carried out at Darjeeling

(altitude : 2200 meters) and at Calcutta (sea level) to the study of underground mu-mesons. The main difficulty associated with the experimental studies of mu-mesons at sea level or at a higher altitude lies in the fact that the beam of mu-mesons obtainable at these heights also contains a small number of strongly interacting particles (π, P) whose presence in the beam may result in a badly distorted picture of mu-meson interactions. At great depths underground, however, the beam is practically free from these undesirable particles and the processes involving mu-mesons can easily be recognized.

A big multiplate cloud chamber of dimensions $(90\times80\times50)$ cm^3 constructed in our Institute was shifted in August 1960 to a place 138 m.w.e. (metres water equivalent) under ground inside the hydroelectric power house of the D.V.C. at Maithon. After its successful installation and trial runs the chamber was ready to be used in experiments within two months in Oct. 1960. The first experimental study taken up using the cloud chamber is on the production of penetrating showers by fast mu-mesons. This experiment is progressing now. A brief analysis of the data already collected in the experiment is given below.

In about 1300 pictures taken so far with a three-fold coincidence shower trigger for the cloud chamber, 7 cases of mu-meson induced penetrating showers were identified. In all of these 7 events the primary had entered the cloud chamber singly and produced inside one of the plates of the chamber of shower containing two or more penetrating particles which had travelled at least 4 cascade lengths in the plates without multiplication. With these 7 events a value of the cross section for shower production by mu-mesons at the depth of the experimental station (138 m.w.e.) of $\sigma=(4.7\pm2.2)\times10^{-30}$ cm^2/nucleon was obtained. This investigation is being continued to improve the statistics of the experimental data.

A.4.2. *Energy dependence of the decay asymmetry of cosmic ray muons*

Cosmic ray muons are expected to have a longitudinal polarization of about 30% assuming that they are generated by pion decay (π-μ decay) in flight in the upper atmosphere. As a result of this polarization the electrons arising from the subsequent μ-e decay will show an asymmetry in the distribution of their directions of emission. The experiment of Clark *et al* (Phys. Rev., **108**, 1538, 1957) and many other subsequent experiments have revealed an asymmetry which is consistent with that anticipated for a 30% polarization of muons. Alikhanyan *et al.* (Proc. Moscow Conf., 1960), on the other hand, have found a higher asymmetry when they concentrated their observations to muons having energies greater than about 1 BEV. These authors have expressed the conclusion that in the energy region above 1 Bev the muons originating from K-μ decay, in which they are nearly 100% polarized contributes appreciably to the beam of particles so that the observed asymmetry is that due to a mixture of two muon currents, one having 30% and the other having 100% polarization. We have studied the decay asymmetry of cosmic ray muons at Darjeeling (Altitude 2200 metres) using a multiplate cloud chamber in two momentm regions, 0.35 and 1.3 Bev/c. Our observations with the higher momentuam group of

muons have been aimed at studying the dependence, if any, of the asymmetry on the momentum of muons. The results of our measurements are given below.

The cloud chamber fitted with eleven one inch thick copper plates was operated by an anticoincidence trigger. In about 36 thousand pictures taken with this arrangement out of 1500 cases of muon decay, 1021 cases in the momentum region 0.35 Bev/c and 479 cases in the region 1.3 Bev/c, were obtained. These numbers gave the values of asymmetry as 1.17 ± 0.05 and 1.18 ± 0.08 and of polarization as 33% and 34% in the momentum regions 0.35 and 1.3 Bev/c respectively.

These results differ from those reported by Alikhanyan in that they do not show any dependence of polarization on the momentum of muons. Our results are, however, slightly higher than the predicted value of 30% polarization for cosmic ray muons. This may be an evidence for the existence of a small contribution of muons from K-μ decay which seems to have affected the experimental results in two different monentum regions equally.

A.4.3. *Scattering of mu-mesons by copper nuclei*

Experiments on the measurements of ionizarion, on the production of knock on electrons, bremsstrahlung, mesic X-rays and electron pairs by mu-mesons and on the creation of mu-meson pairs by energetic photons have revealed that the mu-meson interacts with matter mainly through the electromagnetic field like a heavy electron of mass 207 times the electron mass. On the other hand, there exists quite a large number of experimental results on the measurement of scattering of mu-mesons by atomic nuclei which are not consistent with a purely electromagnetic interaction—the experimental scattering distributions have shown an excess of large angle scatterings over the number expected on the coulomb scattering of mu-mesons. The experimental evidence for the existence of an excess scattering has been obtained mainly from the work with high energy mu-mesons (>600 Mev). In the few low energy experiments carried out so far the statistical precision of the data is too poor for a definite conclusion. We, therefore, decided to investigate the scattering of mu-mesons in the low energy region. The photographs of the mu-mesons tracks obtained during the decay asymmetry experiment have been found to be very suitable for scattering measurements. We selected 450 pictures in which the muons after traversing four or more plates of the cloud chamber stopped and decayed in one of the remaining plates. The momentum of an indivial mu-meson after each scattering was determined from the estimation of the residual range of the particle from the centre of the plate in which the scattering occurred upto the point of stopping. This estimation showed that the mu-mesons selected for measurements had momenta in the region $40 \angle p\beta \angle 170$ Mev/c.

Altogether 2690 scattering angles were measured with the help of a goniometer eye piece. The angular distribution of these 2690 scatterings was found to be in satisfactory agreement with the distribution expected on the basis of an electromagnetic interaction alone between the mu-meson and nucleus. This result, therefore, indicates that there exists no anomalous scattering of mu-mesons in the momentum region below about 200 Mev/c.

We intend to continue our studies on mu-mesons in the underground laboratory recently set up at Maithon.

A.5. Nuclear Physics

A.5.1. *Studies on the Differential Coherent Scattering Cross-section of Gamma -rays using a Large —efficiency Compton Spectrometer*

An experimental arrangement has been setup to study the coherent (Rayleigh) scattering cross-section of different elements, e.g. Cu, Hg, Bi, Pb etc. using as sources of gamma -rays, Co^{60} and Cs^{137}.

A gamma ray beam scattered by a material contains two kinds of photons : (*i*) a large fraction of photons scattered by Compton process, and (*ii*) a smaller number scattered by coherent scattering processes (namely. Rayleigh, Thomson and Dehlbruck scatterings). The latter kind has the same emergy as that of the primary gamma ray, while the former suffers a change in energy as a function of the scattering angle. This difference in energy between the two kinds is the basis by which the photons suffering coherent scattering may be distinguished from a large background of Compton scattered photons.

Our investigation aims at studying the variation of the coherent scattering cross-section for a given gamma ray with atomic number of the scatterer and also variation with gamma ray energy. In order that the photons suffering coherent scattering may be distinguished from a large number of Compton scattered photons, the detection system must possess good energy resolution along with large efficiency. The usual Na I/Ti total absorption gamma ray spectrometer has a very large efficiency but suffers from a rather poor energy resolution. It was decided therefore, to use a Compton gamma ray spectrometer arrangement as a detector of the scattered photons. This possesses a much better energy resolution and to increase the efficiency some modifications have been incorporated into the usual two crystal arrangement of Hofstadter.

The Spectrometer arrangement. This consists of (*i*) a plastic phosphor (6″ long and 1″ diameter). This is the central phosphor and serves as the scatterer of beam to be analysed, and (*ii*) a large volume liquid scintillator with a central hole allowing the passage for the beam. The geometric arrangement between the two scintillators (which is symmetric with respect to the axis of the central phosphor) is such that only those photons are detected by the liquid scintillator as are scattered by the central plastic phosphor by more than 135°. By a coincidence between the two, therefore, we select from the incident beam (in this case this is already a mixture of the Compton and coherent scattered photons from a heavy element scatterer) the back scattered photons, the angle of scattering lying within 135°-180°. The idea behind restricting the scattering angle from the central phosphor is that the energy of the scattered photons (and consequently the energy of recoil of the electrons in the plastic phosphor) in this range is practically constant. A pulse height selection from the central phosphor together with a coincidence between the plastic and liquid scintillators will give the number of photons (of the beam being analysed) in the desired range of energy, and hence the number of photons suffering coherent scattering. Since the energy spread of such photons is very small, a pulse height analysis of the central detector (pulse resulting from recoil electron which is a function of the incident photon

energy) together with a coincidence between the two detectors gives the spectrum of the original gamma-ray beam. Such an experimental arrangement gives better resolution and at the same time higher efficiency. Calculations show that for a separation of 4″ between the plastic phosphor and the liquid scintillator, the efficiency $\sim 4\%$.

During the period under report work has been mainly confined to (*i*) the construction and testing of electronic equipments required for the investigations, (*ii*) long term stability testing of the photomultipliers and accessory circuits (*iii*) preparation of liquid scintillator.

The electronics employed in the present investigation consists of two amplifiers which feed the pulses to the usual slow-fast coincidence circuits. A pulse height analyser is incorporated in the slow circuit.

The amplifiers used have been constructed following models given by W. A. Higginbotham in the report BNL 234 and its supplements. The over all rise time of the amplifier is 0.5μ sec. The maximum linear output, however, is only 10 volts and this has caused some trouble in pulse height analysis.

The fast coincidence circuit is based on the multi-vibrator operated 6BNσ gated beam power tube for series coincidence (Smaller and Avery—R.S.I. 22, 341). The resolution of the circuit is 5×10^{-8} sec.

The slow coincidence circuit is a conventional Rossi circuit with resolution in the micro second range.

For pulse height analysis an Atomic single channel pulse height analyser is being used. For satisfactory pulse height analysis, the pulse should be in the 50 volt range. However, this is much larger than the range of the N.O.L. amplifiers already constructed. A large dynamic range asymmetrically biased L.T.P. is now being incorporated to the existing system to obviate this difficulty.

A long term stability testing is being made. Work in this direction has been hampered due to accidental failure of the air-conditioning equipment and power failure in C.E.S.C.

The liquid scintillator used is a mixture of toluene and diphenyl oxazole ($\sim$ 3 gms/litre) with POPOP ($\sim$ 0.1 mg/litre) as spectrum shifter. The present scintillator has been made from locally available toluene the purity of which is barely adequate for our purpose. Possibility of improving the purity of toluene is under consideration. The largearea liquid scintillator arrangement has poor light collection property. A thin walled glass container is under design and we are presently exploring the possibility of manufacturing it locally.

A.5.2. *Radio-carbon Dating*

Work on radiocarbon dating could not be pursued regularly as an essential equipment (a large volume deepfreeze) is being constructed in the workshop.

Our method of radio-carbon dating consists in converting the sample material into acelytene and then counting this acelytene by the liquid scintillation technique utilising coincidence between two photomultipliers set at the opposite faces of the scintillation cell.

As the solubility of acelytene in the scintillator (toluene+diphenyl oxazole+POPOP as spectrum shifter) is large only at low temperatures, it is necessary to maintain the scintillation cell and the associated part at about –80°C for a period of about twelve hours. The deepfreeze designed and being constructed in the workshop is expected to provide a constant temperature of –20°C; the scintillation cell and the photomultipliers will be housed inside the deepfreeze. Additional lowering of the temperature will be achieved by controlled flow of liquid oxygen cooled gas. The entire electronics has already been constructed and regular work is expected to be resumed after the deepfreeze construction has been completed.

A.6. Neutron Generator

The final phase of the construction of the new high power neutron generator being completed, the machine has been put on operational basis. 14-Mev neutrons have been generated in this remodelled, high efficiency neutron-source, by accelerating to 175 KV, more than 400μa of deuterium beam current, and focussing it on the tritium target, placed at a distance of 3 meters from the ion-source.

The accelerator tube is of vertical construction and has a total length of 3 meters excluding the ion-source. Total accelerating potential is being divided between 4 cylindrical electrodes, of which the first one next to the ion-source probe is the fosussing electrode. The accelerating tube is made up of 4 glass cylinders, flanged at the ends, and internal diamater of each is 6″. These have been specially manufactured by M/s. Sigcol Ltd. The ion-source is a Moak type R.F. ion-source, consuming a power of nearly 40 watts for delivering an ion-current of 400μa to the target. The ion-source is kept at the highest potential and the target is earthed. Pumps are being connected to this target end of the tube. The power for running the circuits for ion-source as well as for the probe and focussing voltage supply, is obtained from a 3KVA, 50 cycles 1450 RPM motor generator, which is insulated for 400 KV and run by a 5 H.P. motor—the connecting shaft being insulator perpex tube of 4′ in length. The above circuits are housed inside an aluminium shelf, which is also insulated from the ground for a potential of 400 KV.

The large diameter of the accelerator tube has been chosen from the consideration of high pumping speed. To ensure high pumping rate, a 6″ diameter, 3 stage, oil diffusion pump has been designed and it is fabricated by a local workshop. This pump is working satisfactorily, with a calculated unbaffled pumping speed of nearly 400 litres/sec. at 10^{-4} mm of Hg.

The generator has been housed in the newly constructed neutron laboratory building. It is a large room ($22'\times15'\times18'$) with an underground basement ($18'\times16'\times9'$). The laboratory is completely air-conditioned. The Cockroft-Walton high-voltage circuit, the insulated motor-generator, and the insulated shelf housing the ion-source oscillator, probe and focussing voltage supply circuits are installed in the upper room, while the grounded target end of the accelerator tube has been projected to the centre of the basement room

through an opening (4′×4′) in the floor of the upper room. The pumping unit is in the basement room. Neutron detectors like (*i*) scintillation counter with photomultiplier and (*ii*) BF_3-long counter are also housed in the basement. There is a control room (17′×7′×8′) built at the western side of the laboratory from which all the operations necessary for running the neutron generator can be remotely controlled. There is a shielded viewing window in between laboratory and the control room. The control room is sufficiently shielded against fast neutron flux exceeding 10^{11}n/sec.

The Cockroft-Walton circuit has been modelled to raise the accelerating potential to 320 KV. This nominal value has not yet been obtained, but the R.F. power supply is now being remodelled to yield more power so that this target is soon achieved.

Work is now in progress for setting up experiments for (*i*) absolute determination of neutron flux, (*ii*) total cross section determination of various materials and (*iii*) angular distribution measurement with a directional neutron detector.

Two deuterium absorbed targets have been ordered from Harwell, which will be used for the production of (d, d) neutrons. For this again, larger voltage are required to be generated in the C.W. circuit.

B. CHEMISTRY

B.1. Analytical and Physical Chemistry

B.1.1. *Vapour phase chromatography*

With the help of the following columns prepared in the laboratory, namely the didecyl phthalate, tricresyl phosphate, silicone oil, diglycerol, apiezon grease L and apiezon grease M, and squalane, it has become possible to separate a large number of different types of compounds such as hydrocarbons, alcohols, esters, fatty acids, ketones, aldehydes etc. and to study the different conditions of the best separation of the members of a particular series and to choose the most suitable liquid phase for the series.

Experiments at different temperatures with the above mentioned columns have enabled us to choose the optimum temperature for the operation of a particular column.

These studies have given us the clue for the sequence of the columns to be used in the separation of a mixture of different types of compounds (such as the neutral essential oils) with the help of multiple column arrangement.

A carrier gas by-pass system for the multiple column arrangement has been designed and constructed.

Preliminary separations of low boiling fractions of Indian petroleum have been achieved. Six different peaks are obtained corresponding to C_4, C_5, C_6 hydrocarbons. Experiments for the further resolution of these fractions with the help of multiple column arrangements using didecyl phthalate, squalane, and diglycerol columns in series have been taken up.

The preliminary fractionation of the essential oils extracted from a sample of tea has been done. Five broad peaks were obtained corresponding to six and seven carbon alcohols, aldehydes, ketones and phenolic bodies. These broad peaks are to be further analysed with the help of other columns using multiple column system.

It has become possible with the multiple column arrangement to separate two pairs of alcohols having almost identical boiling points. The pairs are ter. butyl alcohol, isopropyl alcohol (B.P. 83°C) and allyl alcohol, sec. butyl alcohol (B.P. 97°C).

B.1.2. *Paper electrophoretic studies on some coumarins*

The coumarins in general dissolve in alkaline solution due to the opening of the lactone ring followed by ionic dissociation. The electrophoretic behaviour of some of the coumarins was studied by means of paper electrophoresis technique. The coumarins used were coumarin, psoralene, umbelliferone, dalbergin, marmesine and wedelolactone. The alcoholic solutions of the samples were applied at the centre of 40 cm.×6 cm. Whatman no. 1 filter-paper strips. The strips were then sandwiched between two glass plates and electrophoresis was carried for about an hour with an applied potential gradiant of 10 volts/cm.

The electrolytes were : (1) *N*/10 NaOH solution and (2) Gylcine-NaCl-NaOH buffer system of varying pH. The results obtained are presented in the following table. The investigation is not complete.

Coumarins	Mobility (cm^2/volt × hour)		
	N/10 NaOH solution	Glycine buffer pH 12.7	Glycine buffer pH 8.45
1. Coumarin	—0.90	—0.90	+0.05
2. Psoralene	—0.24	—0.76	0
3. Umbelliferone	—0.33	—0.43	—0.33
4. Dalbergin	—	—0.18	0
5. Marmesin	—	—0.40	+0.20
6. Wedelolactone	—	0	0

B.1.3. *Paper electrophoresis of some metallic ions in lactic acid-sodium lactate buffer*

Paper electrophoretic mobilities of the following metal ions in the form of their lactates in lactic acid—Na lactate buffer were studied in order to investigate into their complex forming abilities with the buffer system.

The metal ions chosen were Cu^{++}, Zn^{++}, Mn^{++}, Cd^{++}, Co^{++}, Mg^{++}, Pb^{++}, $(UO_2)^{++}$, Al^{+++}, Cr^{+++} and Ag^{+}. The complex lactates of the metals were produced by precipitating the corresponding hydroxides and dissolving them in the buffer. Paper electrophoresis of these complex ions was then undertaken using (40 cm. × 6 cm.) strips of Whatman no. 1 filter paper sandwiched between two glass plates. The buffer system was a mixture of equal volumes of 4*N* lactic acid and *N* NaOH solutions. The voltage applied was 400 volts for one hour in each case. The solutions were applied along straight lines across the centre of the strips.

Cu^{++}, Zn^{++}, Al^{+++}, and $(UO_2)^{++}$ were found to remain immobile, indicating that they are forming such complexes which either remain undissociated or have very low mobility in the electrolyte under the experimental conditions; Cd^{++}, CO^{++}, Pb^{++}, and Cr^{++} ions most probably formed complex cations as their mobilities were found to be much less in comparison with those of other bivalent cations like Mg^{++} and Mn^{++}.

The work is being continued.

B.1.4. *Immunoelectrophoretic behaviour of proteins*

Immuno-electrophoretic technique has been adopted in order to study immunological behaviour of known proteins and also to identify unknown proteins. Preliminary experiments were done with immune sera from typhoid patients. Immunological behaviour of β-lactoglobulin is being studied. Toxin-antitoxin systems, employing tetanus antitoxin and toxoid and diptheria antitoxin and toxid, are being investigated.

As a part of the above work, a method is being standardized to estimate the separated proteins after electrophoresis. Protein-binding dyes, such as Amidoschwartz and Azocarmine, are being employed, the gel being formed over thin sheets of glass. The colour is read by densitometer and automatically recorded.

B.1.5. *Uranium and thorium uptake by plants grown on monazite sand.*

In order to investigate the uranium and thorium uptake by plants grown on monazite-bearing soil 10 different samples of plants have been collected from monazite areas in Kerala by the Dept. of Botany of the Institute. The collection contains 4 types of plants namely (1) *Crotolaria striata* (2) *C. verrucosa* (3) *Borassus flabellifer* (4) *Cocos nucifera*. Each type is associated with a sample of the same plant grown on non-monazite area. The analysis of uranium and thorium uptake of these plants has been handed over to the Chemistry Department.

Washing of the plants. In order to get rid of the radioactive dusts deposited over the plant each batch of the plants was washed several times and washings were filtered in each operation. Both the residue and the filtrate were counted for radio-activity by a scintillation counter after proper evaporation and drying in each case. The washing operations were continued till the filtrate and the residue showed very low count.

Ashing. The washed plants were then carefully and thoroughly dried in air oven, cut into small pieces and were ashed in a Muffle furnace at 600°C to 700°C.

Counting. The ashes of three *C. striata* (monazite) samples have been counted along with ashes of one *C. striata* (non-monazite) sample. The counts indicated activity in the monazite sample and no activity in the non-monazite sample. The seed pods from *C. striata* (monazite) samples had been collected and seeds were taken out of them carefully so that they remain uncontaminated from the dust of the pod surfaces. The seeds were ashed in the usual way and the ash was counted. The count indicated radioactivity.

Preparation of "I.R.A. 400" resin columns for the concentration and separation of uranium and thorium from solution. Two columns each containing about 30 gm. of "I.R.A. 400" resin were assembled. The resins were throughly washed with about 2 litres of distilled water, and then with 1 litre of 10% Na_2CO_3 solution. The treated resins were washed with distilled water and then packed in the columns (18 cm. high × 1.5 sq. cm. bed).

Standardization of the columns. From standard solutions of $UO_2(NO_3)$, and $Th(NO_3)_4$, $UO_2(OH)_2$ and $Th(OH)_4$ were precipitated by NH_4OH. These hydroxides were filtered, washed and dissolved in 500 ml of a mixture of 5% Na_2CO_3 and 5% $NaHCO_3$ solutions. This resulting solution was passed through the columns at a very slow rate. 100 ml more of the 5% Na_2CO_3+$NaHCO_3$ solution was then passed through the columns. The effluents contained all the thorium. These were analysed for thorium. 150 ml of 1 M NaCl solution was then passed through the columns. The effluents contained all the uranium. These were analysed for uranium. The analyses showed that over 99% of thorium and uranium taken was recovered.

B.1.6. *Physico-chemical studies on proteins of buffalo's milk*

(*a*) *Investigations on the probability of occurrence of different β-lactoglobulins and α-lactalbumins in buffalo's milk.* Preliminary results of these investigations reported in the previous Annual Report (1959-60, p. 30) indicated that no evidence of the genetic control of

occurrence of β-lactoglobulin and α-lactalbumin as observed in the cow, was found in the case of the buffalo. Further typing experiments carried out by the method of Aschaffenburg and Drewry (Nature, 1955, *176*, 218) with milk from about 100 buffaloes confirm these observations. Buffalo's milk is found to contain only one type of β-lactoglobulin which corresponds closely to the β-lactoglobulin-B of cow's milk and a single α-lactalbumin of higher mobility than that found in European breeds of cow.

The presence of the single α-lactalbumin of higher mobility in buffalo's milk was suggested as a possible basis of a test to detect adulteration of cow's or buffalo's milk by mixing one with the other. Recently, however, in 3 samples of cow's milk, two lactalbumin bands have been observed, of which the faster moving component migrates to the same position as that of the α-lactalbumin of buffalo. This observation is in agreement with the α-lactalbumin polymorphism first observed by Blumberg and Tombs (Nature, 1958, *181*, 683) in Nigerian White Fulani or lyre-horned Zebu cattle. The occurrence of α-lactalbumin polymorphism in cow's milk renders differentiation on this basis uncertain. The faster and slower moving α-lactalbumins of cow's milk have been designated by Blumberg and Tombs as α_A and α_B respectively and when they occur in combination, as α_{AB}. In about 30 cows so far examined (randomly chosen) types with α_A and β_{AB} or β_B were most common. Two or three animals contained α_{AB} and none β_A. The α-lactalbumin polymorphism has been found to be fairly widespread amongst the African Zebu cattle (Aschaffenburg, private communication) and animals of this breed are believed to have originated in India. It is likely therefore that types with α-lactalbumin containing α_A, α_B or combination of both would be more common than has been observed here. A more systematic examination of lactalbumin and lactoglobulins of native breeds of cow is under investigation.

(*b*) *Physico-chemical studies on crystalline β-lactoglobulin of buffalo's milk.* Preparation of crystalline β-lactoglobulin and preliminary results of its electrophoretic and sedimentation behaviour were described in the last Annual Report (1959-60, p. 31). The results indicated that in crystalline form, electrophoretic and sedimentation properties, β-lactoglobulin-B of cow's milk and buffalo β-lactoglobulin showed striking similarity. These investigations have been carried out in greater details under different experimental conditions in order to obtain a better understanding of the properties of the protein molecule. Diffusion coefficient of buffalo β-lactoglobulin has been measured and its molecular weight and axial ratio have been calculated. From measurements of ultraviolet absorption spectra, the specific extinction coefficient and tyrosine and tryptophan contents have been determined. The results described below serve to a great extent in characterization of the protein as well as in establishing points of similarity or otherwise between buffalo β-lactoglobulin and cow β-lactoglobulin-B.

Moving boundary electrophoresis. The comparison of the electrophoretic patterns of buffalo β-lactoglobulin at different pH's and concentrations with those of cow β-B shows some minor differences in degree of heterogeneity and electrophoretic mobilities. The isoelectric point of buffalo β-lactoglobulin is 5.28, which is close to the value of 5.23 for cow β-B

(Klostergaard and Pasternak, J. Am. Chem. Soc. 1957, *79*, 5671). The pH *vs.* mobility curves of the two lactoglobulins intersect near the isoelectric point below and above which the negative or positive mobilities of cow β-lactoglobulin-B show progressively higher values than buffalo β-lactoglobulin with change of pH. In the pH range 4.64 to 5.6 the net charge does not differ by more than one electronic unit. While the nature of the descending patterns of buffalo β-lactoglobulin is remarkably similar to that of cow β-B below the isoelectric point, above it the correspondence is somewhat lost. Buffalo β-lactoglobulin does not show hypersharp descending patterns above the isoelectric points as found in the case of cow β-B by Tombs (Biochem. J. 1958, *69*, 491).

Sedimentation. Sedimentation analysis of buffalo β-lactoglobulin shows that like cow β-B, it does not show tendencies to association or dissociation at low temperature in the region of pH and protein concentration where it is electrophoretically heterogeneous. Buffalo β-lactoglobulin appears to satisfy the tests of homogeneity in the ultracentrifuge at pH 5.10 and exists as a monodisperse solute at this and near about pH's. This is shown from the fact that single symmetrical boundaries are obtained throughout the experiment at pH 5.10 at two different concentrations and the area under the boundary after correction for radial dilution due to sector shape of the cell accounts within 2-3% for all the materials in solution. A straight line plot obtained by application of Fujita's equation (J. Phys. Chem. 1959, *63*, 1092) for computation of diffusion coefficients also indicates homogeneity of the solute.

The dependence of sedimentation coefficient of buffalo β-lactoglobulin on concentration has been studied in acetate-NaCl buffer of pH 5.1, ionic strength 0.2. The value of $s_{20\,w}$ at zero protein concentration is found to be 3.00×10^{-13} sec. which is close to the value of 3.04×10^{-13} sec. for cow β-B (Townend *et al.*, J. Am. Chem. Soc. 1960, *82*, 3161).

Studies on the effect of pH on sedimentation coefficient of buffalo β-lactoglobulin in the pH range 1.6 to 10.0 at room temperature show that it is stable in the pH range of approximately 4.5 to 7. Below pH 4, the buffalo β-lactoglobulin undergoes reversible change and above pH 8, irreversible. Such reversibility at low pH's and irreversibility at alkaline pH's have been observed in the case of cow β-lactoglobulins. Townend *et al.* have shown it to undergo reversible dissociation into subunits of 17,000-18,000 molecular weight at low pH values. It is likely that buffalo β-lactoglobulin would also show similar properties. These points are under investigation at present.

Determination of diffusion coefficient. Diffusion experiments have been carried out in the moving boundary electrophoresis apparatus with buffalo β-lactoglobulin in acetate-NaCl buffer of pH 5.10, ionic strength 0.2 at 20.6 ± 0.03°C. A standard 11 ml capacity cell was used as a diffusion cell and the boundary was sharpened by siphoning technique of Kahn and Polson (J. Phys. and Coll. Chem. 1947, *51*, 816). The value of $D_{20,\,w}$ of buffalo β-lactoglobulin has been found to be 8.48×10^{-7} cm^2 /sec. at 1.g/100 ml. protein concentration. This value is higher than the value of 7.85×10^{-7} cm^2 /sec. for cow β-lactoglobulin.

Molecular weight and frictional ratio. Molecular weight of buffalo β-lactoglobulin calculated from sedimentation and diffusion data has been found to be 34,400 g./mole, which is close to the value of 35,000-36,000 g./mole of cow β-lactoglobulin. The frictional ratio of buffalo β-lactoglobulin has been calculated as 1.16. This value is somewhat lower than the value of 1.33 for cow β-lactoglobulin.

Ultraviolet absorption. Both cow β-B and buffalo β-lactoglobulin have the same extinction coefficients of 9.4 at 278-279 mμ in the neutral pH range. Buffalo β-lactoglobulin contains 7.6 g. moles of tyrosine and 3.9 g. moles of tryptophane per 35,000 g. of protein. These values are fairly close to those of cow β-B (Ogston and Tombs, Biochem. J. 1957, *66*, 399).

Results of electrophoretic and sedimentation experiments show that the electrophoretic heterogeneity of buffalo β-lactoglobulin is not due to molecular association. Similar behaviour is shown by cow β-B and attempts have been made to exaplain its electrophoretic behaviour as being due to interaction with buffers (Timasheff *et al.* J. Am. Chem. Soc. 1961, *83*, 464). It is apparent that although cow β-B and buffalo β-lactoglobulin have almost the same molecular weights, specific extinction coefficients and tryptophane and tryosine contents and the differences in electrophoretic and sedimentation behaviour may be due to the presence of different number and types of ionisable groups and minor structural differences.

(*c*) *Studies of crystalline α-lactalbumin from buffalo's milk.* The preparation of crystalline α-lactalbumin from buffalo's milk was reported in the last Annual Report (1959-60 p. 32.)

Moving boundary electrophoresis. Moving boundary electrophoresis of crystalline α-lactalbumin of buffalo's milk was carried out in a Tiselius apparatus with 11 ml cell at 1°C in the pH range 3.2-8.6 at 0.1 ionic strength and at different protein concentrations at potential gradients of 5-6 volts/cm.

Electrophoresis experiments were carried out in lactate, acetate, phosphate and veronal buffers. The protein was found to show a single symmetrical peak in acetate buffer of pH 4.45 and at other pH's it was found to be heterogeneous. Maximum heterogeneity was observed in lactate buffer pH 3.2 and in phosphate buffer, pH 7.58. The ascending and descending patterns in lactate buffer, pH 3.2, showed three peaks. The fastest ascending pattern was hypersharp while the descending patterns were diffuse. In phosphate buffer pH 7.58, the descending pattern was skew and did not resolve while the ascending pattern showed one main component and two minor components which did not separate completely from the main peak. At pH's 4.85, 5.48 and 8.60 it was found to show two peaks of which the slower moving component represented the major part. The mobilities of buffalo lactalbumin at various pH's were calculated and found to be different from that of cow lactalbumin reported by Klostergaard and Pasternak (J. Am. Chem. Soc. 1957, *79*, 5674). The electrophoretic patterns also differed from those of cow lactalbumin. The isoelectric point of the slower moving component (major fraction) of the buffalo milk lactalbumin was determined from mobilities in acetate buffers of 0.1 ionic strength and was found to be 4.92. The isoelectric point of cow lactalbumin has been reported to be 5.1 by Klostergaard and Pasternak.

Ultraviolet absorption. Ultraviolet absorption of buffalo lactalbumin has been measured in the region of 250 to 370 mμ in acetate buffer, pH 5.1, ionic strength 0.1 and in 0.1N NaOH. Buffalo lactalbumin showed two absorption maxima, one at 281-282 mμ and the other 289-290 mμ. The specific extinction coefficient at 280 mμ in acetate buffer pH 5.1, ionic strength 0.1 was found to be 18.0.

Spectrophotometric determination of tyrosine and tryptophane in buffalo lactalbumin. Tyrosine and trytophane contents have been determined on different samples of buffalo lactalbumin. The average values of tyrosine and trytophane were found to be 5.94% and 4.92% respectively.

B.1.7. *Physico-chemical studies on proteins of goat's milk*

(*a*) *Investigations on the protein constituents of goat milk whey and their occurrence in the milk of individual goats of different breeds.* The investigation arose as a result of the findings with whey proteins of buffalo's milk (see previous section). Whey of goat's milk obtained after removal of the casein by precipitation with Na_2SO_4 (20 g./100 ml.) at 37-40°C, gives a precipitate on treating it with $(NH_4)_2SO_4$ (23 g./100 ml.) containing β-lactoglobulin, lactalbumin and a third component (component 3). The dialysed aqueous solution of the ammonium sulphate shows the presence of only one band in filter paper electrophoresis at pH 8.6 using veronal buffer of 0.1 ionic strength. At lower pH's however, separation occurs and at pH 4.6, in acetate buffer of 0.1 or 0.05 ionic strength, applying a voltage of 240 volts, three well separated bands are obtained, the fastest moving one of which has been identified with the β-lactoglobulin obtained by Askonas (Biochem J. 1954, *55*, 332). The same bands are obtained from the whey fraction, if the milk is treated by Askonas' method.

Milk from more than forty goats belonging to black Bengal and mixed Jamnapari breeds have been examined with the same results. The animals belonging to the former breed are small and have poor milk yield while the latter are large animals with fairly high milk yield. Of the 23 breeds known (Kaura, *Indian Breeds of Livestock* 2nd Ed., Prem Publishers, Lucknow, 1957) only these two breeds are commonly found in this part of the country. So far, no polymorphism has been detected any of the protein constituents and it seems that in goats, like the buffalo and unlike the cow, the occurrence of whey proteins is not genetically controlled. More experiments are being carried out to confirm this observation.

Attempts have been made to separate the three protein constituents and crystallize them. The β-lactoglobulin can be obtained from the filtrate after precipitating the lactalbumin together with the component 3 by acidification of the Na_2SO_4 containing whey to pH 3. From the filtrate, the β-lactoglobulin can be precipitated by addition of ammonium sulphate (23 g./100 ml.) at pH 6. Microcrystals are obtained upon making a paste of this with water. This paste when left in the cold room forms large crystals. Further addition of water dissolves the precipitate and uniform crystals are obtained by dialysing the solution saturated to 50% with ammonium sulphate against $(NH_4)_2SO_4$ solution, the concentration of which is increased slowly from 50% to 70%. The crystals are hexagonal bipyramids as obtained by Aksonas.

The mixture of acid precipitated lactalbumin and component 3 can be separated to a fair degree from a solution at pH 6 by fractionation with ammonium sulphate. Attempts are being made to purify and crystallize these fractions.

B.1.8. *Isolation and characterization of seed proteins*

(*a*) *Studies on sesame seed proteins.* In previous Annual Reports (1958-59, p. 29 and 1959-60, p. 32), it has been shown by sedimentation study and variable-solvent solubility test that crystalline α-globulin contains two distinct components. Electrophoretic analysis, which was limited over the pH range 2.45 to 4.0, gives a single peak at pH's 4.0 and 3.23 and two partially resolved peaks at pH 2.45. At pH's below 4.5, protein undergoes denaturation as indicated by its solubility behaviour in a solvent of low pH. In the year under review, ultracentrifuge experiments have been extended to study particularly the nature of the pattern of crystalline α-globulin at low pH and to confirm the heterogeneity of amorphous α-globulin which was claimed by Nath and Giri (J. Sci. Indus. Res. 1957, *16C*, 51) to be electrophoretically homogeneous protein in the pH range 3.0 to 12.0.

Sedimentation studies on total proteins precipitated with $(NH_4)_2SO_4$, at pH 6.92 reveal the presence of four distinct components having $s_{20,w}$ values of 1.8, 6,88, 12.35 and 18.14×10^{-13} sec. These are designated as S-1, S-7, S-13 and S-19 components. In the neutral range of pH, crystalline α-globulin as well as amorphous α-globulin (prepared by Giri and Nath's method) contain two sedimenting species corresponding to S-13 and S-19 components. In one preparation, a third component having a $s_{20,w}$ value of 23.69×10^{-13} sec. was observed. The result does not agree with Giri and Nath's conclusion of homogeneity of α-globulin. The value of $s_{20,w}$ of S-23 component at zero protin concentration is found to be 13.39×10^{-13} sec., indicating a molecular weight of about 300,000, as is the case with other oil-seed globulins. The specific extinction coefficient of crystalline α-globulin is found to be 10.8 at 279-280 mμ.

Studies on the effect of pH on sedimentation coefficient of α-globulin show that it is stable in the pH range 4.5 to 9.0. At pH 10.0, part of the S-19 component dissociates giving a new molecule having a $S_{20,w}$ value of 8.26×10^{-13} sec. At pH 4.0, α-globulin breaks down into an uneven mixture of closely sedimenting particles which undergo further subdivision into smaller but more well defined units as the pH is lowered to 2.45. The solubility behaviour at low pH's also indicates that irreversible configurational changes take place in the molecule.

The denaturation of many seed globulins at low pH is a general phenomenon first investigated by Osborne (*The Vegetable Proteins*. 2nd ed. Longmans, Green and Co., London, 1924). The denaturation of globular proteins of animal origin, as a function of pH, has received much attention recently, but not much investigation appears to have been done on acid denaturation of seed globulins. Further work on the denaturation of α-globulin of sesame seeds is being taken up.

(*b*) *Studies on* Abrus *seed protein.* Separation of three protein fractions from the extract of seeds of *Abrus precatorius* was reported in the Annual Report of 1959-60 (p. 83).

Preliminary experiments on toxicity have been carried on white mice. Each fraction containing 10 mg. of protein in 0.8 ml phosphate buffer, pH 6.78, was injected intraperitioneally on three white mice. Controls were maintained by injecting equal volumes of buffer on three mice. The supernatant (S), obtained by dialysing the solution of total protein against water, after purification showed strong haemagglutinating activity and strong toxicity on mice. All the three mice injected with this fraction died within 1-3 hr. The solution in 2% NaCl of the residue (R) obtained after dialysing the total protein against water on further dialysis against acetate buffer pH 5.2 gave a supernatant (SR) which showed very strong haemagglutinating activity and found to be equally toxic. All the three mice injected with this fraction died within 1-3 hrs. The residue left after dialysing the solution of R against pH 5.2 on further purification was found to be non-agglutinating and showed less toxicity. The three mice injected with this fraction died after 12-14 hrs.

B.2. Plant Chemistry

B.2.1. *Chemical investigations on* Murraya koenigii.

In continuation of previous work (Annual Report 1959-60, p. 39) it has been observed that the stem-bark contains two crystalline substances, m.p. 176-7° and 166-7° respectively. Both of them could be sublimed unchanged in high vacuum, and are apparently homogeneous.

The compound (m.p. 176-7°) contains nitrogen (Found : C 79-59; H, 5.67 and N, 5.07%). The UV spectrum showed absorption maxima at 238 and 288 mμ.

The second compound showed UV absorption bands at 238, 274 and 332 mμ and gave characteristic colour reaction for coumarin.

B.2.2. *Investigation of* Swietenia mahogani

The decorticated and powdered seeds of *S. mahogani* yielded to petroleum ether (40-60°C) a thick yellow oil (33%). From the chloroform extract a crystalline product was obtained following the method of fractional crystallisation, purification by $Ba(OH)_2$ and finally by chromatography over alumina. It has m.p. 206-7 and sublimes at 200° in high vacuum. The UV curve is similar to that of swietenine, with which it may be structurally related.

B.2.3. *Survey of saponin-bearing plants of India*

B.2.3.1. (*a*) *Barringtonia acutangula* Gaertn. Preliminary investigation on the constitution of barringtogenol D showed it to be a triterpene alcohol containing three hydroxy groups and an oxide linkage (*vide* previous Reports). Further studies on this triterpene establishing its constitution are now described.

The presence of the oxide linkage in barringtogenol D was also established by opening the oxide ring to give the previously described tetraacetate, $C_{38}H_{58}O_8$, m.p. 284-85°, which on hydrolysis furnished a tetrol, $C_{30}H_{50}O_4$, m.p. 293-95°, $[\alpha]^{28°}+48°$.

It has been shown that the ethylenic linkage in barringtogenol D is extremely hindered. Its triacetate did not react with SeO_2 under conditions where all known triterpenes of the β-amyrin series are converted to $\Delta^{11,13(18)}$ dienes. Evidence for the presence of the typical 12:13-double bond was, however, obtained by oxidation of the triacetate with CrO_3 to yield an $\alpha\beta$-unsaturated keto compound, $C_{36}H_{52}O_8$, m.p. 256-58°, having an unusual UV maximum at 241 mμ that is, at a lower wavelength than is uaually observed for 11-keto Δ^{12}—triterpenes. The double bond estimation of tetraacetate obtained after opening of the oxide linkage showed the presence of the original hindered double bond intact, which consumed perbenzoic acid slowly but the uptake was typical of a β-amyrin compound. This proved barringtogenol D to be a β-amyrin derivative and the shielding effect on the double bond was ascribed to the oxide bridge in the compound.

The ready formation of the triacetate indicated the hydroxyl groups to be primary and/or equatorially oriented secondary alcoholic functions. Barringtogenol D contained no α-glycol group but the presence of a 1:3-glycol was suggested by the formation of a crystalline monoacetonide, $C_{33}H_{52}O_4$, m.p. 233-36°, $[\alpha]^{24°}+33°$. Oxidation of this acetonide furnished a colourless product, $C_{33}H_{50}O_4$, $[\alpha]^{25°}+57.2°$, which has been shown by Zimmerman test and optical rotatory dispersion curve to be a 3-keto derivative. Failure of this product and of other oxidation products obtained later to give any colour with ferric chloride (test for diosphenol group) and the absence of 3 : 23-diol system (copper-bronze oxidation) clearly indicated that barringtogenol D contained no other substituents except the hydroxyl group at C_3 in ring A. From the ease of acetylation of the triterpene and the molecular rotational differences (ΔM) of the 3-keto-acetonide and 3-hydroxyacetonide, the secondary hydroxyl at C_3 has been assigned an equatorial β-configuration.

The secondary hydroxyl group of the 1 : 3-glycol, situated in other part of the molecule (D/E ring, there being no other site for 1 : 3-glycol in rings B and C) was unusually hindered towards chromic acid reagents. The sapogenol when oxidised with CrO_3-pyridine complex gave a hydroxy ketone (IR spectrum) and with CrO_3-sulphuric acid in acetone neutral and acid products. The ester of the acid on chromatographic resolution furnished two colourless crystalline compounds, characterised as methyl diketo ester, $C_{31}H_{44}O_5$, m.p. 217-18° and methyl hydroxy keto ester, $C_{31}H_{46}O_5$, m.p. 274-77°, $[\alpha]$ +77.45, which formed an orange red crystalline DNP derivative. A band at 1765 cm^{-1} in IR spectrum of the methyl diketo ester suggested the presence of a five-membered ring ketone involving the oxide function. The neutral fraction obtained as a crystalline product melted with a range of 272-88°, which on further oxidation and esterification furnished the above two esters. These reactions of barringtogenol D towards chromium trioxide find a remarkable parallel in the case of aescigenin and offer considerable support to similar disposition of the groupings in barringtogenol D.

From the consideration of all the available positions for the seat of 1 : 3 glycol group in the β-amyrin nucleus and by process of elimination in the light of various reactions described above, it is suggested that barringtogenol D also has its 1 : 3-glycol group at

position 28, 22. This is further supported by the fact that barringtogenol D is accompanied by barringtogenic acid, 2 : 3-dihydroxy-olean-12-ene-23, 28-dioic acid and since the triterpenes so far isolated from different *Barringtonia* species have invariably oxygen function at position 28, it is assumed that barringtogenol D also has a primary hydroxyl group at this position which has been demonstrated by the formation of methyl diketo and methyl hydroxy keto esters. Thus, a 3β, 22, 28-trihydroxy-olean-12-ene, x,y-oxido structure is tentatively suggested for barringtogenol D.

A direct correlation of barringtogenol D with a triterpene of established constitution was confirmed in the following manner.

Barringtogenol D was oxidised with chromium trioxide in acetic acid and benzene and the neutral oxidation product on reduction by Wolff-Kishner method gave a compound, $C_{30}H_{48}O_2$, m.p. 198-200°, $[\alpha]^{25°}+61°$, identical with 22β-hydroxy-16α, 21α-oxido-olean-12-ene, prepared by O. Jeger *et al.* from aescigenin (*Helv. Chim. Acta*, 1957, *40*, 2390). Our compound gave an acetyl derivative, m.p. 201-203°, $[\alpha]^{25°}+79°$ also identical with the acetate of Jeger's compound.

This conclusively proved the structure and stereochemistry of barringtogenol D as 3β, 22β, 28-trihydroxy-16α-21α-oxido-olean-12-ene.

Further proof of barringtogenol C being a member of the β-amyrin group of compounds (*vide* previous Report) was obtained by isolating the SeO_2 oxidation product which gave characteristic triple absorption in the UV for the $\Delta^{11:12,13:18}$ diene. Two of the hydroxyl functions have been shown to be primary alcoholic by preparation of a crystalline triphenyl methyl carbinol derivative, $C_{49}H_{64}O_5$, m.p. 247-48°, $[\alpha]^{25°}+28.22°$.

The work on further elucidation of the structure of barringtogenol C is being continued.

B.2.3.2. (*b*) *Albizzia procera* Benth. The neutral sapogenin fraction obtained from the beans of *Albizzia procera* (*vide* previous Report) on chromatographic resolution gave the following three triterpenoid fractions :

Fraction A, m.p. 260-70°; acetate, m.p. 300-305°.

Fraction B, m.p. 198-99°; acetate, m.p. 220-23°.

Fraction C, m.p. 290-300°; acetate, m.p. 242-44°.

All these products gave pink colour in Liebermann-Burchard test and a yellow colour with tetranitromethane. Further work will be continued as soon as fresh supply of raw material is available.

B.2.4. *Chemical investigation of Indian plant resins.*

In continuation of the previous work, the chemical examination of the resins of *Jatropha curcus*, *Shorea robusta* and *Commiphora mukul* have been undertaken.

(*a*) *J. curcus*. The petroleum ether extract of *J. curcus* stem bark yielded a thick reddish brown resin. The volatile constituents were removed by steam distillation and the residue was examined for acid fraction. The latter was found to be practically absent. Preliminary paper chromatography of the neutral fraction did not indicate the presence

of triterpenoid type of compounds. β-Sitosterol was isolated. It was chracterised through its benzoate m.p. 141°-143° and acetate m.p. 127°-128°.

(*b*) *Shorea robusta*. Since this resin contains about 65-70% essential oil it was subjected to steam distillation for a long period. The residue was extracted with petroleum ether and benzene successively. On removal of the solvents gummy residues were obtained. The acid fraction was very small. The neutral fraction on paper chromatography indicated the presence of triterpeniod type of compounds.

However on column chromatography over neutral alumina and acid washed alumina the neutral fraction failed to resolve itself into crystalline fractions. The acid fraction was esterified and the ester subsequently chromatographed over neutral and acid washed alumina. These attempts to obtain crystalline fractions were unsuccessful. Various glassy resinous fractions were obtained. These displayed a brilliant violet colour in Libermann-Burchard test.

(*c*) *Commiphora mukul*. The commercial resin was extracted with ether over a period of 60 hr. and the ethereal extract was treated with 10% alkali leaving a reddish brown neutral fraction.

The neutral fraction was chromatographed over neutral alumina. The petrol ether eluate which was gummy in nature solidified on treatment with methanol; m.p. 129-136°.

The benzene eluate was also reddish brown and gummy in nature but had slight fluorescence in solution. Recrystallisation of the latter from benzene yielded a colourless crystalline solid, m.p. 230-239°. This compound did not respond to Libermann-Burchard reaction. It is suspected to be a diterpenoid compound. Further work is in progress.

(*d*) *Canarium euphyllum*. An authetic sample of this resin is under investigation.

The inhibitory action of the resins of *J. curcus*, *S. robusta*, *S. tambuggia* and *C. euphyllum* against some test bacteria and fungi has been studied.

B.2.5. *Chemical investigation of lac resin*

Under a project on investigation of the constitution of lac, palas seed lac was fractionated by temperature-phase separation in acetone. Fraction (I) separated at 30° C as a spongy mass. Ether extraction of the latter removed a soft reddish resin (13%) which gave intense purple colour with caustic soda. The residue left after ether extraction, on fractionation at 30°C from acetone, gave a light brown amorphous powder.

Study of its characteristic properties, *viz.*, melting point, acid value, saponification value, hydroxyl value etc. is being carried out.

B.3. Radiochemistry

B.3.1. *Photosynthesis*

(*i*) *The role of iron-containing compounds in photosynthesis*. The role of iron-containing compounds in photosynthesis has been investigated further using Fe^{59} as a tracer. Detailed fractionation of the cell constituents has revealed an accumulation of Fe^{59} in chloroplasts, mitochondria and acetone precipitable substances which according to some Russian workers

are concerned with the early CO_2 fixation reactions in photosynthesis. With a view to isolating a relatively large amount of the substance to permit chemical studies, several twigs of *Xanthium strumarium* were allowed to take up 20μ C of $Fe^{59}Cl_3$ for 24 hr. followed by photosynthesis in $C^{14}O_2$ for 15 min. The leaves were then extracted in 0.5m sucrose. The extract was treated with 1.5-2 volumes of cold acetone or with $(NH_4)_2SO_4$ to 50% saturation. Both the acetone and $(NH_4)_2SO_4$ precipitates revealed Fe^{59} and C^{14}-activity which were discriminated through the use of aluminium absorbers. Specific activity of the acetone precipitate was much higher than that of the $(NH_4)_2SO_4$ precipitate. Part of the Fe^{59} and C^{14}-activity could be extracted with water, phosphate buffer or 10% NaCl. The Fe^{59} : C^{14} ratio of the water extractable acetone precipitate and in the chloroplast was 5 : 1 and 6 : 1 respectively.

(*ii*) *Photosynthesis in variegated leaves*. An investigation has been undertaken to study photosynthesis in variegated leaves. Intact leaves and isolated sections of white apparently non-photosynthetic regions of *Scindapsus officinalis* were allowed to fix $C^{14}O_2$ in high intensity light for 15 min. and the products analysed by paper chromatography and autoradiography. The green region of the leaves revealed the conventional pathway of photosynthetic CO_2 fixation. In isolated non-green regions dark CO_2-fixation products, were prominent. C^{14}-activity in the isolated sections was higher than that in the non-green region of the intact leaf, indicating a rapid translocation of CO_2-fixation products.

B.3.2. *The action of plant growth hormones*

(*i*) *The binding of auxin to protein*. The interrelationship between the process of binding of auxin to protein and protein synthesis has been studied. Sections of pea internodes or *Avena* coleoptiles were incubated with naphthalene acetic acid-1-C^{14} and chloramphenicol or 8-azaguanine—well known inhibitors of protein synthesis, and the trichloroacetic acid precipitable proteins isolated. None of these compounds had any inhibitory effect on the binding process; on the other hand a promotive effect was observed, which was more prominent when lower concentrations were used. However, a pre-incubation in chloramphenicol before addition of auxin had some inhibitory effect. Chloramphenicol was also found to be harmless to the growth of *Avena* coleoptiles whether in presence or absence of indole acetic acid. Chloramphenicol also did not inhibit the incorporation of amino acids—glycine-2-C^{14} and *l*-leucine-C^{14}—into protein. There was some stimulation at lower concentrations.

The auxin-protein binding does not appear to be controlled by RNA. Pre-incubation with 100 μg./ml RNase showed no inhibitory effect on the binding process. On the other hand some stimulation was observed which is probably due to the liberation of nucleotides utilized in the binding process.

(*ii*) *Auxin-induced stimulation of amide synthesis*. If the contention that auxin induces stimulation of ATP synthesis (suggested previously), is correct it may be predicted that application of IAA would stimulate such ATP consuming reactions as amide synthesis. This has been found to be so. Thus, coleoptile tissues, when pre-incubated with IAA, showed an increased conversion of aspartic acid-C^{14} to asparagine-C^{14} and glutamic acid-C^{14} to glutamine-C^{14}.

(*iii*) *The relationship between malic acid and auxin action.* On the basis of the data obtained in previous experiments concerning the effect of IAA on CO_2 fixation in *Avena* coleoptiles, it was suggested tentatively that application of IAA stimulates metabolic changes leading to the generation of DPNH which is utilized for the formation of malic acid etc. The occurrence of a DPN-linked malic dehydrogenase in coleptile tissues has since then been established and it has been found that the equilibrium of the reaction involving the conversion of oxaloacetate to malate is far towards the formation of malate involving the utilization of DPNH.

The fate of malic acid has been studied by incubating coleoptile sections with malic acid-1, 4-C^{14} (prepared in this laboratory) in presence and absence of auxin. It was observed that a significant part of the malic acid-C^{14} metabolised could be recovered in respiratory CO_2 and in the ethanol-insoluble cell-wall components.

B.3.3. *Biosynthesis and metabolism of gibberellic acid*

Attempts have been made to isolate gibberellic acid-C^{14} by incorporating acetate-1-C^{14}, acetate-2-C^{14}, glucose-C^{14}, fructose-C^{14}, sucrose-C^{14} and extracts of C^{14}-labelled soyabean leaves in the culture media on which *Gibberella fujikuori* was grown. In most of these cases, gibberellic acid was found to be tagged with C^{14}. The yields however were low and attempts are now being made to improve them.

With a view to studying the biosynthetic steps in the formation of gibberellic acid, a number of variants have been isolated through ultraviolet irradiation. Suspension of very fine fragments of mycelia were obtained by using a Nossal disintegrator or by vigorous shaking of the culture fiasks during the incubation period. The latter procedure yielded better results. At 100 erg./sec./mm^2., ten minutes were completely lethal. Variants were obtained by irradiating the suspension for 2.5, 5 and 7.5 minutes.

Acetone extracts of seeds of several plants belonging to *Cucurbitaceae* and *Leguminoseae* have been tested for the occurrence of gibberellin type compounds. Partially purified extracts were tested by the dwarf maize bioassay method of Phinney and West. In preliminary experiments extracts of unripe green gram (*Cicer arietinum*) and *Cucumis melo* var. *momordica* had revealed marked growth promotive properties characteristic of gibberellin type compounds.

B.3.4. *Interrelationship between the synthesis of DNA and RNA in coconut milk*

Interrelationship between the synthesis of DNA and RNA is a matter of considerable interest. Coconut milk with its free nuclei seems to be ideally suited for this type of investigations. Young green coconut fruits were injected with a solution of phosphate-P^{32} without breaking open the nuts and with the inner environment least disturbed. After incubation for 18-20 hr. the milk was decanted and centrifuged at low speed to separate the nuclei. The milk from another coconut which had received no P^{32} was similarly centrifuged and the supernatants in the two tubes were interchanged after washing the nuclei in 0.5m sucrose. The tubes were incubated for 2.5-3.5 hr. The contents were then centrifuged the nuclei and the supernatant separated, treated with cold perchloric acid and

the nucleic acid fractions isolated. If the phosphate residue of RNA is transferred to DNA and *vice versa*, nucleic acids of the unlabelled nuclei and 'supernatant' were expected to be tagged with P^{32} after the interchange. This was found to be so. The contribution of non-metabolic exchange reactions was obtained by incubating boiled unlabelled preparations in the reaction mixtures and necessary corrections were applied.

B.3.5. *Mechanism of action of antibiotic substances*

(*i*) *Preparation of S^{35}- and H^3-labelled penicillin.* An investigation has been undertaken to study the site of action of antibiotic substances. Different antibiotic substances are proposed to be prepared marked with suitable radioactive isotopes, which may then be used as a convenient tool to trace the path of such substances inside the cell. S^{35}- and H^3-labelled penicillin have already been isolated. Penicillin-S^{35} was obtained by growing a penicillin-producing strain of *Penicillium* sp. on a medium containing $Na_2S^{35}O_4$ and the penicillin isolated by solvent extraction and chromatography. H-^{3}labelled penicillin was prepared by incubating a solution of the commercially available sodium salt of penicillin in tritiated water for several days. Penicillin exchanged its hydrogen for tritium and was highly radioactive.

(*ii*) *Mechanism of action of penicillin.* It is geneally believed that one of the major sites of action of penicillin is the cell-wall. This contention has been confirmed by incubating *Staphylococcus aureus* cells in presence of labelled metabolites and penicillin. Penicillin-treated cells absorbed more P^{32} than the untreated ones. Sulphate-S^{35} was absorbed very poorly with or without penicillin.

In view of certain structural similarities between penicillin and nucleic acids the effect of penicillin on nucleic acid metabolism in *Staph. aureus* was studied using P^{32} as a tracer. The cells in their logarithmic phase of growth were incubated with phosphate-P^{32} and different concentrations of penicillin for 4 hr. The cells were then ruptured in a Nossal disintegrator and subcellular particles isolated by differential centrifugation in a refrigerated centrifuge at 3,000 and 12,000$\times g$ and the nucleic acids of each fraction isolaced. These were then hydrolysed and chromatographed in 69% butyric acid in 0.85% NaOH. At concentration of and lower than 0.5 U/ml penicillin interfered only slightly, if at all, with the incorporation of P^{32} in the nucleic acids. At 1 U/ml, however, nucleic acid metabolism in most of the fractions was affected, the most remarkable of which was the RNA-fraction of particles sedimentable at 12,000$\times g$. Hydrolysis of the RNA fraction revealed very little P^{32}-activity in the constituent nucleotides. Investigations are now in progress in which C^{14}- labelled formate, acetate, bicarbonate and glycine are used to tag the carbon skeleton of the nucleic acids.

B.4. Enzyme Chemistry

B.4.1. *Microbial protein synthesis with relation to the biogenesis of nucleic acid*

Biosynthesis of protein in relation to the biogenesis of nucleic acid is being studied in this laboratory using *Azotobacter vinelandii* as the study organism. An active particulate preparation from *A. vinelandii* capable of amino-acid incorporation into protein was reported

last year. This preparation consisted of disintegrated cell wall and cell membrane fragments sedimenting between 2,000 and 30,000 $\times g$. Studies have since been made with smaller particles sedimentable at ultracentrifugal speeds (105,000 $\times g$.). The smaller particles or the ribosomes also incorporate C^{14}-labelled amino-acids although to a less extent than the heavier particles. The requirements for amino-acid incorporation were the same as reported for heavier particles.

B.4.2. *Interrelationship between the synthesis of particulate and soluble RNA.*

Kinetic studies on P^{32}-incorporation with growing cells of *A. vinelandii* reveal that different particulate fractions are much more rapidly saturated with tracer than the soluble fraction. Thus the particulate RNA seems to be synthesised first which then finds its way into the soluble supernatant. P^{32}-incorporation experiments with a cell-free 30,000 $\times g$ supernatant fraction, however, indicate predominant labelling of the soluble RNA which is dependent on Mg^{2+} and active phosphate donor like phosphoenolpyruvate or phosphoglycerate. The incorporation is inhibited by ATP, F,-DNP, cell wall or membrane fraction and on anaerobiosis. Thus the exchange due to polynucleotide phosphorylase can be ruled out. As the 30,000 $\times g$ supernatant contains ribosomal particles as well, the site of synthesis of soluble RNA is not known at present. This point is now under investigation.

B.4.3. *Studies on subcellular fractions with respect to their RNA content and functions in protein synthesis*

Different particulate and supernatant fractions obtained by differential centrifugation were assayed for their protein and RNA contents. The ratio of RNA to protein is found to be highest in the smaller particles or the ribosomes. The heavier cell wall or membrane fraction also contains a considerable amount of RNA a part of which is soluble RNA. This may well explain why the preparation is capable of incorporating amino-acids without any added soluble RNA which has an intermediary role in protein synthesis as carrier of activated animo-acid. A ribonucleoprotein (RNP) has been isolated from *A. vinelandii* by salt fractionation method. It contains about 50% of RNA expressed as weight per cent of RNA plus protein. This RNP is not, however, able to incorporate amino-acids. This may be due to the loss of structural integrity of the particles during the relatively drastic isolation procedure. The properties of this RNP preparation are being compared with those of one isolated by milder centrifugal methods. Although RNP particles are thought to be the sites of protein synthesis in mammalian systems, the present studies with *A. vinelandii* indicate that the sites of protein and particulate RNA syntheses in bacterial cells seem to be the cell wall or membrane fragments which have the ribosomal particles attached to them.

B.4.4. *Separation of soluble and particulate RNA by anion exchange chromatography and by starch gel electrophoresis*

When RNA prepared from different subcellular fractions are adosrbed on DEAE-cellulose and eluted with gradually increasing concentrations of NaCl in neutral phosphate buffer, a part of the RNA can be eluted with the buffer up to NaCl concentration

of 1M. The remaining RNA can be eluted with NaCl only at alkaline pH (0.1M NaOH or NH_4OH). The part eluted with neutral NaCl, designated as RNA I, has properties like soluble RNA. The other part, RNA II, is particulate RNA and consistitutes the main part of 30,000 $\times g$ particulate RNA and almost the whole of ribosomal RNA. In *in vitro* P^{32} incorporation, RNA I takes up label at more than ten times faster rate than RNA II. The sequence of labelling of these two metabolically different RNA fractions both *in vivo* and *in vitro* is under studies in detail.

Starch gel electrophoresis is found to be the most suitable method for resolving RNA preparations that apparently seem to be homogeneous. Whole cell RNA and soluble RNA both contain molecules of different sizes and or charge distributions. They are partially fractionated on starch gel due to difference in mobility. The method is now being standardized.

B.4.5. *Studies on the biochemistry of rice plant*

Preliminary studies with germinating rice seedlings show the presence some of the key enzymes of the pentose phosphate pathway, e.g. glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase and transketolase. Transketolase activity seems to be very high. This enzyme is being purified. Studies on other enzymes of the pentose phosphate as also of glycolytic pathways are in progress.

B.4.6. *Studies on whole-body irradiation of small animals*

Earlier work on irradiated animals showed the presence of ammonia in animal systems suffering from acute radiation syndrome. Estimations of blood ammonia and also the amount of free ammonia in individual organs are expected to be rewarding. With this end in view a method has been devised to estimate microquantities of ammonia in blood. One to two c.c. of blood, collected from heart puncture, diluted with normal saline, is deproteinised with alkali and zinc sulphate. The centrifuged supernatant is then treated with small portions of Nessler's reagent and the resulting colour is matched against standards.

Six albino rats were irradiated from a Co^{60} 20-curie source (600 r) and the urinary pH was noted. The urine, after irradiation, becomes alkaline and then gradually becomes acidic (normal) in reaction. The general toxic features are closely associated with pH changes in urine.

B.5 Plant Biochemistry

B.5.1. *Studies on Crown Gall Problem*

B.5.1.1. *Graft implantation of bacteria-free crown gall tissue in healthy plants.* The most distinguishing and characteristic property of crown gall tumour tissue is its autonomous growth potentiality. The tissues are capable of growing in purely synthetic medium devoid of any exogenous growth promoting substance such as IAA, NAA or coconut milk. However, the autonomous growth potentiality is only confirmed on successful implantation

of bacteria-free tissues in healthy host plant. Bacteria-free gall tissues of *Vicia faba*, *Coreopsis lanceolata* and *Ixora* sp., which were isolated previously, were grown in synthetic medium for more than two years in shake culture method, standardised in this laboratory. Since their isolation from primary galls no exogenous growth promoting substance was used in the medium. The tissues maintained a more or less uniform growth rate during this period and in no case differentiation of the cultures was observed. The tissue pieces were grafted into respective healthy host plants using both single and double slit method as in case of hollyhock gall tissue grafts. Typical undifferentiated tumours developed from the implants in all the three species. When the tumours attained a moderate size tissues were isolated from these galls and in all the cases were found to be bacteria-free. These new strains were maintained in synthetic medium and were found to grow without any signs of differentiation.

B.5.1.2. *Nutrient medium.* In order to find out best suited medium for crown gall tissues of *Vicia faba* and *Coreopsis lanceolata*, the experiments undertaken and reported in last year's report were continued. In addition to these two species bacteria-free crown gall tissue of *Ixora* sp. was also included in the studies. The tissues were grown for a period of 42 days in shake culture method and six nutrient media recommended by different authors for tissues of crown gall origin were used. Initial and final fresh weights of individual tissues were recorded. The growth was expressed in terms of 'growth value'—a ratio of final fresh weight to initial fresh weight. Average growth value in each case was compared with that of control in which the tissues were grown in tobacco medium of Hildebrandt, Riker and Duggar (1946). For *Vicia faba* gall tissue tobacco medium and Gautheret's medium, for Coreopsisg all tissue, Gautheret's medium and for Ixora gall tissue, modified hollyhock medium were found to give the best growth. Of the accessory growth factors tested nicotinic acid and calcium pantothenate stimulated the growth of *Vicia faba* tissue and pyridoxine stimulated the growth of Coreopsis gall tissue. None of these substances had any influence on the growth of Ixora gall tissue.

B.5.1.3. *Normal Callus tissues.* Normal callus tissues of hollyhock, Coreopsis and Nasturtium were so far maintained in tobacco medium agar slants. The nutrient medium for hollyhock tissue was supplemented with a mixture of 2,4-D (5×10^{-7}) and coconut milk (15%); for Coreopsis tissue, with a mixture of NAA (5×10^{-7}) and coconut milk (15%) and for Nasturtium tissue with 2,4-D (5×10^{-7}). Higher concentrations of growth substances are generally used at the time of isolation and early maintenance of normal tissues and suitably reduced concentrations are used for continued maintenance and growth of the tissues. Concentrations of growth substances higher than 10^{-7} were initially used for these tissues.

From the results obtained in these studies it was observed that concentration of 2,4-D for hollyhock tissue could be reduced to 1×10^{-7}, of NAA for Coreopsis tissue to 2.5×10^{-7} and of 2,4-D for Nasturtium tissue to 2×10^{-7}. In addition to these substances the presence of calcium pantothenate, nicotinic acid and pyridoxine was found to be necessary for rapid tissue growth in all the three species.

B.5.1.4. *Habituated tissue.* Normal tissues grown in synthetic medium are incapable of continued growth in absence of any externally added growth promoting substance. Gautheret, however, first reported that in certain cases such as Scorzonera, the normal tissues having undergone numerous transfers in media containing added growth substance, acquire the faculty of growing without such a substance and furthermore become completely insensitive to this substance. Gautheret proposed the expression anergie a l' auxine. This has been translated by White as habituation. Apart from this particular behaviour towards auxin the anergized tissues possess a group of properties which allow them to be distinguished from the corresponding normal tissues and also exhibit some properties in common with that of the corresponding gall tissue. For these reasons such a tissue has become important in comparative studies on crown gall tissue and normal tissue. The method adopted to isolate such tissues consists of growing the normal tissues in the presence of an auxin such as IAA or NAA for about 3 to 6 months. Small bits of these tissues are then transferred to the simple salt-carbohydrate medium devoid of auxin. Within first two to three transfers the non-habituated normal tissues generally cease growing and ultimately die. But if this process of testing is repeated month after month, there ultimately may come a point at which some of the tissues on such a transfer would no longer die but continue to develop in a manner similar to that observed on the auxin supplemented medium. In some plant species such as carrot, habituation is reported to have been accomplished in a very short period while for others isolation requires a very long period and failures have also been reported. Attempts were made to isolate such habituated tissues from hollyhock and Coreopsis normal tissue grown in presence of IAA and NAA for about six months. Tests were carried out at 60 days' interval throughout the year. After transferring the tissues to medium devoid of growth substance observations were made up to a period of 2 months. So far habituated strains have not been isolated from either of the species tried.

B.5.2. *Glycolytic enzymes in higher plants*

B.5.2.1. *Purification of aldolase.* Certain results relating to isolation and crystallisation of the enzyme, aldolase, from mung bean seedlings were described in last years report. Ammonium sulphate fractionation of a 0.1% potassium carbonate extract of mung bean acetone powder yielded a fairly active fraction precipitating between 0.5 and 0.55 saturation of the salt. When a solution of this fraction in veronal buffer, pH 8.65, was cautioulsy treated with saturated solution of ammonium sulphate at the same pH till faintly turbid and left overnight, a crystalline precipitate, showing aldolase activity, formed the next morning. On a careful investigation during the year under review, it was found that the crystalline material was not a homogeneous protein. Hence a modification of the method of preparation was attempted.

The commissioning of the Beckman recording spectrophotometer (model DK—2) during the year permitted the adoption of optical methods of Warburg and Christian for the assay of aldolase activity and for the estimation of protein. For the former, the increase

in optical density at 340 mμ, on addition of fructose diphosphate to the test system containing DPN, sodium arsenate, crystalline triosephosphate dehydrogenase (prepared from rabbit muscle) and the enzyme preparation under assay, was followed against time and that quantity of the enzyme solution bringing about the conversion of 1.0 μmole of FDP per minute was taken as an unit of enzyme. Protein content of the enzyme solutions was determined by measuring the optical density of a suitably diluted aliquot at 280 mμ and 260mμ, and calculated by the method of Warburg and Christian from the 280/260 ratio. This ratio also served as a rough indicator of the nucleic acid content of the solution.

Various solutions, namely 0.1% potassium carbonate, 0.03 M potassium hydroxide, 0.05M Tris buffer of different pH, were tested as medium for extraction of the enzyme from fresh mung bean seedlings or from mung bean acetone powder. It was found that 0.1% K_2CO_3 solution was the best extracting medium. The crude extract, however, was considerably rich in nucleic acid (280/260 ratio=about 0.7). A high nucleic acid content generally interferes with fractionation of enzymes and hence the extract was treated with 0.05 volume of 1.0M manganese chloride solution to precipitate the nucleate. The pH of the solution was maintained at 7.5 during the precipitation. After standing for 10 minutes, the precipitate was removed by centrifugation at 8000 × *g* for 10 minutes and the supernatant (280/260 ratio=about 1.2) was subjected to fractionation with ammonium sulphate. This was done by careful gradual addition of powdered ammonium sulphate, with stirring. Calculated quantity of the salt to obtain the desired degree of saturation was used, and the pH of the solution was maintained at 7.5 during the operation by cautious addition of dilute ammonia. The precipitates were allowed to stand for 45 minutes after which they were collected by centrifugation at 10,000 × *g* for 15 minutes and dissolved in minimum quantity of Tris buffer, pH 8.4 and subjected to enzyme assay. The protein content of the solutions was also determined. The specific activity i.e. the units of enzyme per mg. protein and the total activity of each fraction were calculated. Thus the fractions precipitating at 0-0.35, 0.35-0.45, 0.45-0.55, 0.55-0.65, 0.65-0.75 saturation of ammonium sulphate were examined.

The aldolase activity in the 0.35 precipitate and the 0.75 supernatant was negligible. Of the other fractions, each showed aldolase activity, but the 0.45-0.55 fraction showed the maximum specific activity as well as the maximum total activity. The specific activity of this fraction indicated that the enzyme was 20 times concentrated.

The 0.45-0.55 fraction yielded a bright clear solution in Tris buffer pH 8.4 (280/260 ratio=about 1.32). Further purification of this enzyme by calcium phosphate gel treatment was attempted. At pH 5.0, the enzyme was readily adsorbed by the gel and was completely adsorbed from a diluted solution when the gel : protein ratio was 3 : 1. The adsorped enzyme could be eluted from the gel by extraction with sodium carbonate-bicarbonate buffer, pH 9.2. The specific activity of the enzyme in the eluate increased three fold thus resulting in a net 60 times concentration of the enzyme. The enzyme however could not be crystallised even at this stage. The purity of the enzyme in this eluate and possibility of further purification is now under investigation.

B.5.2.2. *Aldolase activity in whole seedlings and in different organs at different ages of plants.* The relative activity of the two major pathways of carbohydrate metabolism, namely the Embden-Meyerhof-Parnas (EMP) pathway and the hexose-monophosphate (HMP) pathway, in plants has been an important question in plant biochemistry. It is believed that while the EMP pathway is almost the exclusive mechanism of carbohydrate metabolism in young seedlings or growing regions, the HMP pathway becomes considerably active with the age of the plant. The evidence in support of this view was derived from the relative rate of $C^{14}O_2$ production by plant tissues of different age in presence of glucose-1-C^{14} and glucose-6-C^{14} and also some observations on the activity of some of the key enzymes of the two pathways. The relative activity of the enzymes in very young seedlings or the different organs of such seedlings during the early period of growth is not known exactly and it was thought worthwhile to undertake a study of the activity of some of the key enzymes of the two pathways in such cases. During the period under review, the aldolase activity in young seedlings of mung bean (*Phaseolus aureus*, strain N.P. 28, from I.A.R.I., New Delhi) varying from 24 hours to 120 hours in age, and also in the roots, hypocotyls, and cotyledons of such seedlings has been thoroughly investigated.

The mung beans were germinated and grown in the dark at 25°C±1°C. Healthy whole seedlings or the different organs from such seedlings were collected at the end of every 24 hours from the time of soaking and weighed quantities of the samples were used to extract the enzyme. The samples were chilled for one hour and thoroughly macerated with 10 volumes of 0.1% K_2CO_3 solution. The macerate was filtered through two layers of cheese cloth and the filtrate was centrifuged at 20,000 × *g* for 15 minutes. The residue was discarded and the supernatant was freed of nucleic acid by treatment with 0.05 volume of 1.0M $MnCl_2$ solution. The nucleic acid free supernatant was made 0.35 saturated with ammonium sulphate and the precipitate was removed after half an hour by centrifugation. The 0.35 supernatant was made 0.75 saturated with ammonium sulphate and the precipitate was allowed to stand for half an hour. The pH of the solution was maintained at 7.5 during all these steps. The 0.35-0.75 precipitate was collected by centrifugation and dissolved in minimum quantity of Tris buffer pH 8.4. This solution was designated as R_2 solution. All operations were carried out at 2°C in the cold room. It was found that under the procedure described above, the 0.35 saturation precipitate or the 0.75 supernatant contained little aldolase activity and practically the whole of the extracted activity was found in the 0.35-0.75 precipitate. Hence the activity of the R_2 solutions, both total and specific, could be taken to represent the activity of the respective samples.

The experiments revealed that in whole seedlings, the aldolase activity reached an appreciable level in 24 hours, and the maximum activity was reached in 48 hours. Thereafter, the activity decreased gradually with the age of the seedlings to reach almost a constant minimum value by the 4th day. Considering the individual organs, it was found that in 24 hours the cotyledons recorded a much higher activity than the roots. Thereafter the activity in cotyledons gradually decreased with progressive age. In 48 hours, the activity in roots increased sharply followed by a gradual drop in activity upto the 96th hour and a steady value thereafter. In the hypocotyl tissues, the activity was rather low

in 48 hours, but thereafter the activity increased steadily up to the 5th day. The specific activity of the R_2 solution from each sample also followed the same trend of total activity as described above. Moreover, the mean specific activity of the R_2 solutions of root, hypocotyl and cotyledon on any day agreed fairly well with the specific activity of the R_2 solution of the whole seedlings of the corresponding age.

At the time of writing, the triosephosphate dehydrogenase, triosephosphate isomerase and α-glycerophosphate dehydrogenase activities in young mung seedlings and the different organs are under investigation.

C. BOTANY

C.2. Plant Physiology

C.2.1. *Electrical correlate of Desmodium pulsation*

It has been shown that the electrical correlate of Desmodium pulsation is not an exact replica of the mechanical movement. It is composed of a central spike with some small waves on either side of it. It was mentioned in the previous report that attempts were being made to interpret the complex electrical phenomenon of Desmodium pulsation by simultaneous recording of impulses from different quadrants of the pulvinus.

Attempts were first made to simultaneously record the electrical impulses from the opposite sides of the pulvinus by kaolin connections. But correct recording of the impulses was difficult in this method as the Kaolin paste covering a larger area when applied on two sides probably affected the motility of pulvinus and reduced the electrical potential difference of the two sides.

Attempts were then made to connect the different quadrants by fine platinum pins. But this was abandoned afterwards because, due to want of rigidity, the pins could not be made sufficiently thin for insertion into the tissue of the pulvinule without injury. It has later been substituted by tempered steel pins. These pins can be made very thin without losing its rigidity. Slight penetration of such pin into the pulvinule could be effected without much injury, which was indicated by quick recovery of the leaf. By this method it has been easier to record simultaneously the impulses of the upper and lower sides of the pulvinule.

The records so far obtained show that the lower side of the pulvinule is the main seat of electrical activity. The electrical phenomenon of the upper side of the pulvinule seems to play only a secondary role. It is much feebler in comparison to that of lower side and its pattern is also different.

Further details are expected to be obtained in the coming monsoon and autumn seasons when the plants will be in optionum condition.

C.2.2. *Biphasic electric response of Mimosa during the contraction of the pulvins.*

It has been reported previously that a biphasic electrical response appears at different points of the leaf away from one another, almost simultaneously, during the contraction of the pulvinus. Such simultaneous appearance of the impulse at different points indicated that it is transmitted at a very high speed. It has also been previously reported that the electrical response precedes the mechanical fall of the leaf by 1/20 to 1/10 of a second.

Further investigation was undertaken to determine whether the short time-difference between the electrical impulse and the mechanical fall of the leaf is due to the latent period as such or it is associated with the inertia of the leaf. Investigation was also undertaken to understand the mechanics of the high speed transmission of the impulse.

In order to observe how the time-difference between the electrical impulse and the mechanical fall of the leaf is dependent on the inertia of the leaf an experiment was undertaken with the help of Bose's Resonant Recorder. If the inertia of the leaf be the sole cause of this time-difference it is expected to be modified by altering the weight of the leaf system.

In this experiment a stem piece of Mimosa with a leaf attached to it was used. The leaf was first relieved of the weight of the subpetioles by cutting out at the junction of the petiole. The lower end of the stem was placed in water and the upper cut end of the stem and the petiolar cut end were sealed. The petiole recovered and took up its normal position after a time. The petiole was attached to the recording lever of the Resonant Recorder which inscribed twenty dots per second on the recording smoked plate when the recorder was started. A direct electrical stimulus was applied to the pulvinus at a fixed position of the moving plate by an automatic arrangement in the recorder. The number of horizontal dots beyond the fixed line of stimulation indicated the latent period of fall of the leaf.

By this method observations were made on the latent period of fall of the petiole under the following conditions :

(1) free from the weight of the subpetioles;

(2) by addition of equivalent weight of the subpetioles;

(3) by addition of weight double that of the subpetioles.

A number of experiments performed with different specimens indicated no difference in the latent period of fall of the leaf under the varying conditions. This indicates that in the time difference between the electrical impulse during pulvinar contraction and fall of the leaf, if the inertia of the leaf has played a part it may be considered quite negligible.

Simultaneous appearance of the biphasic response at different regions of the leaf is another problem that requires a suitable explanation. It has been previously investigated how the pulvinar sap during excitatory contraction is translocated through the petiole and the stem. If the biphasic response be due to the transmitted hydrostatic pressure from the pulvinus then it can appear simultaneously at different points. In order to be definite about such a possibility an experiment was undertaken in the following manner.

A stem piece of Mimosa was cut out and soaked in water. After that it was clamped vertically and the basal end was placed in water, the upper end being connected with a rubber pouch filled with water, through glass tube with rubber joints. Two different points in the stem, away from each other, were independently connected with two amplifiers for simultaneous study of the electrical changes at the two points.

Under the arrangement, just with the application of a pressure at the rubber pouch a biphasic response was found to appear at both the points of the stem quite simultaneously. This is indicative that the biphasic response simultaneously occurring at different points in Mimosa leaf may be the result of a hydrostatic pressure initiated at the pulvinus.

It may reasonably be assumed from the above observations that the biphasic response is initiated in the pulvinus with the contraction of the active cells. The hydrostatic pressure produced by the impact brings about electrical changes simultaneously throughout the channel, only diminishing in magnitude with the increase of distance and resistance. The collapsing of the reacting cells, however, does not bring about instantaneous contraction of the pulvinus. The latent period of contraction of the pulvinus may be mainly due to the resistance of other tissues in the pulvinus, the inertia of the leaf playing only an insignificant role.

C.2.3. *Respiration of Desmodium pulsating against a load*

In a previous report it has been shown that the amplitude of Desmodium pulsation is considerably increased when a pull is applied towards the abaxial side of the leaf by hanging a certain load. It was also mentioned in the same report that attempts were being made for correct determination of the rate of respiration of the leaf under the condition to ascertain whether this manifestation of increased activity of the leaf is accompanied by increased rate of respiration.

Some difficulties encountered in the manipulation of the load within the closed respirometer from outside, had been mentioned. Further attempts towards solution of those difficulties by modification of the device mentioned therein having failed, the introduction of the desired weight was made by opening the respirometer after the normal record had been taken for a time. In this method as the respirometer had to be taken out of water bath, a time was allowed after replacing it into water for adjustment of temperature before recording was restarted.

Some records taken in this way show marked increase of the rate of respiration along with the increase of the amplitude of pulsation by addition of load. More work has to be done to correlate the additional work done by the leaf with the increased rate of respiration. It has also to be seen how the respiration rate changes when the amplitude of pulsation is reduced under heavier weights.

C.2.4. *Summer oscillation of Desmodium pulsation*

A peculiar oscillation has been found to be developed in the leaflet of Desmodium when dry heat prevails in summer. The pulsations become reduced and irregular and they do not maintain the same base line. Along with pulsation the angular position of the leaf with the petiole gradually changes and as a result the base line of pulsation is continuously shifted up and down in an oscillatory manner, making two to three such oscillations in about an hour.

A few preliminary observations recently made on this summer oscillation of the leaf are given as follows : (1) the amplitude of pulsation is comparatively larger when the

leaf is in its downward position, (2) the oscillation is abated and the amplitude of pulsation is increased when the specimen is enclosed in an ice chamber. (3) Addition of weight at the abaxial side increases the amplitude of pulsation but it does not affect the oscillatory movement.

It may be noted that the leaf of Mimosa also acquires such an oscillatory behaviour when enclosed in a constant condition of moderately high temperature. The condition was found to be detrimental to the plant. In Desmodium too this character develops in the leaf when the condition of the plant sufficiently deteriorates under protracted drought condition in high summer. The plants cannot stand this condition for a long time and most of them die out every year.

Further details of this particular behaviour of the leaf will be studied next year.

C.3. Cytology

C.3.1. *Cytological studies on pulvini and petioles in allied species of plants with motile and non-motile organs*

Investigations on sensitive plants were directed to establishing the points of similarity and differences between sensitive and non-sensitive species of *Mimosa*. The touch sensitive or seismonastic species available were several :

Mimosa pudica Linn (indigenous)

Mimosa Spegazzinii Pirotta (collected by Dr. J. C. Bose from Europe)

Mimosa sensitiva Linn (collected by Dr. P. K. Bose from Assam)

The systematic identity of the latter two are in doubt and are being determined with help from the authorities at Kew Gardens, England, as well as the Botanical Gardens, Howrah. As noted previously, these three plants exhibit different grades of motility and their internal modifications can be correlated to their functions. Such structural adaptations as emerged were found to be common to those of other groups of sensitive plants, namely,

Biophytum sensitivum Linn

Averrhoa Carambola Linn and

Oxalis repens Thumb.

belonging to the family Oxalidaceae. Certain details of structure had previously been correlated with the motile function. The sensitive *Mimosa* was compared to the non-seismonastic *Mimosa hamata* Linn, collected from the Ridge, at New Delhi. Certain striking features of dissimilarity were at once apparent. The pulvini of the latter were semisolid in structure and showed none of the modifications associated with contractility. The most significant feature that has developed is the presence of a continuous system of tubes in all the aerial parts of the motile species, and which structure is completely absent in the non-motile species. This "system of tubes" is nothing more than the modification of one of the regular features of plant structure, namely the protoxylem. In the seismonastic

Mimosa the protoxylem remains unlignified and develops an uneven thickening along the walls which in transection gives it the appearance of collenchyma. However, the elements are not unduly elongated, have flat as opposed to tapering end walls devoid of chloroplasts and are rich in living cell contents. The contents are viscous in nature and carry other elements besides tannin which latter is very faintly distributed. These cells are interconnected by numerous plasmodesmata which pierce through the thick side walls.

These protoxylem tubes are the innermost vascular tissue and border either on the pith, or if the latter is excluded, are carried in the centre of the organ. In addition to the *Mimosa* sp. this "internal collenchyma" is present also in *Averrhoa carambola* but totally absent in *M. hamata* in which the protoxylem has the usual character. It is evident that the "internal phloem" of Acharya Bose is this tissue, for it well resembles the phloem in having living, non-lignified, tubular elements, although in point of origin it is in fact part of the xylem tissue. The role it plays in the conduction of stimuli is exemplified in its being internally situated as well as to the fact that it is developed only in seismonastic plants.

It is proposed to refer to this specialized tissue adapted for the conduction of stimuli in motile plants as "Bose's tubes" in the memory of their discoverer, Acharya Bose.

Microchemical and ontogenetic studies of this special conducting tissue are under way.

RADIATION AND CHEMICAL MUTAGENESIS, CYTOGENETICS, PLANT BREEDING, AND ANATOMY

C.4. Radiation mutations

C.4.1. and *and* 2. *Effects of X-rays, beta rays and gamma rays in Til (Sesamum) and Rape and Mustard (Brassica)*

This is a research project which is being financed by the Indian Central Oil-seeds Committee since 1950, for inducing mutants with desirable economic characters in Til (*Sesamum*) and Rape and Mustard (*Brassica*) by using ionizing radiations. Both the dominant and recessive mutants of economic importance viz. increased number of fruits, weight of seeds, oil content and/or early flowering etc. isolated from the irradiated progenies every year, are being studied in their succeeding generations.

C.4.1.a. *Effects of X-rays in Til (Sesamum)*

The season was favourable for the growth of *Sesamum* although a few of the experiments were affected by phyllody and early rains.

Sesamum orientale var. *T.* 10, *T* 12, *T* 16 and *T* 20 from West Bengal and *TMV* 1 from Madras were studied during the year under report.

C.4.1.a.1. *Breeding behaviour of the recessive mutants.* During the current year, the behaviour of *White flowered mutant* and *Small seeded mutant* which were isolated from *T* 16 in the X_3 and X_4 generations from 20,000 r and 14,400 r X-ray treatments respectively were studied with respect to their oil content and peroxide values which are included in Table 1.

TABLE 1

Control and mutants	Mean percentage of oil ± S.E.	Peroxide value	
		Fresh	One month old
T 16 (West Bengal)	40.0 ± 0.46	3.61	23.66
White flowered mutant	43.5 ± 0.46	3.58	20.23
Small seeded mutant	50.9 ± 0.46	3.26	18.16

It will be seen from the above table that *White flowered mutant* and *Small seeded mutant* gave about 4 per cent and 11 per cent more oil respectively than the control and this difference was found to be highly significant.

The keeping quality of the oil of the mutants continued be to better than the control as indicated by their peroxide values 3.61, 3.58 and 3.26 in the freshly extracted oils of the control, *White flowered* and *Small seed mutants* respectively. Oils kept for one month gave peroxide values 23.66, 20.23 and 18.16 respectively hence the oils of the mutants show better keeping quality.

The *White flowered mutant* started flowering 6 days later while the *Small seeded mutant* was 1 day earlier than the control.

A new *Small seeded mutant* (*"SSM"*$_2$) isolated in the X_3-1959 generation from *T* 10 after 1,25,000 r X-ray treatment was found to breed true during the current year. The mean percentage of oil of the control and the mutant is given in Table 2.

TABLE 2

Control and mutant	Mean percentage of oil + S.E.	Difference ± S.E.
T 10 (W. Bengal)	47.8 ± 0.18	—
SSM_2	55.5 ± 0.41	7.7* ± 0.47

* Significant at 1% level.

From the above table it will be seen that the mutant was giving about 8 per cent more oil than the control.

Multicoloured seeds mutant. The oil contents of the variously coloured seeds of the Multicoloured seeds mutant isolated in the X_1 generation of *T* 12 are given in Table 3.

TABLE 3

Control and mutants	Percentage of oil
T 12	43.15
Dull brown	43.36
Brownish black	45.41
Brown	45.93
Blackish Brown	45.96
Black	46.29
Grey	54.03
Ash	55.72
Dull white	56.44

It will be noted from the above table that all the differently coloured seed mutants yielded higher percentage of oil than the control, the maximum being observed in the *dull white* where the difference against the control was more than 13 per cent.

C.4.1.a.2. *Breeding behaviour of the higher yielding selections.* In the X_{11} generation, selections in 14,500 r and 20,000 r X-ray treatments of *T* 12 and *T* 16 gave higher fruit number than the control.

Table 4 gives the yield behaviour of *T* 12 and *T* 16 in X_9 generation during the year under report.

TABLE 4

Type and Locality	Control and X-ray treatment	Selection No.	No. of fruits	Weight of seeds in gm.
T 12 (West Bengal)	Control	II/2/2/4	192	15.80
	14,500 r	I/4/3/18	264	29.00
	,,	IV/2/3/16	275	23.74
T 16 (West Bengal)	Control	II/5/1/11	237	30.30
	14,500 r	IV/1/1/12	522	23.50
	,,	VI/4/4/6	377	24.20

It will be seen from the above table that the selections in 14,500 r X-ray treatment of *T* 12 and *T* 16 gave higher number of fruits and weight of seeds in the former but only higher number of fruits in the latter than their respective controls.

In the X_8 and X_7 generations during the current year, the selections in the different doses of X-ray treatments of *T* 10, *T* 12 and *T* 16 gave higher fruit number and seed weight than their respective controls.

In the X_6 generation, selection I/11/2/14 in *T* 20 after 80,000 r and III/24/1/19 and II/11/4/6 in *T* 12 after 24,000 r and 80,000 r X-ray doses respectively gave higher fruit number in the former and higher fruit number and seed weight in the latter, than their respective controls.

In the X_5 generation of *T* 10, the selection in 1,20,000 r X-ray treatment gave 376 fruits with 24.32 gm. of seeds as against control with 288 fruits and 22.10 gm. of seeds.

In the X_4 generation during the year under report, selections from 75,000 r, 1,00,000 r and 1,25,000 r X-ray treatments in *T* 10, *T* 12, *T* 16, *T* 20 and *TMV* 1 gave higher fruit number and/or seed weight than their respective controls.

In the X_I generation, most of the selections in treatments 10,000 r, 20,000 r, 30,000 r, 40,000 r and 50,000 r X-ray in *T* 10, *T* 16, *White flowered mutant* and *Small seeded mutant* were lower in seed weight although many selections gave higher number of fruits than their respective controls.

C.4.1.a.3. *Flowering behaviour.* In the X_{11} generation, the treatments 14,500 r and 20,000 r X-ray in *T* 12 was 1 day earlier and 14,500 r in *T* 16 was 2 days earlier than their respective controls.

In X_9, X-ray treatments in *T 16* started flowering earlier than the control, but no difference was noted in *T 12.*

In X_8 no marked difference was noted in the different treatments of *T* 10, *T* 12, *T* 16 and *T* 20.

In the X_7 generation of *T* 10, the treatments in 10,000 r, 12,500 r and 56,000 r X-ray treatments started flowering 1 day earlier than the control.

In the X_4 generation, the treatments 75,000 r and 1,00,000 r X-rays in *T 10* was early as against the control.

In the X_1 generation, the treatments in *T 10*, *T* 16 and *White flowered mutant* were earlier by 2 to 6 days than their respective controls, while in *Small seeded mutant* no earliness was noted in the different X-ray treatments.

C.4.1.a.4. *Pollen sterility and diameter.* In most of the current X-ray treatments, higher percentage of sterile pollen grains was noted against the control. No distinct variation was observed in the mean size of the pollen grains of the control and treatments.

C.4.1.b. *Effects of radioactive sulphur* (S^{35})

C.4.1.b.1. *Breeding behaviour of the higher yielding selections.* In the S_5 generation during the year under report, the selections in 89μc and 123μc/48, 72, and 96 hours treatments were higher yielding than the control.

In the S_3—1960 generation of *Small seeded mutant,* the selections in 630μc/24 hours, 1960 μc/48 hours and 72 hours durations gave higher yield than the dry control.

In the S_1 generation of *T* 20 during the current year, one selection each in 1892μc/24 hours, 1892μc and 2937μc/48 hours treatments gave higher yield than the dry control.

C.4.1.b.2. *Flowering behaviour.* Variations were observed in the time of flowering in the different varieties and generations of the treatments. In the S_1 generation of *T* 10 during the year under report, earliness from 2 to 6 days has been noted in the treatments as against the dry control.

C.4.1.c. *Effect of radioactive phosphorus* (P^{32})

C.4.1.c.1. *Breeding behaviour of the higher yielding selections.* In the P_5 generation of *T* 12 during the current year, treatment 104μc/48 hours gave 213 fruits with 30.05 gm. of seed against 284 fruits with 28.20 gm. seeds in the control, while in *T* 16, 750 μc/24 hours and 300 μc/48 hours durations gave 150 fruits with 11.25 gm. and 464 fruits with 25.00 gm. of seeds respectively as against 146 fruits and 10.20 gm. of seeds in the control.

In the P_3 generation, 1830μc/48 hours in *T* 16 and 1830μc/24 hours and 48 hours in *White flowered mutant* and 720μc/48 hours in *Small seeded mutant* gave higher fruit number and/or seed weight than their respective dry controls.

In the P_1 generation during the current year, all the selections from the different treatments of *T* 10, *T* 16 and *T* 20 were lower yielding than their respective dry controls.

C.4.1.c.2. *Flowering behaviour.* In the P_5 generation of *T* 16 during the year under report earliness was observed in the treatments upto 4 days against the control.

In the P_1 generation during the current year, treatments in *T 10* were found ta be about 3 to 9 days earlier than the dry control. Earliness from 2 to 4 days was also noted in treatments of *T 16.*

C.4.1.d. *Effects of gamma rays* (Co^{60}) *on Sesamum*

C.4.1.d.1. *Breeding behaviour of higher yielding selections.* In the G_2 generation of *T 12* and *T 16,* the selections, where the seedlings were treated with 16,524 r; 4,135 r; 1,304 r and 662 r were found to have higher fruit number and greater seed weight than their respective controls except the selections in *T 12* after 4,135 r gamma ray treatment. A few selections in *T 12* and most of selections in *T 16* in the G_2 generation where they were exposed to chronic gamma radiation were higher in fruit number and/or seed weight than their respective controls.

In the G_1 generation during the current year, the selections in *T 10* and *T 16,* where the dry seeds were exposed to 5,133 r; 1,283 r and 321 r gamma rays gave higher yield of fruits and seeds than their respective controls.

In the G_1 generation of *T 10, T 16, White flowered mutant* and *Small seeded mutant,* a few selections in the treatments 10,000 r and 30,000 r and 50,000 r gave higher number of fruits than their respective controls.

C.4.1.d.2. *Flowering behaviour.* The progenies of the treatments of the seedlings in the G_2 generation of *T 12*, flowered upto *7* days earlier than the control.

In the G_2 generation of the chronically irradiated progenies of *T 12* and *T 16*, the flowering was earlier by 1 to 3 days than their controls.

In the G_1 population of chronically irradiated *T 10* and *T 16*, earliness was noted upto 6 days, as against their respective controls.

C.4.1.d.3. *Growth response to chronic gamma radiation—Sesamum.* Table 5 gives the mean height and mean number of branches of *T* 16 in the G_1 generation during the current year, where the plants were exposed to chronic gamma irradiation.

TABLE 5

Type and Locality	Control and total gamma ray treatment* in mr.	Mean height in cm.	Mean number of branches.
T 16 (W. Bengal)	Control	84.1	15.9
	31,05,075	94.0	16.5
	3,44,931	107.5	19.9
	1,24,209	122.6	19.9
	63,390	130.6	21.4
	38,348	127.3	16.9
	25,718	139.4	17.5
	18,432	145.7	17.3
	13,799	147.8	17.3
	8,654	138.3	18.1
	S.E.	3.99	0.57

* Chronic treatment

It will be seen from Table 5 that there is significant increase in height in all the gamma ray treatments except 31,05,075 mr. So also stimulation in the mean number of branches was observed in 3,44,931 mr; 1,24,209 mr; 63,390 mr and 8,654 mr gamma ray treatments as against the control.

C.4.1.e. *Cytological observations*

Flower buds were fixed from the fresh treatments and the meiotic abnormalities were studied. The aberrations noted include, clumping, early separation, non-disjunction, bridges and laggards. Very rarely univalents were also observed.

C.4.2. *Effects of X-rays, beta rays and gamma rays in Brassica*

Irradiation experiments were continued during the year under report with the following types of *Brassica*, *B. campestris* varieties *T 10* and *T 151* (Kanpur), *Tori 5* (W. Bengal) *Brown sarson* (East Punjab), *B. juncea* var. *Rai 5* (W. Bengal), *R. L.* 9 (East Punjab), *T 185* (New Delhi) and the X-ray isolated *Appressed pod mutant*-"*APM*" and *Thickened pod mutant* —"*TPM*".

The season was not fabourable for the growth of *Brassica* as most of the plants under experiments could not survive due to unexpected rains and attack of Brassica aphids.

A comparative yield trial experiment was carried out with the stabilised mutants and some high yielding selections along with their respective controls. The behaviour of these are under observation.

C.4.2.a. *Effect of X-rays in Brassica*

C.4.2.a.1. *Yield behaviour of selections.* Table 6 shows the yield behaviour of the selections in *Tori 7* which are in the X_2 generation during the year under report.

TABLE 6

Type and Locality	Control and X-ray treatment	No. of days taken for first flowering	Selections No.	Yield	
				No. of fruits	Weight of seeds in gm.
Tori 7 (W. Bengal)	Control	47	2/13	207	1.850
	40 000 r	32	3/38	337	2.960
	60,000 r	29	6/5	207	0.670
	1,00,000 r	41	9/2	140	2.090

It will be seen from the above table that the selections in treatments 40,000 r and 1,00,000 r X-ray treatments gave 337 fruits with 2.960 gm. seeds and 140 fruits with 2.090 gm. seeds respectively against the control with 207 fruits and 1.850 gm. seeds.

Table 7 gives the behaviour of the selections in *Rai 5* which are in X_1 generation during the year under report.

TABLE 7

Type and Locality	Control and X-ray Treatment	Selection No.	Yield	
			No. of fruits	Weight of seeds in gm.
Rai 5 (W. Bengal)	Control	2/19	207	1.715
	10,000 r	3/31	240	0.650
	20,000 r	4/7	206	2.360
	30,000 r	5/19	275	2.785

It will be seen from the table that *Rai 5* control gave 207 fruits and 1.715 gm. of seeds. Three selections, one each in 10,000 r; 20,000 r and 30,000 r X-ray treatments were made of which the selections under 10,000 r gave higher number of fruits (240), the selection under 20,000 r gave higher seed yield (2.360 gm.) while the selection under 30,000 r gave higher yield both in fruits (275) and seeds (2.785 gm.).

C.4.2.a.2. *Flowering behaviour of selections.* From table 6 it will be noted that the selections in the treatments are early in flowering than the control. The control flowered after 47 days whereas the selections under 40,000 r, 60,000 r and 1,00,000 r X-ray treatments flowered after 32 days 29 days and 41 days respectively.

C.4.2.b. and c. *Effect of radioaative phosphorus (P^{32}) and radioactive sulphur (S^{35}) in Brassica*

Two types, viz. *T 10* and *T 151* of *B. campestris* were irradiated with P^{32} and S^{35} during the year under report. The activity of the treatments were as follows :

P^{32} (*i*) 1504.2 μ c and (*ii*) 2256.3 μ c 24 and 48 hours

S^{35} (*i*) 1191.6 μ c and (*ii*) 1787.4 μ c 24 and 48 hours

All plants under treatments failed to survive. This may be due to the lethality of the dosages or heavy rains. However germination behaviour has been observed.

C.4.2.b. and c.1. *Yield behaviour of selections.* Most of the selections of *Rai 5*, *APM* and *TPM* referred to in the last years' report continued to give increased yield of fruits and seeds against their respective controls.

C.4.2.b. and c.2. *Flowering behaiour of selections.* Some of the early flowering selections of the last year were early during the year under report. Some new early flowering selections have been isolated from the second generation during the current year.

C.4.2.d. *Effect of chronic gamma ray—Brassica*

C.4.2.d.1. *Yield behaviour of selections.* Table 8 shows the yield behaviour of the selections in the G_1 generation of *T* 10 (Yellow sarson) and *Brown sarson.*

TABLE 8

Type and locality	Control and total gamma ray treatment* in mr.	Selection No.	Yield	
			No. of fruits	Weight of seed (gm).
Yellow sarson T 10 (Kanpur)	Control	5/6	39	0.390
	3,11,414	I/2/14	120	2.625
	18,405	II/2/9	42	0.770
	12,460	III/3/9	140	3.560
	6,388	IV/6/16	324	4.590
	3,844	V/4/14	162	5.615
	2,574	VI/3/11	135	1.955
	1,844	VII/4/17	190	7.240
	1,385	VIII/12/14	220	4.555
Brown sarson (E. Punjab)	Control	5/3	717	8.090
	18,405	II/2/9	407	11.355
	12,460	III/5/9	565	19.690
	6,388	IV/2/8	713	14.875
	3,844	V/7/5	430	14.670
	2,574	VI/3/3	498	15.320
	1,844	VII/3/11	623	18.700

* Chronic treatment.

It will be seen from the table that all the selections in the treatments of *T 10* gave higher number of fruits and weight of seeds than the control. It will also be noted that the selections in *Brown sarson* were higher in seed yield although they showed lesser fruit number than the control.

One selection in the re-irradiation of G_1—seeds of *Rai* 5 under 6,388 mr treatment gave 13.910 gm. seeds against the control with 9.740 gm.

C.4.2.d.2. *Flowering behaviour.* All the selections in the treatments of *T* 10 were earlier than the control. In *Brown sarson*, the selections from treatments 18,405 mr; 6,388 mr; 3,844 mr; 2,574 mr and 1,844 mr were found to be earlier than the control. In *Rai* 5 selections from treatments 3,11,414 mr and 18,405 mr were also earlier than the control.

C.4.2.d.3. *Pollen grain sterility.* In the current treatments of X-ray and gamm ray in *Rai 5*, *T* 10 and *Brown sarson*, no appreciable changes in the pollen sterility have been observed.

C.4.2.e. *Cytological observations.*

In the current treatments, meiotic irregularities such as, univalents, trivalents, bridges and laggards have been noted.

C.4.3. *Radiation mutation in Jute (Corchorus olitorius and C. capsularis)*

This scheme is financed by the Indian Central Jute Committee for inducing favourable mutations using different ionizing radiations like X-rays, beta rays from radioactive phosphorus (P^{32}) and Sulphur (S^{35}) and gamma rays from Co^{60}.

C.4.3.1. *Sowing season.* The sowing was started at the end of April and due to late rain and irregular irrigation, germination of seeds was affected.

C.4.3.2. *Treatment.* The varieties T.M., R-26, C. G. from *Corchorus olitorius* and D. 154 from C. capsularis were selected for the mutation work in jute. The dry seeds of the varieties T.M., R-26, C.G. were treated with 1.5 mc and 3.0 mc of P^{32} and in D-154 only one dose i.e. 9.0 mc of P^{32} was used. The dry seeds of T.M., R-26, C.G. and D-154 were also treated with 3.0 mc, 6.0 mc and 9.0 mc of S^{35}. The doses mentioned above indicate the initial activities of the isotopes used for the experiment and the seeds were treated for 12 hours and these with their respective controls were sown in replicated plots at Falta Experimental Station. Some dry seeds of T.M., R-26, C.G. and D-154 were sent to the U.S.A. Pavilion at the National Agricultural Fair held in Delhi in the year 1960 for treatment with chronic gamma radiation, and the doses in this case were 10,000; 20,000; 30,000; 40,000 and 50,000 r units respectively. Again in the same year some dry seeds of the above mentioned varieties were treated with X-rays and only 20,000 and 40,000 r were used. All the treated seeds with their respective controls were sown in replicated plots at Falta Experimental Station of the Bose Institute.

C.4.3.3. *Germination.* The effects of treatment with different ionizing radiation were noted first during the germination of seeds in the field; out of these the percentage of germination was calculated and the data are given in Table 9-12. In the Table it can be seen that after P^{32} treatment the percentage of germination was very irregular in the varieties T.M., R-26 and C.G.; only in D-154 the percentage was 15.8 in control and 30.2 in 9.0 mc treatment which signified that 9.0 mc treatment has a stimulating effect on the germination of seeds. The seeds treated with S^{35} did not show any good results. But the seeds treated with γ-rays and X-rays showed very interesting results in germination.

In both types of irradiation a gradual fall of percentage of germination was observed with the increase of doses and these are depicted in Table 11 and 12.

TABLE 9

GERMINATION PERCENTAGE OF SEEDS AFTER TREATMENT WITH P^{32}

Date of sowing 28.4.1960

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	35.8	39.2	34.2	15.8
1.5 mc	28.0	36.0	35.7	—
3.0 mc	33.5	27.2	33.3	—
9.0 mc	—	—	—	30.2

TABLE 10

GERMINATION PERCENTAGE OF SEEDS AFTER TREATMENT WITH S^{35}

Date of sowing 28.4.1960

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	34.7	27.8	34.1	13.3
3.0 mc	26.2	27.7	33.2	17.7
6.0 mc	26.0	29.2	32.2	22.3
9.0 mc	28.2	28.7	30.5	20.5

TABLE 11

GERMINATION PERCENTAGE OF SEEDS AFTER TREATMENT WITH Co^{60} (FROM DELHI)

Date of sowing 2. 6 1960

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	20.0	34.6	25.2	22.4
10,000 r	14.6	14.2	12.8	11.8
20,000 r	10.6	8.0	12.0	10.6
30,000 r	14.8	7.4	8.8	9.4
40,000 r	11.8	7.0	9.0	6.6
50,000 r	8.4	5.4	10.0	6.6

TABLE 12

GERMINATION PERCENTAGE OF SEEDS AFTER TREATMENT WITH X-RAYS

Date of sowing 19.5.1960

Control and Treatment	Variety T. M.	R—26	C. G.	D—254
Control	86.7	78.3	85.0	85.7
20,000 r	89.7	75.3	84.3	81.3
40,000 r	78.7	63.0	71.7	80.3

C.4.3.4. *Flowering.* The flowering behaviour of the P_1, S_1, γ_1, X_1 population was studied and it was found in most of the cases that the time taken for the first flowering in the treated plants was late with respect to their respective controls.

Average height and basal diameter, fibre and wood weight of the control and treated plants in the first generation

TABLE 13

AVERAGE HEIGHT AND BASAL DIAMETER (IN BRACKETS) IN CENTIMETRES IN CONTROL & P_1 POPULATION

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	338.3 (1.29)	325.5 (1.37)	330.2 (1.53)	276.0 (1.76)
1.5 mc	320.2 (1.23)	309.7 (1.35)	295.0 (1.55)	—
3.0 mc	330.7 (1.28)	325.3 (1.57)	293.2 (1.40)	—
9.0 mc	—	—	—	257.3 (1.70)

TABLE 14

AVERAGE HEIGHT AND BASAL DIAMETER (IN BRACKETS) IN CENTIMETRES IN CONTROL & S_1 POPULATION

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	357.5 (1.52)	315.8 (1.49)	288.5 (1.31)	285.7 (2.01)
3.0 mc	355.3 (1.42)	317.7 (1.47)	291.2 (1.29)	287.5 (2.15)
6.0 mc	338.7 (1.36)	316.0 (1.45)	299.0 (1.39)	302.8 (1.93)
9.0 mc	352.7 (1.49)	334.3 (1.64)	291.2 (1.33)	291.5 (1.97)

TABLE 15

AVERAGE HEIGHT AND BASAL DIAMETER (IN BRACKET) IN CENTIMETRES AFTER TREATMENT WITH X-RAYS

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	239.5 (0.98)	163.2 (0.67)	199.0 (0.82)	156.0 (1.06)
20,000 r	181.2 (0.81)	170.2 (0.72)	162.0 (0.68)	140.0 (0.97)
40,000 r	156.0 (0.66)	154.2 (0.73)	170.2 (0.80)	107.7 (0.74)

It is seen from table 15 that the average height in the varieties T.M., R-26, C.G. and D-154 decreased after P^{32} treatment; in basal diamater there was also a decrease

with the exceptions of R-26 after 3.0 mc treatment and C.G. after 1.5 mc treatment. Again from table 14 it can be seen that after S^{35} treatment the average height and basal diameter of the treated population in variety T.M. decreased in comparison to the control whereas in the varieties R-26, C.G. and D-154, the average height of the population increased by the application of different treatments. Table 15 shows the average height and basal diameter of the control and treated population after X-rays irradiation. It can be seen from table 18 that in the variety T.M. the height and basal diameter decreased gradually with the increase of doses, but in R-26, there was an increase in height in 20,000 r treatment and then a fall observed in 40,000 r treatment. In C.G. with 20,000 r treatment the height and basal diameter of the population decreased whereas in 40,000 r treatment an increase in height and basal diameter was noticed. In D-154 the effects of treatments manifested as a decrease in the height and basal diameter of the treated population with gradual increase of doses. The effects of X-rays were very much pronounced whereas they were not so in the case of P^{32} and S^{35} as no significant results were obtained after their application.

C.4.3.5. *Fibre and Wood weight.* The average weight of fibre and wood of X_1 population were shown in table 18. In X-ray treatment a gradual fall in weight of fibre and wood was observed with increase of dose. This type of behaviour was not noticed in plants after P^{32} and S^{35} treatments. Moreover no significant result was observed after P^{32} and S^{35} treatments.

TABLE 16

AVERAGE WEIGHT (IN GMS) OF FIBRE AND WOOD (IN BRACKETS) OF P_1 POPULATION

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	11.9 (37.7)	9.5 (31.9)	12.6 (38.9)	13.6 (33.7)
1.5 mc	11.5 (32.4)	9.7 (30.8)	9.9 (29.9)	— —
3.0 mc	12.1 (36.9)	10.1 (33.7)	9.4 (29.1)	— —
9.0 mc	—	—	—	10.5 (26.6)

TABLE 17

AVERAGE WEIGHT (IN GMS) OF FIBRE AND WOOD (IN BRACKETS) OF S_1 POPULATION

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	15.1 (45.2)	14.1 (48.1)	9.2 (30.9)	19.1 (50.9)
3.0 mc	14.6 (42.4)	10.8 (40.7)	8.7 (29.7)	14.1 (34.8)
6.0 mc	13.2 (40.6)	11.0 (38.0)	9.6 (31.8)	17.0 (41.6)
9.0 mc	14.9 (46.2)	13.2 (47.6)	8.5 (28.6)	13.7 (33.4)

TABLE 18

AVERAGE WEIGHT (IN GMS) OF FIBRE AND WOOD (IN BRACKETS) IN THE X_1 POPULATIONS

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	5.1 (14.5)	2.6 (7.9)	3.0 (8.7)	3.0 (10.2)
20,000 r	2.5 (7.1)	2.3 (6.8)	2.1 (5.4)	2.1 (7 2)
40,000 r	1.8 (5.5)	2.2 (6.8)	1.6 (6.3)	0.9 (3.7)

C.4.3.6. *Pollen sterility.* The pollen grains of controls and treated plants were collected from the first generation of the population and out of these the percentage of pollen sterility was calculated. The pollen sterility of P_1 population with the control are given in table 19. From the same table it is seen that in all the varieties the percentage of pollen sterility increased with increase of doses. Similar increase was observed in S_1 population, but in the both P_1 and S_1 population the increase in pollen sterility was very slow, in comparison to pollen sterility from the population raised from Co^{60} and X-ray treatment. In the table it was found that the population raised after X-ray irradiation from the varieties T.M., R-26, C.G. and D-154 had a gradual increase of pollen sterility with the increase of doses irrespective of the varieties.

TABLE 19

PERCENTAGE OF POLLEN STERILITY AFTER TREATMENT WITH P^{32}

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	1.1	0.3	0.8	0.4
1.5 mc	2.9	2.4	4.4	—
3.0 mc	5.1	5.7	9.3	—
9.0 mc	—	—	—	8.4

TABLE 20

PERCENTAGE OF POLLEN STERILITY AFTER TREATMENT WITH S^{35}

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	1.1	0.8	0.5	0.4
3.0 mc	1.1	1.6	1.0	1.2
6.0 mc	1.6	1.9	1.3	2.2
9.0 mc	2.2	3.3	1.9	3.3

TABLE 21

PERCENTAGE OF POLLEN STERILITY AFTER TREATMENT WITH Co^{60} (FROM DELHI)

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	1.6	0.5	0.7	0.7
10,000 r	4.5	16.2	28.6	36.8

TABLE 22

PERCENTAGE OF POLLEN STERILITY AFTER TREATMENT WITH X-RAYS

Control and Treatment	Variety T. M.	R—26	C. G.	D—154
Control	1.6	0.5	0.7	0.7
20,000 r	8.8	8.0	9.6	21.4
40,000 r	22.8	17.0	16.7	27.9

C.4.3.7. *Cytology.* The cytological observations were made both from root tips and flower buds of the controls and treated plants and only anaphases were counted during cell divisions. Anaphase abnormalities were found in most of the cases where treatments were applied.

C.4.3.8. *Morphology.* The morphological peculiarities such as cut formation in leaves, cup leaves, double leaves (back to back, face to face etc.) crinkled leaves and forking of leaves were observed in the early stages of plants raised after different treatments. These characters did not persist long, but the peculiarities like branching of stem and fasciation when once appeared, persisted till the harvesting period. Some trailing plants in the variety D-154 treated with 7.5 mc segregated in the P_2 generation. Most of these plants died out during the different stages of growth and only 2 plants survived and yielded very few seeds.

C.4.3.9. *Gamma-irradiation.* In the year under report some dry seeds of T.M., R-26, D-154 were sown in different sectors at the Nilgunge Experimental Station for chronic gamma irradiation from Co^{60} source. Each sector was divided into 9 equal beds and the length of each bed was 5 metre. The plants in the field were subjected to irradiation for about 2030 hours. The germination of seeds was counted in the field but no conclusive results were obtained. The time taken for the first flowering was noted for individual plant and it was found that in all the varieties the plants in the first bed flowered later than the plants in the subsequent beds which signified that the plants further way from the source had a stimulating effect in early flowering than the plants nearest the source. Apart from the first bed there was no definite sequence of flowering of the plants in between second to ninth beds.

TABLE 23

PERCENTAGE OF GERMINATION OF SEEDS AFTER TREAMENT WITH γ-RAYS

Bed No.	Variety T. M.	R—26	C. G.	D—154
1	24.4	43.7	57.8	31.8
2	20.4	53.6	43.9	48.9
3	12.9	46.4	51.5	50.7
4	27.7	29.6	65.3	48.5
5	26.0	36.2	43.7	28.8
6	24.4	34.6	39.9	30.0
7	27.6	40.8	40.0	8.0
8	31.8	46.1	38.0	23.0
9	34.9	42.8	44.1	30.1

The average fibre weight of γ_1—population was taken and from table 26 it is seen that in the varieties T.M., C.G. and D-154 the maximum weight of fibre was observed from bed No. 2 whereas in R-26 it was in bed No. 3.

TABLE 24

Bed No.	Varity T. M. First flowering time (days)	T. M. Mean flowering ±S. E. (days)	R—26 First flowering time	R—26 Mean flowering ±S. E.	C G First flowering time	C G Mean flowering ±S. E.	D—154 First flowering time	D—154 Mean flowering ±S. E.
1	109	119.9±2.05	80	103.2±2.87	74	99.3±2.51	99	108.2±2.08
2	90	118.8±1.58	63	96.2±2.11	74	87.2±1.43	93	102.9±0.70
3	81	117.7±1.41	63	102.4±1.25	68	89.7±1.00	95	106.0±0.59
4	79	120.3±0.94	63	100.6±1.36	67	91.6±1.03	73	115.4±0.47
5	92	121.7±0.71	63	102.7±1.14	68	92.3±0.80	93	104.5±0.41
6	79	121.6±0.66	64	103.6±0.85	67	90.2±0.72	93	105.7±0.36
7	80	122.3±0.51	63	105.9±0.70	67	93.8±0.65	93	104.9±0.47
8	67	130.6±0.47	65	106.7±0.68	73	98.5±0.57	93	104.8±0.94
9	69	91.1±3.30	63	104.7±0.48	63	93.5±0.38	90	103.7±0.18

The average height in cm of γ_1 -population was entered in table 25 and it is clear from the table that in variety T.M. the maximum height was observed in bed No. 5 whereas in the varieties R-26, C.G. and D-154 the maximum average height was observed in bed No. 4.

TABLE 25

AVERAGE HEIGHT (IN CMS) OF γ_1 POPULATION
Total number of plant studied = 360

Bed No.	Variety T. M.	R—26	C. G.	D—154
1	381	365	347	301
2	438	376	317	334
3	415	382	405	350
4	425	422	409	361
5	442	398	377	343
6	432	404	391	346
7	440	385	374	305
8	433	340	385	341
9	432	409	358	337

TABLE 26

FIBRE WEIGHT (IN GMS) γ_1 POPULATION

Bed No.	Variety T. M.	R–26	C. C.	D-254
1	25.8	33.3	21.3	49.6
2	41.3	28.8	42.9	53.2
3	27.0	32.5	34.5	38.0
4	32.0	47.8	34.8	32.0
5	35.1	25.9	28.1	39.7
6	38.7	27.1	27.2	39.4
7	34.9	17.6	29.5	37.4
8	32.8	16.1	34.0	40.3
9	35.2	25.1	26.2	33.3

In order to study the segregation 2nd generation population was raised, but no conclusive result has been obtained. Some plants with thick and shortened pods were however obtained and these have been kept for further investigation.

C.4.4. *Radiation mutation in Groundnut*

This scheme is financed by the Indian Central Oil seeds Committee for inducing mutants with X-rays and radio-active isotopes. The year under report is the 3rd year of investigation.

C.4.4.1. *New treatments.* Several fresh treatment were made during the year under report. Seeds of varieties of AK. 10, TMV 3 and Small Japan were treated with 2.5, 5 and 7.5 mc of radio-active phosphorus (P^{32}) and radioactive sulphur (S^{35}) for 24 and 48 hours.

X-ray doses of 60,000, 70,000 and 80,000 r were also given to seeds of the above varieties.

C.4.4.2. *Morphological variations.* No abnormal plants were recorded in the 1st generation treatment. Chlorophyll mutants were isolated in the S_2 generation (var. Small Japan) but no seeds could be obtained. Some spreading plants were obtained (var. AK. 10) in the P_2 generation, having small seeds, some of which seemed early maturing. In the variety Small Japan progenies of the four dwarf plants which were isolated in the previous year, bred true this year also in the P_3 generation. However one (P_3) plant was found to be a chimaera—some branches and leaves resembling control plants—other branches having the typical mutant form.

C.4.4.3. *Flowering behaviour.* Flowering behaviour was studied in the P_3 generation, in the variety Small Japan. The flowering period was recorded as the number of days from seeding to the day of the 1st flower of a plant. The data of the two treatments (P^{32}, 24 and 48 hours) and their control were subjected to statistical analysis and the results are shown in Table 27 and 28.

It can be seen (1) that there is no change in the meantime of flowering in the three populations.

(2) Between lines variations are significantly greater than within lines variations showing that lines within each population are not homogeneous with regard to flowering time.

(3) No increase in (genetic) variability was observed in the treatments as compared to the control—i.e. no change has been brought about (in the flowering time) by irradiation.

C.4.4.4. *Studies on Quantitative characters.* S_3 progenies from seeds of variety TMV-3 treated with S^{35} for 24 hours (A) and 48 hours (B) together with their untreated control (C) were sown in compact family blocks and the following characters were statistically analysed : (*i*) pod weight (gms) (*ii*) pod number (*iii*) spreading habit (cms) and (*iv*) time of maturity (number of days from emergence of seeds to the date when half of the plants in a sub-plot got yellow in colour).

From tables 29-30 it can be seen that though there were no differences in the mean values, genetic variability increased with respect to pod weight and pod number after S^{35} treatment and that this variability was more pronounced after the 24 hours treatments.

C.4.4.5. *Gamma radiation* (Co^{60}). Plants of all three varieties (AK. 10, TMV-3 and Coimbatore) were grown under continuous irradiation, in a Co^{60} field operated by the Institute, at Nilgunge. Plants were in different sectors and subjected to doses varying from 4863 r to 13.6 r during the growing season (June to October, 1960).

Results of these experiments are being analysed.

TABLE 27a

ANALYSIS OF VARIANCE (24 HRS. P^{32})

Variation due to	d. f.	m. s.	e. m. s.
Between lines	24	64.58**	$\sigma e^2 + 27.15\ \sigma i^2$
Within lines	654	20.05	σe^2

TABLE 27b

ANALYSIS OF VARIANCE (48 HRS. P^{32})

Variation due to	d. f.	m. s.	e. m. s.
Between lines	24	73.87**	$\sigma e^2 + 28.74\ \sigma i^2$
Within lines	694	22.67	e^2

TABLE 27c

ANALYSIS OF VARIANCE (CONTROL)

Variation due to	d.f.	.m.s.	e. m. s.
Between lines	14	79.71**	$\sigma e^2 + 25.67 \sigma i^2$
Within lines	371	18.91	σe^2

** denote significant beyond 1% level.

TABLE 28

SHOWING MEAN TIME (IN DAYS) OF FLOWERING, GENETIC VARIABILITY AND INTRA CLASS CORRELATION CO-EFFICIENT

Population	A*	B	C
Mean time of flowering ($\bar{x}$)	30.4	30.0	30.4
Genetic variability (σg^2)	1.64	1.78	2.37
Intra class correlation (=Heritability) $\left(\frac{\sigma g^2}{\sigma g^2+\sigma e^2}\right)$	0.0756	0.0728	0.114

A = 24 hours P^{32} treatment
B = 48 hours P^{32} treatment
C = Control.

TABLE 29a

ANALYSIS OF VARIANCE (24 HOURS S^{35})

Source of variation	d.f.	Pod weight		Pod number		Spreading habit		Maturity	
		m.s.	F	m.s.	F	m.s.	F	m.s.	F
Selection	19	180.0	2.42**	234.7	2.09*	28.68	1.84	4.16	—
Error	38	74.3		112.4		15.58		4.92	

TABLE 29b

ANALYSIS OF VARIANCE (48 HOURS S^{35})

Source of variation	d.f.	Pod weight		Pod number		Spreading		Maturity	
		m.s.	F	m.s.	F	m.s.	F	m.s.	F
Selection	19	131.0	1.16	183.5	1.09	25.84	1.45	7.00	—
Error	38	113.0		168.4		17.79		10.50	

TABLE 29c

ANALYSIS OF VARIANCE (CONTROL)

Source of variation	d.f.	Pod weight		Pod number		Spreading habit		Maturity	
		m.s.	F	m.s.	F	m.s.	F	m.s.	F
Selection	9	105.1	—	142.9	—	15.78	1.66	9.33	1.47
Error	18	134.8		204.5		9.50		6.33	

* denotes significant at 5% level.
** denotes significant at 1% level.

TABLE 30

SHOWING THE COMPARISON OF PARAMETERS OF FOUR CHARACTERS IN THREE POPULATIONS

Parameters	Pod weight (gms)			Pod number			Spreading habit			Maturity (days)		
	*A	*B	*C	A	B	C	A	B	C	A	B	C
Population mean ($\bar{X}$)	55.2	55.6	56.2	72.2	74.1	72.9	35.0	34.5	34.8	123.2	123.4	123.6
Genetic variance (σg^2)	35.23	6.01	—	40.78	5.03	—	4.37	2.68	2.09	—	—	1.00
Error variance (σe^2)	74.34	112.99	134.84	112.36	168.42	96.85	15.58	17.79	9.50	4.92	10.50	6.33
Genetic coeff. Variation	10.8	4.4	—	8.9	3.0	—	6.0	4.8	4.2	—	—	—
Heritability	58.7	13.8	—	52.1	8.2	—	45.5	31.1	39.7	—	—	—

A = 24 hours S^{35} treatment
B = 48 hours S^{35} treatment
C = Control.

C.4.5. *Investigations on induced mutagenesis in paddy using X-rays and radioactive isotopes*

This is a research project, being financed by the Atomic Energy Commission, Government of India, for studying both the fundamental and applied aspects of irradiation in paddy with X-rays and beta rays from radioisotopes and is in the fifth year of investigation, during the year under report. Different varieties of Aman, Boro and Aus paddy were used during these investigations.

C.4.5.1. *Studies in Aman paddy.* During the year under report, three varieties of winter (Aman) paddy, namely *Rupsail*, *Latisail* and *Patnai* were irradiated with beta rays from radioactive phosphorus (P^{32}) and sulphur (S^{35}). The second generation population of *Rupsail* after beta ray irradiations was studied. Selections for earliness and grain size, were grown to note their behaviour.

C.4.5.1.a. *Fresh Treatments.* Three varieties of winter paddy, namely, *Rupsail*, *Latisail* and *Patnai* were treated with P^{32} and S^{35} by soaking the seeds in isotope solutions for 24 hours in petridishes with initial activities of 6, 12, 18 and 24 μc per seed. Seeds kept in distilled water for the same period served as control. After the treatments seeds were washed in water and transferred to the field for sowing in nursery beds.

C.4.5.1.a.1. *Germination.* The seedlings sprouted after each treatment were noted 14 days after sowing. Tables 31-32 gives the percentage of germination of the three varieties after different P^{32} and S^{35} treatments.

TABLE 31

PERCENTAGE OF GERMINATION P^{32}

Varieties / Treatments	Rupsail	Latisail	Patnai
0 (Control)	34.4	44.8	45.6
6 μc/seed	52.0	46.4	45.6
12 μc/seed	38.4	30.4	48.0
18 μc/seed	40.8	41.6	37.6
24 μc/seed	32.8	30.4	52.0

TABLE 32

PERCENTAGE OF GERMINATION

S^{35}

Varieties / Treatments	Rupsail	Latisail	Patnai
0 (Control)	35.2	45.6	47.2
6 μc/seed	34.4	58.4	43.2
12 μc/seed	39.2	41.6	48.0
18 μc/seed	43.2	62.4	52.0
24 μc/seed	48.8	44.8	56.8

It can be seen from the tables that there was no regular pattern in the sprouting ability of the varieties following P^{32} and S^{35} treatments. The treatments seem to have little inhibitory effect on germination. The variety *Latisail*, however, showed a fall in the percentage of germination after P^{32} treatments excepting 6 μc/seed dosage.

C.4.5.1.b. *P_2 and S_2 generations.* The variety *Rupsail* which was irradiated with 0.5, 1.0 and 1.5 mc (i.e. 2, 4 and 6μc per seed respectively) initial activities of radioactive phosphorus and sulphur in the year 1959 was bulked treatment-wise and grown as P_2 and S_2.

C.4.5.1.b.1. *Chlorophyll mutations.* The chlorophyll deficiencies (mutants) which appeared in the seedling stage in the second generation population after irradiations consisted of albino, xantha, virescent and striped patterns. The albino or lethal white and xantha or lethal yellow do not survive but the virescents or yellow-green change to green gradually. The type with white-green stripes shed off the leaves showing stripes and develop normal leaves.

Table 33 gives the frequency of occurrence of chlorophyll mutants.

TABLE 33

FREQUENCY OF OCCURRENCE OF CHLOROPHYLL MUTANTS IN SECOND GENERATION

VARIETY—*Rupsail*

Treatment	No. of plants observed	Albino	Xantha	Virescent	Striped	Total No. of chloro-phyll mutations	Percentage
			P^{32}				
0 (Control)	1744	0	0	0	0	0	0.00
2 μc/seed	1888	10	1	2	0	13	0.69
4 μc/seed	2056	11	0	4	0	15	0.73
6 μc/seed	1972	12	2	3	1	18	0.91
			S^{35}				
0 (Control)	1850	0	0	0	0	0	0.00
2 μc/seed	2124	2	0	0	0	2	0.09
4 μc/seed	1901	3	0	2	0	5	0.26
6 μc/seed	2309	2	0	0	0	2	0.09

At the time of harvest fifty selections were made by random sampling of each of the controls and treatments and they will be grown as plant progenies in the third generation to compare the genetic variance of the control and treatments.

C.4.5.1.c. *P_3 generation.*

C.4.5.1.c.1. *Chlorina or greenish-yellow forms.* One of the selections made from the second generation following P^{32} treatment (1350 μc or 10.8 μc/ seed) in *Rupsail* showed segregation for chlorina. Out of a total of 225 plants 47 plants were greenish-yellow. The leaves were greenish-yellow, yellow being more prominent towards the margins and apices. The plants were slightly smaller than the normal ones.

C.4.5.1.c.2. *Earliness.* In the previous report mention was made of an early flowering plant in the variety *Rupsail* isolated in the second generation after 1350 μc (10.8 μc per

seed) P^{32} treatment. The plant flowered 28 days earlier than the earliest flowering plant in the control. Maturity was also earlier by one month. Of 81 seeds that were sown to raise the third generation, 34 plants grew. They were transplanted in lines along with progenies of earliest flowering control (B) and the bulked control (C). The selection for earliness (A) was found to breed true. It flowered 27 days earlier than the two controls. Table 34 reveals the mean time of flowering (in days) with standard error.

TABLE 34

MEAN TIME OF FLOWERING (IN DAYS)

Selection	Mean time*+S.E.
A	96.9 ± 0.52
B	124.0 ± 0.27
C	124.4 ± 0.19

(*Number of plants observed in each selection was 34)

From table 34 it will be seen that the magnitude of standard error is more in the selection for earliness than that of the other two. This indicates that the range of flowering is greater in the selection for earliness.

The agronomic features of the three selections including the early flowering mutant are given in the table 35.

TABLE 35

Character / Variety	Plant height (cm)	No. of ears	Ear-length (cm)	Grain yield (gm)	Plant fertility
Early flowering mutant (A)	150.9±1.64	6.6±0.29	31.1±0.25	3.2±0.38	28.95±0.58
Early flowering selection (B)	194.4±1.02	6.9±0.33	33.4±0.26	15.9±0.58	83.55±0.41
Bulked Control (C)	188.4±1.86	7.0±0.25	34.7±0.25	15.4±0.47	93.04±0.28

The early flowering mutant shows very poor grain yeild compared to the other two selections. This can be attributed to the decreased plant fertility. The average height of plants at harvest was also much shorter.

C.4.5.1.c.3 One off-type plant was isolated in P_3 generation in the variety *Patnai* in 1959 which has narrow grain. The grain dimension of the control and the off-type plant are given in table 36.

TABLE 36

GRAIN DIMENSION (mm.). AVERAGE OF 50 GRAINS

Variety	Length	Breadth	Thickness
Patnai (Control)	10.87 ± 0.074	2.35 ± 0.017	1.75 ± 0.019
Narrow Grain Mutant	10.54 ± 0.062	1.95 ± 0.019	1.63 ± 0.017
Difference	0.33* ± 0.097	0.40* ± 0.026	0.12* ± 0.026

*denote significant at 1% level.

It was grown in the P_4 generation and was found to breed true for grain size.

The agronomic characters are shown in table 37.

TABLE 37

Character / Variety	Plant height (cm)	No. of ears	Ear-length (cm)	Grain Yield (gm)
Patnai (Control)	178.6 ± 1.05	6.8 ± 0.13	26.7 ± 0.10	7.16 ± 0.57
Narrow Grain Mutant	179.6 ± 0.98	8.9 ± 0.23	27.2 ± 0.14	13.11 ± 0.72
Difference	1.0 ± 1.44	2.1* ± 0.29	0.5* ± 0.18	6.0* ± 0.92

* denote significant at 1% level

C.4.5.2. *Studies on Aus Paddy.* C.4.5.2.a.

Fresh treatments were made with 2, 4 and 6 mc initial activities of radio-active phosphorus on one variety of Aus Paddy, namely *Marichbati.*

C.4.5.2.a.1. *Growth rate.* Measurements of growth of plants were made from both control and treated population after transplantation to the field for seven consecutive weeks. It was noticed that initially there was an inhibition in height of plants which was not maintained in the succeeding weeks.

C.4.5.2.a.2. *Number of Tillers, number of Panicles and length of Panicles.* Data regarding the number of tillers, number of panicles and length of panicles were studied, but no appreciable change was noticed in the treatment when compared against the control.

C.4.5.2.a.3. *Morphological changes in first generation.* One plant has been isolated from P_1 generation after 6 mc treatment in the variety *Marichbati*, which had long and narrow grains compared to the short and broad grains in the control. L/B ratio of the selected plant S301 is 3.12 and in the control 2.38.

C.4.5.2.b. *Behaviour of Selections in X_3 and P_3 generations.* 300 selections were made in the second generation of three varieties of Aus Paddy viz. *Marichbati*, *Jhanjee* and *Dular.* These selections were made on the basis of morphological, and cytological peculiarities and pollen sterility. These selections were sown in lines in the field along with their respective controls to study their behaviour in the next (i.e. third) generation. Some selections bred true for the characters selected, some showed segregation of the parental and the induced character, while others did not maintain the characters. Behaviour of some important selections in the third generation are given in Table 38.

C.4.5.3. *Studies on Boro Paddy.*

C.4.5.3.a. The variety *Chinsura Boro II* was treated with 2 mc, 4 mc, and 6 mc initial activities of radio-active prophosprus.

C.4.5.3.a.1. *Germination.* The treated and the control seeds of *Chinsura Boro II* were allowed to germinate in a replicated plot and irregularities were noticed in the rate of germination in both the control and treated plants and no correlation was found with the dosages.

TABLE 38

BEHAVIOUR OF SOME IMPORTANT SELECTIONS IN THE THIRD GENERATION

Number of Selection	Variety and treatment	Characters for which the selection was made	Behaviour in the third generation
S240	Dular ; 50,000r (X-rays)	Green tillers and yellow stigma	Breeds true
S247	Dular ; 75,000r (X-rays)	Semi-sterile	Plants dwarf with normal fertility
S222	Dular ; 3 mc P^{32}	Awned	Breeds true
S238	Dular ; 75,000r (X-rays)	Grain colour black	Segregation into different grain colours e.g. yellow, brown, black, purple etc.
S201	Marichbati 75,000r (X-rays)	Broad grains, reddish just before maturity	Breeds true
S268	Marichbati 75,000r (X-rays)	Dwarf with poor seed setting	Character is not maintained but there is development of small leaf at the panicle and in all plants.
S262	Marichbati 50,000r (X-rays)	Tiller deep purple, apiculus red	Segregation into deep purple and green tillers
S275	Marichbati 75,000r (X-rays)	Large glume	Segregation into normal grains, and grains with large glumes.
S204	Marichbati 25,000r (X-rays)	Deep red colour of the seed	Breeds true
S226	Marichbati 75,000r (X-rays)	Purple tiller and black grain with purple stigma	Segregation into black grains, deep red and yellow grains
S221	Marichbati 75,000r (X-rays)	Multiple ovary, double-rice	Segregation study was not possible as only one such plant was obtained.
S254	Jhanjee 3 mc P^{32}	Juncture red	Segregation into white and red.
S11	Jhanjee 75,000r (X-rays)	Abnormal morphology of plants	Segregation in the colour of the grains into black and yellow

C.4.5.3.a.2. *Pollen sterility.* Pollen sterility increased with the increase of dosages. In the control the pollen sterility was 2.02% whereas in the 6 mc P^{32} treatment the pollen sterility was found to be 5.56%.

C.4.5.3.a.3. *Flowering time.* The duration of flowering period in treated plants was changed. In the control population it was 16 days whereas in the treated population it increased with the increased dosages. In the 2 mc, 4 mc, and 6 mc population the duration of flowering period was 19, 22 and 25 days respectively.

C.4.5.3.a.4. *Chlorophyll mutants.* In the second generation the percentage of albino plants was 0.09% in the 6 mc P^{32} treatment, whereas no albino plants were noticed in any of the other treatments including the control.

C.4.5.3.b. *Studies in the 3rd generation.* Some selections were made from the second generation population regarding grain size, awn character, flowering time, dwarf character etc. Their behaviour will be studied in the 3rd generaion.

C.4.6. *Mutation experiments in the tomato and other plants*

In continuation of the previous years investigation in tomato some further studies were made on the effects of induced mutagenesis with ionizing radiations. Observations were extended during the year under report, to two more plant species, namely, *Vicia faba* and *Hollyhock*. A major portion of the work was on the dwarf mutant (tomato) that was induced with X-rays some years earlier.

C.4.6.1. *Similarity between X-ray irradiation and indoleacetic acid induced changes in the tomato.* It was observed earlier, that when tomato seeds were exposed to X-radiation, double fused leaves were commonly formed at the first three nodes. Fresh irradations made with various doses of X-rays, during the year, showed that with suitable doses (about 12,000 r units) as high a frequency as 15% of the treated seedlings may be expected to show such an anomalous leaf structure. Interesting enough, the same abnormality was also observed in rather good frequency, when young seedlings were treated with indoleacetic acid, but not with gibberellic acid. This suggests a common mode of action between the two agents in the production of this type of abnormality.

C.4.6.2. *Indoleacetic acid and gibberellic acid responses of the tomato dwarf and the control.* In furtherance to the studies made in the previous year on the response of the X-ray induced dwarf to three concentrations of indoleacetic acid and gibberellic acid, 30 days old seedlings of the control too were subjected to same treatments. While there was no growth response or only slight growth depression with indoleacetic acid, gibberellic acid produced an increase in growth rate. Thus the mutant and the control responded to gibberellic acid in the same manner, giving rise to a similar degree of growth increase. This leads to the conclusion that the dwarfing locus is not concerned with gibberellic acid response and that the reduction in growth in the dwarf is brought about by affecting some other metabolic process which is important to growth.

C.4.6.3. *Genotypic modification of the expression of the dwarfing gene.* Experiments were initiated to study the expression of the dwarfing locus behind different genotypic backgrounds and to modify the effect of the dwarfing gene by introducing new genes into the genotype. For this, outcrosses with genetically distinct varieties and the isolation of the F_2 dwarf segregants from them is needed. Succeesful crosses of the control and the mutant were made with six cultivated varieties of Sutton's tomatoes, namely, Abundance, Best of All, Early Market, Golden Queen, Harbinger and Perfection, and also with the wild variety of the cultivated tomato, Yellow Cherry (*Lycopersicon esculentum* var. *cerasiforme*). The F_1 hybrids and the parents were grown together in the field, with randomisation within blocks. In a few of the crosses it was observed that the mutant gene was associated with heterosis, in other words, when the mutant was crossed with a particular variety there was better growth and earlier date of flowering, than when the same variety was crossed with the mother line of the mutant.

C.4.6.4. *Characters of breeding value in the dwarf.* At first sight the mutant appears to be of a deleterious nature, with a drastic reduction in growth and yield; however a careful observation shows that there are certain changes associated with it whch may be taken

advantage of in breeding for pactical purposes. For instance, the stem is relatively erect and hard, and it saves the tedious practice of giving a stalk for support. Also it has a more fibrous root system which is better distributed in the soil, which thus give better fixation to the plant in the soil.

C.4.6.5. *Exposure of the dwarf to chronic gamma irradiation.* To back-mutate the dwarfing gene to normal condition, sixty-day old plants (when they were just forming flower primordia), were subjected to continuous gamma-irradiation treatment, by exposing them in the Co^{60} gamma field. They were placed near the source for 10 days and received a total dose of about, 2,600 r units per individual plant.

C.4.7. *Preliminary X-ray irradiation studies on Vicia faba*

In order to obtain lines segregating for dwarfs, chlorophyll lethals and other types of deleterious mutants, for studies on the nature of gene action, dry seeds of *Vicia faba* (obtained from Suttons) were preliminarily treated with an acute X-ray dose of 10,000 r. Although the treatment produced appreciable reduction in the rate and percentage of seed germination and shoot growth, the treated plants appeared healthy and normal. All the plants —both in the control and the treated ones, flowered but there was no pod formation, and hence no seed set. This was attriubted to unfavourable climatic conditions; for succeessful results, sowing in the month of about September is suggested. The study showed further doses may be administered without bringing total inviability.

C.4.8. *Chlorophyll lethal factor in a natural Holly-hock (Althea rosea) population*

To add some more examples on the study of single gene heterosis in plants, with relation to spontaneous mutations, progenies of a large number of plant species were examined for lethal factors. While growing a sample of about 1200 seedlings of Hollyhock (*Althea rosea*, Family Malvaceae) it was observed that about 15% of the seedlings were of uniform yellow colour, both in the cotyledons and the hypocotyl. There was no further development and they proved to be lethal. The rest of the population was normal and green, and upon preliminary testing some of them proved to be heterozygous for the lethal factor and the rest normal homozygotes. A study of developmental comparison between the two types in segregating lines is planned.

C.5. Chemical mutagenesis

C.5.1. Studies on the effect of the chemical Diemethyl sulphate on jute plants were continued in the C_2 generation. Seeds from selfed (C_1) generation were sown in replicated plots. Seeds from open pollinated (C_1) plants were bulked and sown treatmentwise. No special effect was found as result of the treatments and the results are summarised below :

C.5.1.a. *Germination.* There were no marked differences with regard to the time taken for germination and percentage of germination in the different treatments.

C.5.1.b. *Height.* Treated C_2 plants and their controls, were measured at regular intervals (approximately, at one month intervals) and subjected to statistical analysis. The last measurement was made just before harvest and the height up to the bifurcation point was taken. The details are given in Table 39 and Table 40. The coefficient of variation can be seen to be slightly greater in all the treated plants when compared to control plants. From Table 41 and it can be seen that Tr. 3A shows greater genetic variance, and has a larger mean than the control or other treatments though these differences are not of a significant nature.

C.5.1.c. *Flowering.* Investigations on the flowering behaviour of the treated plants with respect to the control were made. No significant differences in earliness, were found.

C.5.2. *Fresh treatment.* Different concentrations of Ethyleneimine were applied on jute seeds D-154 and T.M. and its effects studied. Morphological changes like irregular serration in leaves, double leaves, forking of leaves, twisting nature of stem and irregular distribution of chlorphyll in leaves were observed.

Further observations in the 2nd generation are being made.

TABLE 39

ANALYSIS OF VARIANCE OF HEIGHTS OF PLANTS (UPTO BIFURCATION)

Treatment	Variation due to	Degrees of freedom	Sum of squares	Mean squares	F
Tr. 1	Replication	2	4420.56	2210.28	2.78
	Selection	6	6065.14	1010.85	1.27
	Error	12	9539.44	794.95	
	Total	20	20025.14		
Tr. 2	Replication	2	24055.57	12027.78	7.03**
	Selection	6	1726.57	287.76	—
	Error	12	20514.43	1709.54	
	Total	20	46296.57		
Tr. 3	Replication	2	18377.79	9188.89	3.73*
	Selection	24	43858.39	1827.43	—
	Error	48	118195.21	2462.40	
	Total	74	180431.39		
Tr. 3A	Replication	2	16718.14	8359.07	4.71*
	Selection	6	19397.14	3232.86	1.82
	Error	12	21265.85	1772.15	
	Total	20	57381.13		

* denotes significant at 5% level
** „ „ „ 1% level

TABLE 40

HEIGHTS (UPTO THE BIFURCATION POINT) OF TREATED AND CONTROL PLANTS

Treatment (Tr.)	No. of plants	Mean height (in cms)	Standard deviation	Standard Error	Co-efficient of variation	T—test
1. Tr. 1 (control)	145	266.28	71.24	5.29	26.62	—
2. Tr. 2	132	264.47	74.69	6.50	28.25	0.19
3. Tr. 3	451	259.90	73.87	3.48	28.46	0.93
4. Tr. 3 A	121	277.61	76.28	6.94	27.48	1.24

TABLE 41

SHOWING GENETIC AND ENVIRONMENTAL VARIANCE OF TREATED AND CONTROL PLANTS

Treatment	Parameters Environmental variance (σe^2)	Genetic variance (σg^2)	Mean height (X)
Tr. 1	794.95	71.96	266.28 cms.
Tr. 2	1709.54	—	264.47 ,,
Tr. 3	2462.46	—	259.90 ,,
Tr. 3A	1709.15	486.90	277.61 ,,

C.6. Cytological and cytotaxonomical studies

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

During the year under report (1960-61) cytotaxonomical studies to investigate the somatic chromosome numbers and other details have been carried out in four genera of the family Malvaceae. The following is the list of the materials studied.

A. *Genus—Gossypium* L.

1. G. arboreum L. var. neglectum, Watt.
2. G. arboreum L. var. neglectum, Watt. forma—indica, H. & G., type—7036
3. G. arboreum L. var. neglectum, Watt. forma—indica, H. &. G., type—Karungani.
4. G. barbadense L. var. Karnak.

B. *Genus—Malvastrum. A. Gray.*

1. Malvastrum Tricuspidatum, A. Gray.

C. *Genus—Thespesia, Corr.*

1. Thespesia populnea, Corr.

D. *Genus—Althaea*, L.

1. Althaea rosea, L.

In order to study the karyotype, root tips were pretreated with aesculin and squashed in 2% Aceto-orcein in the usual Aceto-orcein method. Colchicine (0.2% Sol.), Paradichlorobenzene, and 8-hydroxyquinoline were also tried as prefixatives but aesculin was found to give better results in spreading the chromosomes at metaphase stage. Origin of nucleoli was studied by Feulgen-Fast green technique.

C.6.1.1. *Genus Gossypium* L.

C.6.1.1.a. *G. arboreum*, L. var. neglectum, Watt. The species has a 2n complement of 26 medium sized chromosomes of which two pairs are satellited. The connecting arms of one pair of satellites are relatively shorter than those of the other pair. The presence of four small nucleoli observed at the early prophase stage with Feulgen-Fast green technique suggests that the satellited chromosomes are nucleolar in nature.

Abnormalities like bridges and laggards at the anaphase stage and the presence of hyper- and hypoploid chromosome complements at the metaphase stage were observed in this species.

C.6.1.1.b. *G. arboreum*, L. var. neglectum, Watt. forma—indica, H. & G., types—7036. This is a selected strain. It has 26 medium sized chromosomes in its 2n complement, out of which 2 pairs are satellited. One pair of satellite have bigger knobs with shorter arms while the other pair have smaller knobs with longer arms. Both the satellited pairs of chromosomes are nucleolar in nature as observed by the presence of four nucleoli at the early prophase following Feulgen-Fast green technique.

Cytological abnormalities like the presence of haploid and polyploid chromosome complements are frequent, the occurrence of bridges have also been recorded.

C.6.1.1.c. *G. arboreum*, L. var. neglectum, Watt. forma—indica, H. & G., type—Karungani. This is also a selected strain from *arboreum* species. It has 2n chromosome complement of 26 medium sized chromosomes, having 2 pairs of satellited chromosomes, of which one pair have a shorter arm than the other. Feulgen-Fast green technique shows that there are four small nucleoli existing at the early prophase stage, thus indicating their origin from the satellited chromosomes.

Abnormalities like different types of hyper-and hypoploid chromosome complements in the metaphase stage and the occurrence of bridges at anaphase have been recorded in this type.

C.6.1.1.d. *G. barbadense*, L. var. Karnak. This is an Egyptian Cotton. This variety has 2n complement of 52 relatively large sized chromosomes, of which 3 pairs are satellited. The presence of six nucleoli recorded by Feulgen-Fast green technique suggests their origin from the satellited chromosomes

Cytological abnormalites like different types of chromosome complements at metaphase stage have been recorded.

C.6.1.2. *Genus Malvastrum A. Gray*

C.6.1.2.a. *Malvastrum Tricuspidatum, A. Gray.* The 2nd chromosome complement of this species is 24 chromosomes of which 2 pairs are satellited. One pair of staellite have bigger knobs with shorter arms while the other pair have the reverse characters. The chromosomes are relatively bigger. Four small nucleoli found at the early prophase stage with Feulgen-Fast green technique, are taken to be originated from the satellited chromosomes.

Abnormalities like haploid and polyploid cells have been recorded at the metaphase stage.

C.6.1.3. *Genus Thespesia, Corr.*

C.6.1.3. *Thespesia populnea*, Corr. This consists of 26 relatively big chromosomes in its 2n complement, out of which 2 pairs are satellited. Nucleolar study with Feulgen-Fast green technique reveals the presence of four nucleoli at the early prophase which suggests that these originated from the satellited chromosomes.

No cytological abnormality has so far been observed.

C.6.1.4. *Genus Althaea*, L.

C.6.1.4.a. *Althaea rosea*, L. The variety having white petaloid flowers has been studied only. This type has 2n chromosome complement of 42 of which 3 pairs are satellited. Nucleolar study with Feulgen-Fast green technique shows that there are six small nucleoli at the early prophase suggesting that all the three pairs are nucleolar in nature.

Abnormalities like bridges and laggards have been recorded in this type.

C.6.2. *Cytotaxonomical studies in Chilli (Capcisum annum L.)*

During the year under report, the following varieties of Chilli, *Capsicum annum* L., belonging to the family Solanaceae were collected from different sources (Table 42) and were grown at the Experimental Station of the Bose Institute at Shyamnagar for studying their detailed morphological characters namely : height of the plants, nature of branching, size of the leaves, size of the petiole, colour of both the surfaces of the leaves, colour of the petiole, colour of the nodes and internodes; shape of the stem, colour, size and shape of the flowers and their different parts; size, shape, colour and position of ripe and unripe fruits.

TABLE 42

Reference No.	Name of Variety	Source of collection
WB/C — 28	Shikarpuri	Department of Agriculture
WB/C — 30	Akashy	
WB/C — 32	Round (Black)	Government of West Bengal
WB/C — 35	Red long	,, ,,
WB/C — 39	Jalpaiguri Local	,, ,,
WB/C —112	Mukulkapur	,, ,,
WB/C —118	G—2	,, ,,
WB/C —119	Guntur—G—1	,, ,,
	G—2	,, ,,
	Guntur—G—1	,, ,,
WB/C —130	Cherry Chilli	,, ,,
	Cherry Chilli	,, ,,
WB/C —154A	Wild—1	,, ,,
IC—2	R. S. B.	,, ,,
IC—4	—	,, ,,
IC—17	N.P—46A	,, ,,
Bl/C_1	Surjamukhi	Globe Nursery, Calcutta,
Bl/C_2	Chinese Giant	M/S Suttons, Calcutta.
Bl/C_3	Elephant Trunk	,, ,,
Bl/C_4	Ruby King	,, ,,
Bl/C_5	Chilli Yellow	,, ,,
Bl/C_6	Chilli Red	,, ,,
Bl/C_7	Californian Wonder	,, ,,

C.6.2.a. *Cytological investigations.* Cytological studies reveal the chromosome number ($2n=24$) in all the varieties.

For studying mitotic chromosomes, root tips were stained in Aceto-orcein after pretreating in 0.2% colchicine for 2 hours at 14—18°C. Slight variations were noted in chromosome morphology in the different varieties. Somatic complement of all the varieties under study, in general, were observed to consist of (*a*) a pair of large sub-median constricted chromsome, (*b*) a pair of small chromosomes with sub-terminal constrictions and (*c*) the rest medium sized with sub-median constrictions. There are two pairs of secondary constricted chromosomes, of which one pair is satellited.

Meiotic studies from diakenesis to tetrad formation in P.M.C_s were made. Meiosis was found normal in most of the cases. However, laggards, bridges, asynaptic conditions, micro-nuclei formations were noted in small percentages in some of the varieties namely, WB/C-32, WB/C-35, WB/C-112, WB/C-118, and IC—17.

Pachytene studies in some of the varieties were undertaken and are in progress

C.6.2.b. *Hybridization Exeriments.* Varieties having contrasting morphological characters were choosen for hybridization experiment for genetical studies.

C.6.3. *Cytological and Cytotaxonomical studies in Amaryllidaceae*

A large number of bulbs belonging to the different genera of Amaryllidaceae were collected and chromosome numbers determined and karyotype analyses made for about 34 accessions. Of these two different accessions in *Amaryllis* species had $2n=22$ and $2n=44$ chromosomes respectively. In *Hippeastrum equestre* $2n=22$ chromosomes were observed. The karyotype here has similarity with the karyotypes of the *Amaryllis* species mentioned above. In meiosis 11 bivalents were observed. *Cyrtanthus Mackennii* showed a somatic number of 16 chromosomes. Here a pair of long V-shaped chromosome was found. Other chromsomes were of J or rod-shaped types. In *Hyline gardenariana* $2n=20$ chromosome were counted. The karyotype was different from that observed previously for this species. In most of the other accessions confirmations were made of previous cytological observations. Attempts are being made to check the discrepancies in the observations of the chromosome number and karyotypes in different taxa. Work is also in progress with other accessions. Special emphasis is being given to the study of the static (*Amaryllis* and *Crinum*) and the dynamic (*Hymenocallis* and *Zephyranthes*) groups. Attention has also been given to the horticultural hybrids. Efforts are being made to collect bulbs from different geographic regions with a view to sudy the phylogeny, karyotype evolution and interrelationship of different taxa. Cytological confirmation of taxonomy of the different genera of Amaryllidaceae and their evolutionary relationship is being studied. The role of polyploidy, hybridization and chromosome changes in speciation in this family is also being taken into consideration.

C.6.4. *Cytogenetic Aspects of Sex Determination in Spinacia oleracea* L.

This project was started to find out the range of morphological variation in the longest chromosome pair (chromosome 1) in *Spinacia oleracea* L. which contains the XY factor. For

this reason seeds of different varieties were collected from different countries. Plants were raised in pots and also in a small experimental plot.

Somatic chromosome number was 12 and the longest chromosome pair (chromosome 1) was found to be homomorphic with submedian primary constriction for each of the following varieties studied.

"Australian Spinach" (I.A.R.I.); Spinach (Globe Nuyrsery); Long Standing Round (Sutton & Sons); Dark Green Bloomsdale (U.S.D.A.) Long Standing Bloomsdale (U.S.D.A.); Virginia Savoy (U.S.D.A.); Universal (Germany) and 10-44 (Turkey).

Plants were induced to flower early with artificial lighting arrangements and meiotic studies were done in the following three varieties :

"Australian Spinach" (I.A.R.I.); Spinach (Globe Nursery) and Long Standing Round (Sutton & Sons).

Six bivalents were observed in metaphase I and chromosome behaviour was regular in all the stages of I and II divisions.

C.6.5. *Cytological studies in some Acanthaceae species*

Chromosome numbers in some plants of the family Acanthaceae were determined after a study of the meiosis and the mitosis. The names of these plants with their respective chromosome numbers are given below:

Cardanthera triflora	$2n=30$
Justicia gendarussa	$2n=30$
Nelsonia campestris	$2n=32$

These chrosome numbers are reported for the first time.

C.7. Plant anatomy

C.7.1. *Anatomical studies on the abnormal double leaf of Ageratum conyzoides* Linn

During the year under report, the study of abnormal "double leaf" of *Ageratum conyzoides* Linn. was taken as a part of the investigations. External morphology showed that the two leaves of the double leaf are fused by their petiole forming a common leaf-stalk. Their laminae are fused to each other back to back by the midrib excepting for their apical portions. Anatomical studies revealed that the double leaf has six leaf-trace bundles with two common midrib bundles, compared to a normal leaf which has three trace bundles.

These two midrib exarch bundles divide to form four bundles and each move to their distal ends taking a simultaneous torsion. One half of each bundle joins with another half of the other bundle forming two new endarch bundles. These newly formed bundles pass out as the midribs of the free apical regions of the double leaf.

C.7.2. *Effect of irradiation on the Anatomy of Plants*

Studies on the irradiation effect on the anatomy of plants were started with the mutants of *Sesamum orientale*—"Small seeded mutant" (S.S.M.) and "White Flowered Mutant" (W.F.M.) and that of *Brassica juncea* —"Thickened Pod Mutant" (T.P.M.) and "Appressed Pod Mutant" (A.P.M.). All these mutants were obtained by X-irradiation at different doses. S.S.M., and W.F.M., T.P.M., and A.P.M. were obtained at doses 14,500 r, 20,000 r, 40,000 r and 1,00,000 r respectively. Besides their morphological differences anatomical studies revealed that in all the root tips the quiescent zone is considerably changed. For further observation in this line, seeds of *S. orientale* and *B. juncea* were treated with X-rays at 20,000 r, 40,000 r, 60,000 r, and 80,000 r doses. The seeds were germinated and the root tips were studied. These showed that in the root tips of the treated seeds the number of initial layers decreased. And with the increase of radiation there is a remarkable diminution of the quiescent zone.

Internal radiation effect with S^{35} isotope was studied on *Arachis hypogea* (Groundnut). Stem anatomy of Groundnut showed that in the treated plants there is a profuse increase of xylem fibres. Also it was found that in the treated (S_1) plants there is a decrease in the number of vessels and the average lumen of the vessels are narrower. The number of vessels in the leaves also is found to be less. The stomatal size in the treated plants are reduced but their average number per unit are are considerably increased on either surface of the leaves.

Shoot apex anatomy after irradiation is at present under study.

C.8. The effect of natural chronic irradiation on plants

To ascertain the effect of natural chronic irradiation on plants growing in monazite sand a visit to Manavalakurichy (Madras), Chavara (Kerala) and Neendahara (Kerala) was made. With the help of a Philips monitor it was found that Manavalakurichy had a greater background radiation, the maximum being 1.8 mrh. (Gamma) and 3.0 mrh. (Beta), than the other two places. Hence attention was concentrated on certain plants growing in Manavalakurichy. For further cytogenetic investigations fixation of flower buds and collection of seeds from the following plants were made :

Cortalaria striata
Crotalaria verrucosa
Crotalaria medicaginca
Phaseolus trilobus

For a comparative study, flower bud fixation and seed collection from these plants were made from adjacent non-monazite regions also.

No appreciable difference in pollen sterility was found between plants of the monazite and the non-monazite regions.

The leaves of *Cocos nucifera* and *Borassus fiabellifer* are being analysed chemically to find out whether the radioactive elements present in the monazite sand are absorbed by the trees and translocated to the leaves. A similar procedure is being pursued with the shoots of *Crotalaria striata* and *Crotalaria verrucosa*. (For the results of the chemical analysis, see the report of the Chemistry Section—B.1.5).

MICROBIOLOGY

D.1. Production of antibiotic substances by microorganisms

D.1.1. *Studies on a broad-spectrum antibiotic isolated from a Streptomyces sp. A_{14} 475*

A broad-spectrum antibiotic produced from the *Streptomyces sp. A_{14} 475* is found to be basic in character. It produced a number of salts, of which the hydrochloride, sulphate and acetate forms could be used for its characteristion and clinical trial.

The method of isolation was modified by using Amberlite IRC-50 in Na-cycle with advantage that most of the brown-coloured impurities of the fermentation media remained unadsorbed thus making the subsequent stages of purification less troublesome. Elution from the adsorbed column was carried out by $\frac{N}{10}$ HCl, $\frac{N}{10}$ H_2SO_4 or 2% HAc according to the type of salt required for further investigation. The eluted volume was sufficiency large, and to concentrate it was passed through IRC-50 in H cycle and elution was carried out with the same acid as used in previous operation. Repetition of the process of adsorption in the resin column in different cycles and subsequent elutions, reduced the final volume and impurities were removed to a considerable extent.

An attempt was then made to isolate the antibiotic in the form of free base by elution with NH_4OH but on concentration the activity of the compound was observed to decrease appreciably.

In the second phase of adosprtion and elution, the active principle admixed with free acid was neutralised by passing over Amberlite IRC-400 in OH cycle (NH_4OH used) at a comparatively rapid rate of flow so that only the acid would be neutralised. Thus the exchange of negative ion of the active material could not occur in this case. The rate of passing the solution for neutralisation required careful attention. The eluted volumes containing the active material were collected and evaporated to dryness in vacuo.

A_{14} 457—Reineckate salt

A saturated sq. solution of Ammonium Reineckate was added drop by drop to a solution of *A_{14}* 475 hydrochloride until no nore ppt. formed and kept at 4°C for an hour, centrifuged and crystallised at 60°C from distilled water.

p-Hydroxyazobenzene—p'—sulfonate of A_{14} 475

An aq. of solution of A_{14} 475 hydrochloride was added dropwise to a warm aq. solution of the sodium salt of p-Hydroxyazobensene-p'-sulfonic acid with constant shaking until no more precipitate was formed; it was kept at 4°C, centrifuged and the ppt. was dissolved by heating on steam bath.

A crystalline product separated out on slow-cooling.

D.1.1.2. *Countercurrent Distribution.* The crude antibiotic was purified by the method of countercurrent distribution. The two solvent phases used were equal volumes of butanol

and water containing 5% p-toluene sulphonic acid with respect to aqueous phase which acted as a carrier. The distribution was carried out in an all glass 25-tube countercurrent distribution apparatus. The capacity of each tube was 10 ml. in each phase.

Distribution of the antibiotic was estimated by agar-cup assay method against *Staph. aureus* after neutralising the solution in each tube in the presence of alcohol acting as a co-solvent between the two phases, n-butanol and water. The maximum concentration of the antibiotic was obtained in tube no. 7. By this method the antibiotic had been obtained in a more purified form. The results of the countercurrent distribution experiment, moreover, indicated that there was only one active component in the antibiotic.

D.1.1.3. *Physical and chemical proportions.* The purified sample in the form of sulfate was white needle-shaped crystals, decomposing at about 300°C.

Spectrophotometric analysis were carried out in the UV-range in $\frac{N}{10}$ HCl and methanol. No characteristic absorption in the UV range was observed.

The substance was highly soluble in water the form of hydrochloride, less soluble in methanol and ethanol, insoluble in most organic solvents like ether, chloroform, benzene, ethyl acetate, petroleum ether etc. The sulfate derivative of the antibiotic was also soluble in water and insoluble in all other organic solvents.

Results of the colour reaction are represented below

1. Molisch's test	.. ..	positive
2. Anthrone test	.. ..	positive
3. Ehrlich's test	.. ..	negative
4. Ninhydrin test	.. ..	negative
5. Biuret test	.. ..	negative

Paper electrophoresis was studied with pure sample of the antibiotic in the sulphate form in presence of two buffers at pH 4.2 (acetate buffer) and pH 8.6 (vernol buffer). Results are presented below and the displacement of the antibiotic was studied by bioautographic method.

TABLE 1

DISPLACEMENT MEASURED BY BIO-ASSAY AGAINST STAPH. AUREUS

Solvents	Voltage	Current strength	Time	Antibiotic	
				Polarity	Displacement
0.1 M Vernol buffer (pH 8.6)	200	2—3 milliamp	180 mins.	—ve	2.2 cm
0.1 M Acetate buffer (pH 4.4)	150	3—5 milliamp	150 mins.	+ve	2.0 cm

D.1.1.4. *Toxicity test.* Toxicity studies of the antibiotic A_{14} 475 had been performed on white mice at the Central Drug Research Institute, Lucknow, and the Bengal Immunity Research Institute, Calcutta. The results showed that the antibiotic was relatively non-toxic.

The antibiotic was tested for acute toxicity on mice using a dose of 25 mg/kg. body wt., 50 mg/kg. body wt. and 100 mg/kg. body wt. intravenously. There was no untoward symptom or death within 72-hours after injection. From the results it seemed that the antibiotic was a failry non-toxic drug at the dose levels tested.

D.1.1.5. *Mode of Bactereostatic activity.* The mode of action of the antibiotic had been studied in relation to protein and nucleic acid metabolism in *E. coli.* It was observed that RNA synthesis of *E. coli* was immediately stopped due to the activity of the antibiotic resulting in the failure of the growth rate through depleted protein metabolism. The "uncoupling" phenomenon of RNA and protein synthesis was, however, not observed in this case. The mode of action of the antibiotic appeared to be the same at the bacteriostatic and bactericidal concentrations. The antibiotic-sensitive organism *E. coli.* could, however, recover from the effect of the antibiotic at the bacteriostatic level (5 mg/ml.) after about one and a half hour.

It was observed that nucleic acid synthesis of *E. coli* was primarily checked due to the action of antibiotic A 14475.

Biological and physicochemical studies on the stability of the antibiotic A 14475 in neutral soil revealed that the antibiotic was more rapidly inactivated in non-sterile soil than in sterile one.

D.1.2. *Broad-sepctrum antibiotic from a Pseudomonas sp.*

D.1.2.1. *Studies on the production of antibiotics from Pseudomonas sp.* A broad-spectrum antibiotic having remarkable antibacterial activity was produced from a *Pseudomonas* sp. isolated in the laboratory. The active principle was produced in shake flasks and an increased yield was noted as compared to stationary culture conditions.

Of all the media used for antibiotic production, it was found that a medium having the following composition : peptone—1%, glycerol—4%, NaCl—0.5%, KNO_3—0.1% in tap water, there was a fair growth in culture medium and adequate pigment production in shaken condition at 25°C within a period of 72 to 80 hrs. The studies on the ingredients of the above medium showed that glycerol and minerals were indispensable for pigment production. Peptone in the medium could, however, be replaced by defatted mustard cake, while corn steep liquor lowered the yield of the antibiotic production.

The blue-coloured active principle was extracted with chloroform and from the chloroform layer, it was taken up into aqueous layer by shaking with 2(N) HCl solution. By successive repetition of this process, the active principle so obtained after evaporation of the final chloroform layer was fairly pure. Further purification was carried out by dissolving the blue mass in a minimum volume of methanol and then 4 to 5 vols. of water was added. The solution was kept in the refrigerator at 4°C for 2 days, when blue needle-shaped crystals were separated out. The m.p. of the antibiotic was 114°-116°C. Homogeniety of the antibiotic was claimed by paper chromatography and paper electro-phoresis method and as well by the countercurrent distribution technique. It formed picrate, picrolonate, reineckate, hydrochloride; elemental tests indicated that the compound contained C, H, N, and O. Both UV and Infra red spectrophotometric data revealed the presence of conjugated ketone, N—CH_3, C—CH_3, N-substituted benzene ring. The qualitative indication of the presence of quinone moiety and tertiary nitrogen were corroborated by chemical tests.

On an examination of the biological activity of the antibiotic, it was noted that the compound retained its activity at a wide range of pH. Heating the solution of the antibiotic even at 100°C for 1 hr. would not lower its activity appreciably. Even sterilization of the antibiotic solution at 10 lbs. p.s.i. for 30 mins. did not lower the activity to a considerable extent.

TABLE 2

ANTIBACTERIAL SPECTRUM OF THE ACTIVE SUBSTANCE AGAINST BACTERIA

Culture	Min. inhibitory conc. γ/ml.
1. Staph. aureus (non resistant) ..	2.5
2. ,, ,, (Resistant) ..	15
3. B. subtilis	25
4. B. anthracis	50
5. B. megaterium	100
6. P. vulgaris	10
7. S. albus	10
8. E. coli	25
9. E. typhosa	10
10. V. cholereae	10
11. P. aeruginosa	—
12. My. smegmatis	25

D.1.3. *Antifungal activity of the strain Ac_2 435*

D.1.3.1. *Taxonomic character and antimicrobial activity.* The actinomycetes strain Ac_2 435 was tested for identification. Its cultural and biochemical characters were compared with the list of classification as described in Bergey's Manual of Determinative Bacteriology (7th Edition). It seems, this is a new record.

Its antibiotic spectrum is rather broad, the active substance being active against bacteria and fungi. It has marked antifungal activity against 3 saprophytic, 7 plant pathogenic and 5 human pathogenic fungi.

D.1.3.2. *Optimum conditions for the production of the antibiotic.* Stationary culture method was used for the production of the antibiotic. Although the production took place over a wide range of temperature, the optimum temperature was found to be at the region of 28°—30°C. The optimum *p*H was noted to be 6.8—7.2. The yield of the antibiotic and the effect of prolongation of the incubation period was studied in 13 different media. It was noted that antibiotic production begins from the 2nd day of incubation and continues upto the 30th day, attaining a maximum between the 6th and 10th day. Even after 30 days of incubtion, there was no marked decrease in the antibiotic yield.

TABLE 3

ANTIFUNCAL ACTIVITY OF Ac_2 435

Test fungus	Antibiotic zone in mm.
Saprophytic	
Penicillium chrysogenum	27
P. notatum	25
Saccharomyces cerevisae ..	25
Plant pathogenic	
Aspergillus niger	20
A. oryzae	22
Alternaria solani	30
Curvularia spp.	35
Fusarium spp.	20
Helminthosporium sativum ..	22
Glomerella cingulata ..	40
Human pathogenic	
Candida albicans ..	25
Microsporum gypsum ..	25
Epidermophyton floccosum ..	28
Trichophyton rubrum ..	30
T. sulphureum	35

D.1.3.3. *Nutritional requirements.* 17 carbon sources, in the form of sugars, polyhydric and trihydric alcohols and organic compounds were supplied as nutrition for this strain and it was found that except for a few organic compounds the strain grew and produced the antibiotic in all the carbon sources tested. So far as the nitrogen requirement was concerned antibiotic production took place favourably in the presence of amino acids and ammonium salts. The effect of vitamins in various concentrations were tested but there was no remarkable change in antibiotic production.

D.1.3.4. *Chemical purification of the antibiotic: Solvent extraction.* The active principle can be extracted from the culture broth with *n*-butanol. Evaporation of the solvent yielded a dark reddish-brown, gummy mass. The pigment could be removed by adsorbing alcoholic solution of the antibiotic on a column of cellulose powder and eluting it with a mixture of benzene and butanol. The product was almost colourless.

Ion exchange resin. The culture broth was passed through a column of resin (Amberlite IRA-400) and the antibiotic was eluted with alkaline alcohol in the form of sodium salt. Neutralization of the product in water and subsequent shaking with *n*-butanol transferred the antibiotic from the water phase to the butanol phase. Evaporation of butanol and subsequent washing with alcohol to free the antibiotic from inorganic salts gave a light yellow coloured antibiotic.

Countercurrent distribution. The dark, reddish brown gummy material obtained by the solvent extraction method, was distributed in a 24-tube all-glass countercurrent distribution apparatus. A number of solvent pairs were tried, but the methyl ethyl ketone-barbitone buffer (*p*H 9) solvent system and a mixed solvent system in which the upper phase consisted of methyl ethyl ketone with various proportions of *n*-butanol and the lower one, barbitone buffer (*p*H 9) proved to be satisfactory. The distributed material was estimated by the bioassay method using *Curvularia* as the test fungus. Colorimetric estimation was also done in a Klett Summerson colorimeter.

Experiments performed with a mixed solvent pair in which the upper phase consisted of 25% *n*-butanol and 75% methyl ethyl ketone and the lower phase, barbitone buffer brought about the separation of an inactive pigment from an active substance. There was, however, an indication of a second pigment whose distribution band was more or less the same as that of the antibiotic. This idea was strengthened when an experiment with a similar system of solvents, in which the upper phase consisted of 10% butanol instead of 25%, was performed. The antibiotic partially purified in these experiments, when distributed in the solvent system of methyl ethyl ketone and barbitone buffer, brought about the separation of the antibiotic from the second pigment, which was also, inactive. The active principle, thus purified, is light yellow coloured.

D.2. Induced mutation in microorganisms

D.2.1. *Radiation damage recovery in Aspergillus niger*

Experiments were performed with a view to determine the effect of post-treatment of spores with certain nutritive substances leading to the recovery from radiation damage. For this purpose spores of *A. niger* (V_{35}) after irradiation were subjected to treatments for one hour in casein hydrolysate (10%), B-vitamins (2%) and nucleic acid hydrolysate (3%) solutions. After the treatment, the control and as well as treated sets of spores were plated in CM, and survival percentage determined. Percent biochemical mutants were determined by the total transfer method. Table 1 gives the results of the experiment.

It appears from the table that there was slight recovery of damage in the spores treated with B-vitamins.

TABLE 1

TIME OF IRRADIATION—12 MINUTES
Dose—100 ergs/mm²/sec.

Percent	Irradiated but untreated	Irradiated and trated for one hour		
		Casein hydrolysate 10%	Vitamins (B) 2%	Nucleic acid 3%
Survival	3.0	2.9	3.6	2.8
Morphological mutations	10.7	8.8	5.3	9.6
Biochemical mutations	1.7	4.4	12.5	2.8

D.2.1.2. *Utilization of growth factors by different biochemical mutants.* A number of biochemical mutants of *A. niger* were selected and experiments were done to determine the optimum concentrations of growth factors required for their nutrition. Minimal medium was prepared (*p*H at 6.0) and distributed into 100 ml. Erlenmeyer flasks each receiving 25 ml. of the medium. Growth factors at different concentrations were added to these flasks and for each concentration replicates were made.

The flasks were inoculated and incubated at 28°C for ten days, the mycelia were harvested in a weighed dry filter paper, the material was dried overnight at 80°C and weighed again. Tables 2, 3 and 4 will show the growth rate of deficient mutants in the presence of required Amino acids, purines and pyrimidines and vitamins respectively.

TABLE 2

Strain	Amino acids	Concentration in mg/ml 0.01	0.02	0.03	0.04	0.05
		Dry wt. in mgs.	Dry wt. in mgs.	Dry wt. in mgs.	Dry wt. in mgs.	Dry wt. in mgs.
Av_2	Methionine	76	132	168	294	308
Av_3	Arginine	39	50	66	68	70
Av_4	Lysine	310	312	314	315	323
SG_{11}	Leucine	38	68	75	90	113
SG_{12}	Isoleucine	270	300	307	320	372
SG_{34}	Histidine	3	26	111	140	147
I_{10}	Glycine	3	6	7	16	26
L_{15}	Threonine	20	20	22	22	46
L_{15}	Aspartic acid	18	18	20	24	46

TABLE 3

Strain	Growth factor	Concentration in mg/ml. 0.01	0.02	0.03	0.04	0.05
		Dry wt. in mgs.	Dry wt. in mgs.	Dry wt. in mgs.	Dry wt. in mgs.	Dry wt. in mgs.
E_8	Cytosine	87	134	181	190	242
P_1	Xanthine	10	15	16	29	45
J_{25}	Adenine	10	40	92	124	124
J_{25}	Hypoxanthine	34	75	92	98	104
O_3	Cytosine	58	266	286	334	340
O_3	Uracil	122	158	220	252	300

TABLE 4

Strain	Vitamin	Conc. in mg/25 ml. of medium	Dry wt. of mycelium in mg.
SG_{20}	Pyridoxine	0.025	188
		0.050	210
		0.075	285
		0.100	294
		0.125	272
C_{25}	Nicotinic acid	0.005	143
		0.010	164
		0.015	186
		0.020	232
		0.025	197
SG_{10}	Thiamine	0.025	255
		0.050	276
		0.075	313
		0.100	280
		0.125	246
O_{18}	Biotin	0.00001	266
		0.00002	315
		0.00003	306
		0.00004	305
		0.00005	266
N_2	Para-aminobenzoic acid	0.0005	245
		0.0010	258
		0.0015	286
		0.0020	288
		0.0025	285
C_{47}	Choline chloride	0.01	77
		0.02	93
		0.03	216
		0.04	242
		0.05	352

D.2.1.3. *Studies on the growth of Phycomycetes as material for induced mutation.* Work has been undertaken with *Rhizopus oryzae*, a Phycomycetous fungus producing sarco latic acid. It has a fast and spreading growth, and colony counts are difficult to make, as all small colonies merge to form a bigger one. By trying different media and chemicals it has now been possible to get compact colonies of the fungus. The following media was developed.

Sodium chloride	.. ..	90.0 gms.
Glucose	.. ..	40.0 gms.
KH_2PO_4	.. ..	0.3 gms.
NH_4NO_3	.. ..	1.2 gms.
$MgSO_4$	.. ..	0.12 gms.
Ferric tartarate	.. ..	0.003 gms.
Distilled water *p*H adjusted to 6.0	..	1000 ml.

Table 5 gives the results of irradiation experiments with Ultraviolet light.

TABLE 5

Dose 100 ergs/mm²/sec				
	Time of irradiation in minutes			
	1	2	3	4
% Survival	46.3%	20.0%	0.2%	0.2%

A number of biochemical mutants and lactic acid variants have been obtained; detailed studies are in progress.

D.2.2. *Effect of chemical mutagens on Aspergillus niger.*

D.2.2.1. *Studies on biochemical deficient mutants of Aspergillus niger.* The following mutants have so far been isolated in previous experiments:

TABLE 5

SPECIFIC REQUIREMENTS OF THE DEFICIENT MUTANTS

Strain index	Growth factor requirements	Strain index	Growth factor requirements
A 12	Guanine, hypoxanthine	C 16	Lysine HCl
C 1	Methionine, cystine, cysteine	C 24	Adenine, hypoxanthine
		C 32	Aneurine HCl
$C\ 1_2$	Methionine, cystine, cysteine	$C\ 39—20_1$	Methionine, cystine, cysteine
C 3	Methionine, cystine, cysteine	$C\ 39—20_2$	Adenine, hypoxanthine
$C\ 3_2$	Adenine, hypoxanthine	$C\ 39—20_3$	Methionine, cystine, cysteine
C 8	Adenine, hypoxanthine	$C\ 39—20_4$	Adenine, hypoxanthine
C 12	Methionine, cystine, cysteine	$C\ 39—12_1$	Arginine
C 16	Arginine	D 12	Nicotinic acid
		D 20	Methionine, cysteine

Acid production by deficient mutants. The mutants were grown in fermenting medium in conical flasks, to which necessary nutritional factors were added in trace. Total acid production was determined on the 8th day of incubation. Results indicate very low yield of citric acid.

D.2.2.2. *Treatment with nitrogen mustard.* The chemical is in the form of D-(2-chloro-ethyl) methylamine Hydrochloride. To 10 ml. of a suspension of spores of *A. niger* strain C, was added 10 ml. of a solution of the chemical in $NaHCO_3$, and samples were taken out

at intervals and plated out. The experiment was done with three concentrations of nitrogen mustard and results on the percentage of survival are given in Table 2.

TABLE 6

PERCENTAGE OF SURVIVAL OF THE SPORES OF A. NIGER STRAIN C IN DIFFERENT CONCENTRATIONS OF NITROGEN MUSTARD

Time of treatment (minute)	Concentration of nitrogen mustard (percent)		
	0.02	0.04	0.1
0	100	100	100
8	90.6	87.6	80.3
12	82.2	80.1	70.0
16	70.8	67.5	56.0
20	65.2	54.8	46.7
24	62.5	46.8	32.1
28	43.9	38.0	29.5

It is evident from the above table that nitrogen mustard was toxic to the spores of *A. niger*, and that this toxicity increased in intensity with the increase (a) in the time of treatment as also (*b*) in the concentration of the chemical.

But it is interesting to note that these 3 concentrations of nitrogen mustard were ineffective in producing morphological mutants of a significant number. Out of 500 strains studied from each of the 3 experiments, the number of morphological mutants are 1, 1 and 3 in the nitrogen mustard concentration of 0.02, 0.04 and 0.1 per cent respectively.

D.2.2.3. *Biochemical activities of the mutants.* The mutants were grown in synthetic medium and filtrates were titrated on the 8th day of incubation as usual. 500 mutants were studied for each of the 3 sets of experiments, and the rate of appearance of high-yielding strains was found to be of the order of 16.5, 5.4 and 17.2 percent respectively. Results will show that there is no correlation between the concentration of nitrogen mustard applied and the frequency of high-yielding strains.

D.2.2.4. *Treatment with colchicine.* Aqueous suspension of the spores of *A. niger* strain C was treated with phosphate buffer solution of colchicine at pH 6.2 and in the concentration of 1.5%. Samples were taken at desired intervals and plated out as usual. Results of the treatment are given in Table 3.

TABLE 7

RESULTS OF THE TREATMENT OF A. NIGER STRAIN C WITH COLCHICINE

Time of treatment (minute)	Survival (percent)
0	100
8	84.6
16	81.9
24	76.9
28	69.0

It is clear that colchicine also exerts toxic action on the spores of *A. niger*, although its intensity is less than that in the case of nitrogen mustard. Morphological mutants are also very scanty,—numbering only 3 out of 500 cultures studied.

D.3. Microbial Genetics

D.3.1. *Studies on the mutagenic properties of chemicals on Neurospora crassa*

The effects of various chemicals on a methionineless strain of *Neurospora* have been studied. For detection of mutagenic potentialities of chemicals the reverse mutation technique has been employed. The technique consists essentially of plating spores of a high concentration of known strength in the minimal medium completely devoid of organic substances, vitamins (excepting biotin) and amino acids etc. Colonies appearing on such a minimal medium are counted as reverse mutations though genetical tests are necessary before these reverse mutations are taken as true gene mutations. The mutagenic property of a chemical could thus be precisely detected with comparatively simpler means.

Preliminary results of experiments with Dimethyl ethylene immine, Para-amino-hippuric acid and Acridine orange are being presented.

D.3.1.1. *Dimethyl ethylene immine.* Dimethyl ethylene immine synthesised in the chemical laboratory of the Bose Institute has been used as a mutagenic agent.

Spore suspensions varying from 6 to 10 millions per cc. were used for the experiments. Conidiospores were harvested from cultures grown at room temperature of 25° to 30°C, centrifuged at 3000 r.p.m., washed and treated with the chemical for different lengths of time. During treatment, tubes containing spore suspensions were kept agitated on a shaker. After treatment the spores were washed again and diluted to lower strengths of 10^{-5} and 10^{-6} per cc. Spores were then plated, 10 from each lot using 1 cc. in each plate on Fries complete medium with surbose added. At the same time 5 to 10 platings were done on Fries minimal medium with sorbose, 1 cc. of concentrated spore suspension being taken in each plate from each lot.

In preparing the purified agar for the minimal medium G. W. Beadle and E. L. Tatum's method was followed. All mutation experiments have been done in a constant temperature room maintained at 25° ±1°C and for incubation experimental plates were kept at 25° to 30°C.

Results of experiments with different concentrations of dimethyl ethylene immine are given in Table 1.

Dimethyl ethylene immine shows a very strong killing effect on conidiospores when treated for shorter durations. However, with increase in the duration of treatment at conc. 0.001 Mol the survival percentage increases from 25.49% to 54.50% (Table 1). The

drop in survival per cent at 30 mins. treatment may be overlooked. At 10 minutes treatments a higher concentration of 0.01 Mol shows almost similar effect. At a still higher concentration of 0.2 Mol at the same duration of treatment, the survival percentage falls to 14.09%.

TABLE 1

SHOWING PERCENTAGE OF SURVIVAL OF SPORES ON TREATMENT

	Concentration of chemical	Duration of treatment	Number of spores survived at 10^{-5} per cc.	Percentage of survival
Control		—	965	100%
Number of spores per cc. at 10^{-5} dilution: 96.5	0.001 Mol	5 mins	24.6	25.49%
		10 ,,	47.0	48.70%
		15 ,,	52.6	54.50%
		30 ,,	49.3	51.08%
	0.01 Mol	10 ,,	45.3	46.94
	0.2 Mol	10 ,,	13.5	14.09

The experiment was repeated and a similar resistance of spores to increasing duration of treatment with concs. of 0.01 Mol and 0.02 Mol was noted. This behaviour points to a probable decomposition of the chemical during treatment so that it becomes less active on spores while in solution for a longer time.

No colony in the minimal plates could, however, be found thus indicating the non-mutagenic character of the chemical under conditions the same was used on the particular mutant gene of *Neurospora crassa*.

Further experiments with Dimethyl ethylene immine and other immines had to be stopped because of non-availability of some of the constituent chemicals necessary for the synthesis of such chemicals.

D.3.1.2. *Para-amino-hippuric acid.* This amino acid was used in order to study its probable effect on the DNA constituent of the spore cells. The general procedure of the experiments was the same as stated before. Varying treatments with concentrations of 0.025 and 0.05 Mol showed a stronger effect of the latter on the survival of spores.

In another experiment keeping the conc. at 0.05 Mol spores were treated for 2, 4, 8, 16, 24 and 36 hours. Very little effect was found even at 24 hours treatment. At 36 hours, however, the survival was 57.26%.

A few reverse colonies were observed on the minimal platings; but the response was very poor and rather irregular in relation to the degree of treatment.

TABLE 2

SHOWING EFFECT OF PARA-AMINO-HIPPURIC ACID ON NEUROSPORA CRASSA

Control and Treatments	Number of viable spores per cc. $\times 10^6$	Number of spores survived $\times 10^6$	Percent survival	Number of mutations counted	Mutation scored: per 10^6 initial spores	Mutation scored: per 10^6 survived spores
Control	6.95		100%	3	0.086	0.086
2 hours		7.02	100%	1	0.028	0.028
4 ,,		6.91	99.42%	5	0.144	0.144
8 ,,		6.47	93.09%	2	0.057	0.061
16 ,,		6.13	88.20%	5	0.144	0.163
24 ,,		6.42	97.37%	—		
36 ,,		3.97	57.26%	3	0.086	0.151

D.3.1.3. *Acridine orange.* In view of the recently observed mutagenic effect of Acridine orange upon some bacteria the effect of the same was tested upon Neurospora. Technical procedure was the same as followed before. Treatment of spores at concentrations of 50 μg per ml, and 100 μg per ml from 2 hours to 8 hours showed considerable killing of spores. Observations on the survival of spores after treatments are shown in Table 3.

TABLE 3

SHOWING EFFECT OF ACRIDINE ORANGE ON SURVIVAL OF SPORES OF NEUROSPORA CRASSA

	Concentration of chemical	Duration of treatment	Number of spores survived at 10^{-5} per ml.	Percentage of survival
Control Number of spores per cc. at 10^{-5} dilution: 94.7			94.7	100.00
	50 μg/ml	2 hours	48.6	49.64
		4 ,,	55.7	58.80
		6 ,,	36.7	38.75
		8 ,,	37.6	39.70
	100 μg/ml	4 ,,	18.5	19.53
		6 ,,	11.5	12.14
		8 ,,	24.5	25.87

Some anomaly in the killing effect will be observed in the table. This is apparently due to the stickiness of Acridine orange stain which can hardly be removed completely and uniformly from the spores in spite of repeated washings. Moreover, the platings were irregularly exposed to light while kept on the table during incubation and it may be that there was some irregular activation of light on the treated spores. The experiment yielded some reverse mutations on minimal plates, maximum number obtained being 14 at 4 hours —5 μg/ml treatment as against 3 in the control.

In a more detailed and careful expriment some interesting results were obtained; using the conc. of 50 μg per ml and varying the *p*H range from 4.8 to 8.8 treatments were given for 2, 4, 6 and 8 hrs.

As the action of Acridine orange is influenced by light, the spore suspensions during treatments were kept cut off from light while on the shaker and platings done immediately after the treatments were over and plates transferred to a dark chamber. Results of the experiment are summarised in table 4. At 8 hours treatments survival of spores is between 42.39% to 56.76% and the action of the chemical so far as the viability of spores is concerned varies within narrow limits. It is evident that the *p*H of chemical has little effect on the viability of spores.

In about a week's time the reverse colonies grew up on the minimal plates. All the colonies, however, were not equally vigorous. All reverse mutation colonies have been tested on Fries minimal slants. With increase in *p*H of chemical and duration of treatment lesser number of reverse mutations have been observed. At *p*H 6.1, 2 hours and 4 hours treatments gave the maximum number of colonies on the minimal plates. Thus mutations from all the 2 hours tratments taken together number 53 and those from 4 hours 50 while 6 hours and 8 hours treatments yeilded only 24 and 21 mutations respectively.

More elaborate experiments with Acridine orange on the basis of the experimental results obtained so far are being planned.

TABLE 4

SHOWING THE EFFECT OF ACRIDINE ORANGE ON SURVIVAL OF SPORES AND MUTATION RATE AS OBSERVED IN NEUROSPORA CRASSA

	Treatment	pH of spore suspension	Number of spores survived at 10^{-6} per cc.	Percent survival	Number of mutation counted	Mutations scored: per 10^6 initial spores	Mutations scored: per 10^6 survived spores
Control		4.8		100.00	2	0.047	0.047
Number of spores per cc. at 10^{-6} dilution: 8.42	2 hours	4.8	6.34	75.29	6	0.142	0.189
		6.1	5.64	66.98	16	0.382	0.567
		6.65	7.68	91.21	9	0.213	0.234
		7.0	4.96	58.90	15	0.358	0.604
		8.8	5.72	67.93	7	0.166	0.244
	4 hours	4.8	6.87	81.59	11	0.268	0.320
		6.1	6.25	74.22	18	0.425	0.576
		6.65	7.08	84.08	6	0.142	0.169
		7.0	5.45	64.72	5	0.118	0.183
		8.8	6.42	76.24	10	0.237	0.311
	6 hours	4.8	4.56	54.15	3	0.07	0.131
		6.1	5.82	69.12	10	0.237	0.343
		6.65	7.35	87.29	1	0.023	0.026
		7.0	4.44	52.73	2	0.047	0.090
		8.8	5.04	59.85	8	0.190	0.316
	8 hours	4.8	4.28	58.31	1	0.023	0.046
		6.1	3.57	42.39	4	0.095	0.224
		6.65	4.09	48.57	2	0.047	0.097
		7.0	4.13	49.04	9	0.213	0.435
		8.8	4.78	56.76	5	0.118	0.209

D.3.2. *Improved technique for the isolation of Biochemical mutants of a strain of Streptomyces sp, Ac_{21}* (460)

The isolation of mutants was done by the filtration technique in which the UV irradiated spore suspension (1 cc. in each case) was allowed to grow in 20 cc. MM liquid medium. After 24 hours of germination at 28°C the spore suspension was filtered through sintered glass filter no. 4 and the filtrate was then plated out in complete medium; (the germinated spores cannot pass through the filter and only the non-germinated spores can pass). Then the colonies were picked up by total isolation method of Fries transferring the colonies onto minimal agar plates and allowed to incubate at 28°C for 6 days.

It is assumed that the mutation has occurred if there is no growth of an inoculum on minimal medium. The organism in that case is unable to synthesize some of the vital cellular constituents essential for its growth. The nature of the deficiency is now determined by subculturing the probable mutants into the following media given in the Table 5:

TABLE 5

Media	Supplemented with substances
1. Complete ..	—
2. Minimal ..	—
3. ,, ..	Amino acid mixture
4. ,, ..	Vitamin mixture
5. ,, ..	Yeast extract
6. ,, ..	Nucleic acid hydrolysate

For determining the characterisation of the biochemical mutants, auxanographic technique was adopted. Out of a total of 2870 colonies picked up from filtered irradiated spore suspension, finally 22 strains showed nutritional deficiency. The deficiency of a particular compound was demonstrated on further analysis with amino acids and pure chemical constituents of vitamins and nucleic acids. The results obtained showing nutritional deficiency are given below (Table 6).

TABLE 6

(Biochemical mutants) Growth requirements	Number of strains isolated
Glycine/Histidine/Alanine/Ornithine/Methionine	1
Histidine/Valine/Ornithine	1
Tryptophane/Hydroxy proline ..	1
Histidine/Proline/Lysine	1
Valine/Methionine/Cystine/Cysteine ..	1
Glycine/D-L-proline/Histidine/Asparagmic Acid	1
Aspartic acid/Cystine	1
Glycine/Leucine/Arginine	1
Histidine/Tyrosine	1
Aspartic acid/DL-proline/Valine/Cysteine	1
Glycine/Valine/Cystine	1
Lysine/Tyrosine	1
Aspartic Acid/Histidine/Tyrosine ..	1
Glycine/Histidine/Cysteine	1
Histidine/Lysine/Arginine	1
Histidine/Arginine	1
Histidine/Threonine	1
Proline/Asparaginic Acid	1
Glycine	1
Arginine/Tyrosine	1
Biotin	1
Hypoxanthine	1

D.3.3. *Production of Biochemical mutants by UV irradiation of A. chevalieri*

Experiments are being conducted on the effect of UV on *Aspergillus chevalieri* strain No. 88 (obtained from Indian Jute Mills Association Research Institute). The cultures were grown on Czapek Dox agar medium (20 ml) with 2% malt ext. in 100 ml. conical flasks and incubated for 12 days at 30°C.

The growth of the organism was luxuriant and spore production was heavy, the cultures becoming brown within 6-8 days after the production of conidial heads. The spores were harvested in sterile calsolene solution (1:10,0000). The suspension was then shaken for 30 mins. to break spore clumps. This was filtered through sterile absorbent cotton wool to remove any mixed-up mycelium. The spore suspension was plated out after dilution in CDA to determine the spore count.

The spore suspension was irradiated with a 4-watt GE germicidal lamp emiting 90% of the energy in the wavelength of 2537 Å. 5 ml. of the spore suspension was taken of irradiation for each lot. The time of exposure for irradiation was adjusted to 2, 4, 6 and 8 minutes and the dose calculated was 100 ergs/mm /sec.

After irradiation, the spore suspension was diluted in Ringer's solution and plated out in complete medium (CM). The colonies were counted after 2 days of inocubation at 30°C. Table 7 shows the result of the irradiation.

TABLE 7

Time of irradiation	Control	Survival %	Biochemical mutant	Morphological mutant
2 mins.	24.6	11.3%	4.37%	4.37%
4 mins.	,,	2.9%	2.20%	2.20%
6 mins.	,,	5.9%	5.8%	19.6%
8 mins.	,,	0.14%	4.23%	0.13%

The biochemical mutants were isolated by the total isolation method of Fries. The survivors developed on complete medium after UV-treatment were picked up by a forked needle and the inoculum was transferred onto minimal agar medium. Before incubating, the agar plates were placed on a graph paper with intersecting parallel lines at equal distances. The inoculum from complete medium carefully transferred at the counter of each intersections and the plates were kept at 30°C. Strains having nutritional deficiencies failed to grow on minimal medium. Colonies not growing in minimal medium were transferred to complete medium for further study. Strains differing from the parent in morphology were transferred to minimal agar. The biochemical mutants could however be characterised by their failure to grow in minimal medium.

Six mutants were isolated and tests are being carried out for the determination of their nutritional requirements.

D.4. Studies on the Rhizobium strains and related bacteriophage

D.4.1. *Isolation of Rhizobium spp. and their identification*

To isolate the appropriate species of *Rhizobium*, soil pot experiments were done in the laboratory. The pots were filled up with the garden soil of the Bose Institute, Calcutta and seeds were sown without any inoculation.

1. Pisum sativum
2. Phaseolus radiatus
3. Lathyrus sativa
4. Lupin sp.
5. Vigna cajung
6. Trigonella sp.
7. Lens esculentum
8. Phaseolus vulguaris (French bean)
9. Trifolium alexandrianum
10. Dolichos lablab
11. Medicago sativa
12. Cicer arietinum
13. Sesbania sp.
14. Crotolaria juncea
15. Cajanus indicus
16. Arachis hypogea

From soil pot experiments it was found that *Rhizobium* species for *Cicer arietinum*, *Trifolium alexandrianum* and *Phaseolus vulgaris* were absent in the garden soil.

Another experiment was done in the Falta Experimental Station. From this experiment *Rhizobium* species for *Cicer arietinum* was isolated. Here also nodules were not formed in *Trifolium alexandrianum.*

D.4.2. *Studies on the legume seed inoculation in the field*

To study the effect of seed inoculation in the field, an experiment was designed and performed at Falta Experimental Station. Four experimental plots were selected for growing the host plants, two for both inoculated and control lot of the experiment.

For inoculation, the seeds were kept in the suspension of bacteria in sterile tap water made from 2 to 3 days old culure of *Rhizobium.* After 12 hours the suspension was drained off and the seeds were again treated with a freshly prepared suspension of the same bacteria for 1 hr.

The table shows the date for comparative yield of the treated and control legume crops.

TABLE 1

No.	Crop	Yield		Increased in %		Remarks
		Treated	Untreated	Treated	Untreated	
1.	Cicer arietinum	9.6	5.6	67.2%	—	The yield was higher in inoculated plants
2.	Lathyrus sativa	6.6	4.2	56.6%	—	,,
3.	Trigonella sp.	3.3	2.9	14%	—	,,
4.	Vigna catjung	3.5	3.7	—	4%	The yield was higher in control
5.	Phaseolus radiatus	0.95	1.5	—	14.0%	,,
6.	Lens esculentum	15	16	—	6.6%	,,

D.4.3. *Studies on the bacteriophage of legumes and preliminary screening test.*

Bacteriophages isolated from different legume soils were tested by screening test and twenty strains of bacteriophage were isolated of which only nine were selected by the screening test. The results of screening test was also confirmed by the colony count test.

TABLE 2

Index of selected phase	*Rhizobium* spp.	*Corresponding Host plant*
P 1	Rhizobium sp.	Crotolaria juncea
P 4	Rhizobium meliloti	Trigonella sp.
P 6	Rhizobium sp.	Cicer arietinum
P 7	Rhizobium sp.	Glycin hispida
P 9	Rhizobium meliloli	Medicago sativa
P 10	Rhizobium lupinii	Lupinus sp.
P 13	Rhizobium phaseoli	Phaseolus radiatus
P 19	Rhizobium sp.	Arachis hypogea

D.4.4. *Purification of bacteriophage*

9 ml. of broth (YWA) was inoculated with a small dose of the host bacteria and kept at 25°C. After 24 hours the culture broth was inoculated with 1 ml. of phage suspension and incubated at the above temperature.

After 24 hrs. 1 ml. of mixed bacterial and phage suspension was transferred to fresh 9 ml. of broth and filtered through sterilized sintered glass filter no. 5. This process was repeated until a fairly concentrated phage suspension was obtained.

D.4.5. *Phage titre by plaque count method*

This was done by plating 9 ml. of broth culture of *Rhizobium* sp. mixed with phage plus 9 ml. of YWA agar media (concentration of agar was 3%). It was found that the rate of killing of bacterial cell with dilute phage suspension was increased upto 8th hour and after 24 hrs, it was decreased. The following table shows the results.

TABLE 3

Name of phage	Rate of killing of bacterial cell at 2 hrs. interval					
	2 hrs	4 hrs	6 hrs	8 hrs	24 hrs	48 hrs
P 6	53×10^6	50×10^6	63×10^6	12.7×10^7	13.7×10^7	99×10^6
P 7	37×10^6	35×10^6	67×10^6	94×10^6	10.7×10^7	29×10^8
P 10	73×10^6	47×10^6	12.7×10^7	13.1×10^7	36×10^6	14×10^6
P 9	72×10^6	58×10^6	14.0×10^7	12×10^7	19.8×10^7	13.2×10^7
P 12	26×10^6	10×10^6	20×10^6	47×10^6	12×10^7	$77.\times10^6$
P 13	12.2×10^7	39×10^6	16.2×10^7	12.2×10^7	9.8×10^7	16.1×10^7
P 19	72×10^6	58×10^6	14×10^7	12×10^7	19.8×10^7	13.2×10^7

D.4.6. *Development of phage resistant mutants*

An old strain of *Rhizobium* sp. of *Cicer arietinum* was made resistant to the phage T.6. The mutant strains were isolated by double treatment and each treatment continued for 15-20 days. About seven types of colonial mutation were found after phage treatment in *Rhizobium* sp. of *Arachis hypogea.* The colonial morphology was mainained with no apparent change throughout a large number of serial transfers of YWA slant. About seventy colonies were isolated and from these only twelve to fifteen strains were found resistant to

phage. Some were less and others were highly susceptible. From these isolates twentyfive strains were selected including all resistant strains for nodule-forming capacity. It was found that all these twentyfive strains were capable of forming nodule in completely asceptic condition.

D.4. *Lysogenicity test*

A strain of *Rhizobium phaseoli* was found lysogenic by its nature. The phage extract was active against another lysogenic strain *Rhizobium meliloti* which is a widely separated species in the cross-inoculation group.

Phage titre was determined by making *Rh. meliloti* streptomycin-resistant. The plaque number was 86-96.

D.4.8. *Experiment with Ultraviolet radiation*

Rhizobium sp. of *Arachis hypogea* were selected for this purpose for comparison with the phage treated. mutant. In this case ten types of colonial mutants were obtained and only of which a few were found to be resistant to the action of phage.

D.5. Ecology of Soil Fungi

D.5.1. *Yield of Paddy crop in Field experiments*

The field experiments at Falta was continued during this year. Aman Paddy (Patnai-23) was transplated in all the plots as cover crops and fungi were isolated and identified at regular intervals from soil and roots of growing paddy. Soil temperature and rainfall were also recorded.

The average yield of paddy (1960)

Experimental plot		Mds/acre
F_0	..	27.4±1.47
F	..	25.9±1.47
F_D	..	25.1±2.11

The differences in yield between the treaments are not statistically significant.

D.5.2. *Pests and diseases*

Seedlings in the seed bed were attacked by Rice *Hispa* (Hispa armigera) which was quite prevalent in the locality. 50% wettable B.H.C. powder was sprayed twice at the interval of two weeks and pests were brought undr control. Though *Helminthosporoium* leaf spots were noted in some corner tillers, Piricularia was absent throughout the whole growing period. *Ustilagenoidea virgens* was also noted in a few ears of all the plots.

D.5.3. *Isolation and identification of fungi*

Isolation was carried out from soil and roots of paddy plated on malt agar fortified by *streptomycin* at intervals of 1 month.

F_0 = control
F = fertiliser added
F_D = deep ploughed and fertiliser added

The following fungi were recorded :

A. *Fungi imperfecti*

1. Aspergillus niger
2. Aspergillus terreus
3. Penicillium sp. I
4. Penicillium sp. II
5. Trichoderma viride
6. Curvularia sp.
7. Helminthosporium sp.
8. Cephalosporium sp.
9. Alternaria sp.
10. Nigrospora sp.
11. Chaetomella vermicularia
12. Culture No 4 sterile brown by hyphae nodulated
13. Phoma
14. Monotospora
15. Trichothecium
16. Colletotrichum
17. Sclerotium—2
18. Pestalosia
19. Hyalopus
20. Fusarium

* * *

B. *Phycomycetes*

21. Mucor sp. I
22. Mucor sp. II
23. Rhizopus nigricans
24. Cunninghamella sp.
25. Syncephalastrum sp.
26. Phytophthora sp.

C. *Ascomycetes*

27. Arachniotus indica
28. Arachniotus indica var.
29. Culture No. 6
30. ,, No. 7 unidentified
31. ,, No. 8

Though most of the fungi noted above were isolated before, a few types appeared this year e.g. *Alternaria*, *Monotospora* and culture Nos. 6, 7, 8. *Phytophthora*, *Phoma*, *Colletotrichum*, *Sclerotium*-2, *Pestalosia* and *Hyalopus*.

D.5.4. *Aminoacid requirements of some soil fungi*

Czapek Dox's medium prepared from 'Analar' reagents was used as basal medium (*A*) to which casein hydrolysate (*B*) (0.1%) was added. Pyridine-washed agar was used for solidification.

TABLE 1

Organism	Amino acid requirement	Dry wt. of mycelial mat from liquid media in gms.	
		A	B
1. Curvularia sp.	(—)	0.231	0.318
2. Helminthosporium	(—)	0.322	0.391
3. Cephalosporium	(—)	0.222	0.303
4. Mucor sp. I	(+)	0.003	0.362*
5. Rhizopus sp.	(+)	0.0015	0.121*
6. Cunninghamella sp.	(—)	0.275	0.311
7. Aspergillus niger	(—)	0.398	0.555
8. Syncephalastrum sp.	(—)	0.252	0.3895
9. Penicillium sp. I	(—)	0.254	0.338

*Rhizopus and Mucor spp. showed requirement of Casein hydrolysate. In other cases, considerable growth was noted in basal medium though increase in dry weight was recorded in every case.

D.6. Countercurrent Distribution

D.6.1. *Study of the antibiotic principle obtained from a Streptomyces sp.* Ac_{14} 475

The crude antibiotic sample Ac_{14} 475 was distributed between *n*-butanol and 1% HCl solution (24 transfers). Distribution band obtained from the colorimetric estimation revealed a major peak in the 1st tube and 5 minor peaks in the 12th, 14th,

19th and 23rd tubes. Gravimetric distribution band corroborated the major peak in the first tube showing a pseudo-distribution of proceeding type due to salting-out effect. Distribution band from the agar-plate assay showed that the fraction showing the first major distribution band is antibacterial and that showing the last minor band (having peak in the 23rd tube) is antifungal in character. The distribution material from the first major band was recovered and again distributed between *n*-butanol and water containing 5% *p*-toluene sulphonic acid using it as a carrier. A distribution band with a sharp peak in the 7th tube having a good agreement with the theoretical band shows the homogeneity of the antibiotic. As the antibiotic seems to show resemblance to streptomycin produced by *S. lavendulae* in some chemical and microbiological properties, a mixture of these two antibiotics was also distributed between the same solvent pair. A clear-cut separation was effected showing their difference in nature.

D.6.2. *Countercurrent distribution study of the antibiotic principle obtained from a Pseudomonas sp.*

A coloured antifungal and antibacterial antibiotic having acid-base indicating property has been isolated from a *Pseudomonas* sp. The active principle was isolated in a crude form from the harvested broth with chloroform and subsequent evaporation *in vacuo*. The crude antibiotic was distributed in the countercurrent distribution apparatus between the solvent system benzene-water (24 transfers). Distribution band obtained from the colorimetric estimation revealed the major peak in the 7th and two minor peaks in the 12th and 18th tubes. The major fraction is blue in colour and the minor fractions are green and yellow. Distribution band from the agar-plate assay showed that the antibiotic activity is solely due to the major fraction which is blue in colour. This fraction when recovered shows to be constituted of about 95% of the crude sample.

The homogeneity of this major fraction showing the antibiotic activity was further shown by distributing again between chloroform : petroleum ether : water (4:25:4) after recovery from the first distribution. A clear-cut distribution band with a single sharp peak in the 14th tube was obtained both by colorimetric and bio-assay methods of estimation.

D.6.3. *Countercurrent distribution study of the antibiotic principle obtained from Streptomyces sp. Ac_2 435*

A strain of *Streptomyces* sp. designated as Ac_2 435, isolated from the soil samples collected at the Botanical Gardens, Sibpur, showed marked antifungal properties. It was a broad-spectrum antibiotic. A crude form of the antibiotic was obtained by the solvent extraction method. When the fermented broth was extracted with 1/2 volume of *n*-butanol and the butanol was evaporated, a dark brown gummy substance was obtained. A preliminary distribution of the crude sample between aqueous glycerol and acetone indicates the separation of a pale yellow-coloured antifungal antibiotic and an inactive yellow-coloured fraction. This antibiotic fraction was distributed in a countercurrent distribution apparatus (24 transfers) between a mixed solvent system, the upper phase composed of 75% methyl ethyl ketone and 25% *n*-butanol and the lower phase barbitone buffer of *p*H 9. Distribution band estimated by bio-assay method against *Curvularia* as test organism showed that the

antibiotic substance was distributed in a more or less symmetrical way within the tubes 20 to 24 with a sharp peak at the hypothetical tube 21.5. This shows the homogeneous nature of the antibiotic principle.

D.7. Bacterial Transformation

Description of the organisms—The *Micrococcus pyogenes* var. *aureus* (*Staph. aureus*) and *M. pyogenes* var. *albus* (*Staph. albus*) were obtained from stock cultures and were selected for the experimental work. The former was the donor and latter was the recipient strains in this case.

The cells of *Micrococcus pyogenes* var. *aureus* were collected after 24 hours of growth at 37°C. The desoxyribonucleic acid (DNA) contents of the cells were extracted by lysing the cells with sodium deoxycholate (0.1%) at room temperature (32°C), for 30 mins., followed by deproteinization with chloroform and volumes of ethanol. The precipitate thus obtained, dissolved in 0.85% saline. The process of precipitation with alcohol and dissolution in saline was repeated 2-3 times to remove other substances from it.

The NaCl solution of the active substance was subjected to Ribonuclease treatment in a cellophane sac at 37°C against a mixture of equal parts of saline and Vernol buffer at *p*H 7.8 for 6.8 hours and then in the refrigerator overnight. After dialysis, the process of precipitation with ethanol, dissolution with saline was repeated twice and finally the fibrous substance was dissolved in saline.

The potency of the DNA solution was measured spectrophotometrically by recording the optical density at 600 mμ of the colour produced with diphenylamine reagent and compared with a standard DNA solution. The conc. of the stock solution was 0.164 mg/ml. and that of the reaction medium was 0.0328 mg/ml.

The transformation was accomplished by adding 2 ml of stock DNA solution to 8 ml of nutrient broth medium, inoculated, with freshly grown cells of *Micrococcus pyogenes* var. *albus*.

The incubation of the cultures was done at 37°C the reaction time being 15, 30, 45 and 60 mins., 2, 3, 4, 5 and 6 hrs. The culture was chilled after the reaction was complete by immediately placing the tubes at 0°C to stop the reaction. A set of controls was simultaneously run having no DNA in them.

The cells of *Micrococcus pyogens* var. *albus* were plated out on nutrient agar medium and allowed to incubate for 48 hours at 37°C. The no. of colonies both coloured (transformed) and white (*albus*) varieties were examined, and the frequency of transformation induced was calculated.

The coloured colonies appearing on culture plates were isolated in pure culture and their biochemical characters were studied. As a precaution against possible contaminants the isolates were primarily examined microscopically and their gram-characters were determined. About 400 isolated cultures are now studied in detail.

TABLE I

PENICILLIN SENSITIVITY OF MICROCOCCUS SPP
Dilution tube turbidity

Units/ml	M. pyogenes var. aureus	M. pyogenes var. albus	M. citreus
.025	+++	+++	++
0.5	++	+++	+
1.0	—	+	±
2.0	—	±	—
5.0	—	—	—
10.0	—	—	—

+++ = No inhibition, ++ = partial inhibition
+ = incomplete inhibition
± = doubtful, — = complete inhibition

TABLE 2

EFFECT OF TRANSFORMING PRINCIPLE (DNA) ON M. PYOGENES VAR ALBUS CELLS
Inoculum size 3×10^8 cells/ml

Time of reaction (with DNA of Micrococcus pyogenes var *aureus*)	Types of colonies appearing on isolation plates		
	Total number of colonies	Number of Transformants	% of Transformants
15 mins.	1,061	11	1.037
30 ,,	1,633	—	—
45 ,,	7,074	17	0.098
1 hr.	7,937	24	0.302
2 ,,	15,787	71	0.449
3 ,,	4,557	64	1.404
4 ,,	7,382	26	0.352
5 ,,	6,500	12	0.185
6 ,,	6,604	12	0.181

—Not determined

TABLE 3

TRANSFORMATION INVOLVING PIGMENT FORMATION IN MICROCOCCUS SPP. IN COMPARISON WITH TYPES DESCRIBED

Known Types described (Bergey's manual)		Pigment formation	Number of pigmented forms among Transformants	Biochemical group of known type
1. Micrococcus	luteus	citron-yellow	74	A
2. ,,	flavus	,,		
3. ,,	conglomeratus	yellow	101	B
4. ,,	varians	,,		
5. ,,	pyogenes var aureus	orange	12	
6. ,,	pyogenes var albus	white	44431*	C
7. ,,	citreus	lemon-yellow	30	
8. ,,	auranticus	orange	12	D
9. ,,	roseus	rose colour	11	
10. ,,	cinnabareus	cinnabar colour	4	
11. ,,	rubens	flesh colour	4	

*Not transformed
Buff colour 25
Intermediate types (unindentified) colour including mustard cream, vinaceous, ochreceous, cinnamon etc. —64.

E. ZOOLOGY

E.1. *Studies on the metamorphosis of tadpoles (Rana tigrina)*

E.1.1. *Penicillin treatment and intestinal flora of tadpoles.* It was reported that penicillin had an inhibitory action on the growth of bacteria isolated from the intestine of the normal tadpoles. Some of these bacteria produced vitamin B_{12} in a suitable medium, whereas such vitamin B_{12}-producing microorganisms were not found in the intestine of penicillin-treated tadpoles. Penicillin treatment caused complete suppression and ultimate elimination of microorganisms producing vitamin B_{12}. During the year under review, an attempt was made to re-examine the question of inhibitory effect of penicillin on the survival of intestinal flora in a larger population of tadpoles.

The tadpoles were collected from the local tanks and kept in tap water in glass jars containing water weed hydrilla for a few days. They were then divided into batches, each batch containing 50 tadpoles. Ten of these batches were treated with penicillin (80 units per ml.) at every alternate day, seven such treatments being made within fourteen days, after which they were kept in simple tap water containing hydrilla. The control larvae were kept under similar conditions. Out of these ten batches, the result of the four batches are communicated in the following table. The control tadpoles developed into froglets within the usual time (within about 4-8 weeks).

TABLE

Group No.	No. of tadpoles in each group	No. of tadpoles died after penicillin treatment within 8 months	No. of tadpoles metamorphosed within 8 months	No. of tadpoles retarded upto 8 months	No. of tadpoles metamorphosed within 8-9 months	No. of tadpoles died after 8 months
1	50	10	9	31	25	6
2	50	6	12	32	27	4
3	50	5	11	34	20	10
4	50	8	14	28	18	8

Of these, one tadpole in group 2, four tadpoles in group 3 and two in group 4 metamorphosed at the end of ten months.

Five control tadpoles were taken, their alimentary canals were dissected out asceptically as far as possible and crushed in sterile water with a glass rod in separate test tubes to make a suspension of the intestinal mass with the sterile water. This suspension was diluted in stages of ten up to 10^5 and plate cultures were made using nutrient agar as the suitable medium. The bacterial colonies developed in the plates after 24 hours were counted.

Similarly, bacteria were isolated from the alimentary canals of five penicillin-treated tadpoles (8 months old), of which two were again treated with penicillin just before the isolation of bacteria from the intestine. It was observed that the number of colonies developed in the different plates, in which the suspension of the intestinal mass of these two tadpoles (which received fresh penicillin treatment) was given, was markedly less than those developed from the untreated ones. Eighty strains of bacteria from different colonies were isolated from the normal and eighty from the penicillin treated tadpoles and transferred to nutrient agar slants.

These bacteria isolated both from the normal and penicillin treated tadpoles are under investigation. After gram-staining their capability to synthesize vitamin B_{12} in a suitable medium is being studied using mutant E. coli as the test organism. Some of the bacteria isolated from the alimentary canal of untreated larvae produce vitamin B_{12}. The rest of the microorganisms are still under study.

E.1.2. *Iodine treatment of penicillin treated tadpoles (Bufo melanostictus).* As a preliminary study five penicillin treated tadpoles were taken and treated with iodine (50 mg. iodine in 2000 c.c. water). They showed slight change of metamorphosis. The results are not conclusive. Further studies are being made on the effect of iodine treatment on the penicillin treated tadpoles (both Rana tigrina and Bufo melanostictus).

E.2. *Enucleation and nuclear exchange between A. proteus and A. lymax*

Having been successful in establishing homogeneous clones of A. proteus, attempts were being made for obtaining the mass culture of A. descoides and A. lymax, since the middle of last year. For nuclear transplantation two individuals, one of each of different species, are required to be placed side by side, under the moist chamber of the micromanipulator. We have not yet been successful in obtaining mass culture of A. descoides, but in the broth prepared from germinating wheat, mass culture of A. lymax has been possible. Micromanipulation techniques are being used both in hanging drop and conventional methods for pushing the nucleus out of the body of the organism. The newly made apparatus of Peterfi type is being used. Two amoebae of different species are taken side by side under the manipulator, but much difficulty was encountered in manipulating the animals, on account of their fast movements. Their movements become faster by the slightest touch of the needle. A minute drop of chloroform was introduced in the drop of water containing the animals. This slowed down the movement but the animals assumed rounded shape within a few minutes and the nucleus could not be seen through the microscope. Then acetone was introduced which had the desired effect and nucleus could be pushed out of the body by proper manipulation. By repeated trials only in 2 or 3 cases extraction of nuclei was possible but we failed to introduce them in the enucleated organisms. It has not been possible yet to keep the enucleated organisms alive for a long time. However, further attempts are being made for nuclear transfer and for keeping the enucleated organisms alive for a longer time.

F. WORKSHOP

During the year under report the total number of requisitions received from all the departments of the institute was 612. Some of the major works which were taken up are mentioned below :

For Physics Dept.

1. Microwave crystal holders with guides and copper tank
2. Construction of one electromagnet of 1000 gauss is in progress.
3. One rotating table for the above magnet has been completed.

For Cosmic Ray

4. Modification of some accessories of the rectangular cloud chamber.
5. A large number of metal and glass G. M. counters.

For Nuclear Physics

6. Gamma-ray scattering apparatus.
7. Neutron and gamma ray source holders.

For Neutron Generator

8. Erection of Motor Generator : The generator has been mounted on a specially designed 2 ft. high perspex base and is coupled with the motor directly by a perspex tube.
9. Fabrication and assembly of the vacuum system including Wilson-type seals.
10. Rectifier assembly of Cockroft Walton Generator.
11. Corona shield for ion source supply.
12. Fabrication of Electrodes and their supports for positive ray discharge tubes.
13. Water circulating system for the diffiusion pump.

For Chemistry Dept.

14. For Vapour-phase Chromatography Apparatus : fabrication of modified designs of (*i*) thermal conductivity cells thermistor and wire types, and (*ii*) of column heating systems electrical and vapour bath types.

For Botany Dept.

15. G. M. counter casing including perspex shelf, and lead container for Beta counting system.
16. Construction of a 100 K.V. X-Ray unit is in progress.

Microbiology Dept.

17. The steam, water and gas connections to all the apparatus in the fermentation laboratory have been completed.

Zoology Dept.

18. One micromainpulator of an improved design has been constructed.

Besides making the above instruments repair and maintenance of various apparatus were carried out. Diesel pumping units at Shamnagar and Falta experimental sations, and the installation of Cobalt 60 source at Nilgunge were regularly inspected and maintained.

Refrigeration section :

The sector deals with the maintenance of Refrigerators, Deep-freezes and Air cooling systems in the different laboratories. Besides general repairs of and overhauling these machines, the section has completed air-conditioning installations in the following laboratories :

1. Neutron Generator's laboratory in two floors—10 ton water cooled units.
2. Glass House—underground floor—1.5 tons—Air cooled compressor.
3. Growth Room—2 ton—Air cooled compressor.

Arrangements are in progress to air condition (1) the new extensions of 5 rooms of the Plant Biochemistry laboratory by one 3 ton water cooled plant, (2) O° Cold Room and one General Laboratory room on the 1st floor, West Block for the Enzyme laboratory.

Apart from Air-conditioning Works, the department is constructing (*i*) one big Deep Freeze chamber to be maintained at –22°F, (*ii*) one refrigerated chamber for laboratory preparatrory work having arrangements for temperature variations.

The electrical shop besides the usual maintenance works has repaired many electrical machines, installed electrical power and light lines in various laboratories.

G. LIBRARY

The library contains 5364 volumes (2641 books and 2723 bound periodicals). During the year under report 349 volumes (161 books and 188 bound periodicals) were added to to the stock as gainst 209 volumes during the previous year. The total number of periodicals received was 223 (inland 65 and foreign 158) out of which 119 were subscribed and the remaining 104 were received either in exchange of Institute publications or as presentations. The library also received a large number of technical pamphlets, circulars, reprints, annual reports, etc. from various institutions and Government Departments, both inland 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. As a result of the increased working hours more readers not only from the Institute but also from outside institutions use the library particularlary during the evening hours.

According to rules the library is normally meant for Institute research workers. But provision has been made to admit, on the basis of reader's card, bonafide non-institute research workers and scientists for use of the Reading Room; during the year under report 54 reader's cards were issued to outside research workers.

The additional stack-room on the ground floor of the Library building which was made available to the library has been fitted up with steel racks with shelves of 814 running feet; it is being used for storing of the less important periodicals, Institute publications, etc. This has solved, for the time being, the problem of storing the increasing number of books and periodicals purchased for the library.

Due to the increase in the number of research staff in the Institute and to the growing number of outside readers who use the library, the volume of work in the library has increased considerably; to cope with the situation the library staff has been strengthened by the appointment of a junior assistant.

To meet the pressing demands of readers for supply of books and journals and to build up an up-to-date reasearch and reference library, it is necessary to increase the grant which is available to the library from the limited resources of the Instiute. After meeting the mounting cost of periodical subscriptions, specially of American origin, not much is left from the grant for the purchase of books, annual reviews, etc. However, efforts are being made to secure some non-recurring grants in addition to an increased recurring grant, for the purchase of important back numbers of periodicals and books of reference.

PUBLICATIONS

During the year under report Volume 23 (1959-60) of the Transactions of the Bose Research Institute was published. It has been decided that in future the Transactions will be published in four quarterly issues—March, June, September and December; Volume 24. No. 1 (March, 1961, issue) has already been published.

Efforts are being made to reprint some of the important and popular monographs of Sir J. C. Bose, which have been out of print for a long time. The reprinting of 'Response in the Living and Non-Living' has been taken up and is expected to be published in the near future. Other publication during the year was the Report of the Bose Institute for 1959-60.

APPENDIX A

I. Governing Body

Dr. Debendra Mohan Bose
Shri Sudhansu Mohon Bose
Shri Kedar Nath Chatterjee
Shri Saibal Kumar Gupta, I.C.S.
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 Nagchowdhury
Prof. Bhupati Mohon Sen, I.E.S. (*Retd.*)

II. The Council

Shri S. M. Bose
Shri K. N. Chatterjee
Shri S. K. Mandal
Prof. S. K. Mitra, F.R.S.
Shri A. N. Mitter
Dr. B. D. Nagchowdhury
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 Jitendra Nath Lahiri, M.P. (Representative of the Lok Sabha)
Prof. P. Parija, Vice-Chancellor, Utkal University,
Prof. K. Ahmed Chowdhury, Professor of Botany, Muslim University, Aligarh
} Scientists from outside West Bengal, nominated by Govt. of India
Shri Nandadulal Sreemany (Representative of the Calcutta Corporation)

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

APPENDIX B

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

Registrar : Shri S. K. Chatterjee, B.Sc. upto 28.8.60
Shri N. Das Gupta, B.Sc., B.L. from 29.8.60

Department of Physics

Head of the Dept. : The Director
Research Fellow : Dr. T. C. Bhadra, M.Sc., D.Phil.
Research Scholar : Sm. Anjali Chowdhuri, M.Sc. (upto 13.11.60)
Mary & Richard Keating Student : Shri Subhendu Kumar Dutta, M.Sc.
Govt. of India Shri Amalendu Nath, M.Sc. (up to 10.5.60)
Senior Scholar : Shri Arun Kumar Chatterjee (From 18.7.60)
Dept. of Atomic Energy, Junior Fellow : Shri Amalendu Nath, M.Sc. (from 11.5.60.

Department of Chemistry

Head of the Dept : Dr. P. K. Bose, D.Sc., F.N.I.
Research Fellow : Dr. A. Sen, M.Sc., Ph.D. (Leeds)
Dr. S. K. Roy, M.Sc., Ph.D. (Lond), D.I.C.
Dr. D. P. Burma, M.Sc., D.Phil.
Dr. S. P. Sen, M.Sc., D.Phil.
Dr. A. K. Barua, M.Sc., D.Phil.
Dr. A. G. Datta, M.Sc., D.Phil. (from 24.10.60 to 26.3.61)
Pool Officer-C.S.I.R. : Dr. A. G. Datta, M.Sc., D.Phil. (from 27.3.61)
Research Assistant : Dr. D. P. Chakraborty, M.Sc., D.Phil.
Dr. V. N. Gadgil, M.Sc., D.Phil.
Dr. S. B. Sarkar, M.Sc., Dr.rer.Nat.
Research Scholar : Shri Nirmal K. Sinha, M.Sc.
Shri B. K. Burman, M.Sc.
Shri S. K. Chakraborty, M.Sc. (upto 5.3.61)
Shri Radha Kanta Mandal, M.Sc.
Sm. Latika Danda, M.Sc.
Shri Kala Chand Das, M.Sc. (from 13.2.61)
Shri Rabindra Prashad, M.Sc. (upto 8.8.60)
Govt. of India Sm. Archana Sengupta, M.Sc. (upto 25.2.61)
Senior Scholar : Shri S. D. Bhattacharjee, M.Sc.
Sm. Shila Chowdhury, M.Sc. (from 13.2.61)
Shri Deb K. Mukherjee, M.Sc. (from 24.2.61)
Sir J. C. Bose Fellow, Calcutta University : Dr. K. K. Mukherjee, M.B.B.S.
National Institute of Sciences, Junior Fellow : Dr. (Mrs) Mira Sen, M.Sc., D.Phil.

Attached Worker : Dr. S. N. De, M.B., D.T.M., Ph.D. (Lond)
Dr. P. N. Das, M.B.B.S.
Prof. K. C. Basumallick, M.D., Ph.D. (Lond)
Dr. Madhuri Roy, M.Sc., D.Phil.
Sri S. P. Saha, M.Sc.

Department of Botany

Head of the Dept : Dr. K. T. Jacob, M.A., Ph.D. (on deputation leave)
Offg. Head of the Dept : Dr. Sunando Bose, M.Sc., Filosofic Doctor (Lund)
Research Fellow : Shri R. K. Basu, M.Sc.
Statistician : Shri A. K. Roychowdhury, M.Sc.
Research Assistant : Shri B. K. Datta
Shri A. T. Guhathakurtha
Shri A. K. Adhikari, M.Sc.
Shri V. S. Sarma, M.Sc.
Research Scholar : Shri Biswaranjan Banerjee, M.Sc.
Shri Sakhyasinha Sarkar, M.Sc.
Govt. of India Senior Scholar : Sm. Anima De, M.Sc.
Shri Josy Joseph, M.Sc. (from 29.6.60)
National Institute of Sciences-Senior Fellow : Dr. Smritimoy Bose, M.Sc., Ph.D.
Attached Workers : Shri D. K. Chakraborty, M.Sc.
Mrs. Mridula Datta, M.Sc.

Department of Microbiology

Head of the Dept : Dr. P. N. Nandi, M.Sc., Ph.D. (Lond), D.I.C.
Research Fellow : Shri K. L. Chowdhuri, M.Sc.
Research Assistant : Shri A. K. Mishra, M.Sc.
Shri P. P. Mukherjee, M.Sc.
Research Scholar : Sm. Ashalata Paul, M.Sc.
Shri Sankar Lal Chakraborty, M.Sc.
Sm. Gita Bhattacherjee, M.Sc.
Govt. of India Senior Scholar : Sm. Itu Sanyal, M.Sc.
Attached Worker : Shri Narendra Nath Shee

Department of Zoology

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

Workshop

Superintendent : Shri M. L. Chatterjee
Superintendent of Electricals, Electronics and Refrigeration : Shri S. R. Ghosh

Library

Librarian : Shri P. K. Chakraborty
Assistant Librarian : Sm. Asoka Dhar

STAFF APPOINTED UNDER GRANT-IN-AID SCHEMES

A. Department of Atomic Energy, Govt. of India

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

Scheme No. I. Cosmic ray investigation
Dr. M. S. Sinha, D.Sc., F.N.I. (*Research Officer*) upto 31.10.60
Shri Indralal Chakraborty*
Shri S. N. Sengupta, M.Sc. (*Junior Research Assistant*)
Shri N. Chowdhury, M.Sc. -do-

Scheme No. II. Nuclear Physics investigations with Neutron Generator
Shri A. M. Ghose, M.Sc. (*Senior Scientific Officer*)
Shri Bimalendu Mitra, M.Sc. (*Senior Scientific Assistant*)

Scheme No. III. Investigations on the induced mutagenesis in crop plants using X-rays and Radioactive isotope
Shri Amal Kumar Bose, M.Sc. (*Junior Research Assistant*)
Shri Balaram Mazumdar, M.Sc. -do-

B. Indian Central Oilseeds Committee

Inducing mutants with desirable economic characters in til, rape and mustard by X-ray
Investigator-in-Charge : Dr. S. Bose
Shri G. Gopalan Nair, M.Sc. (*Botanist*)
Shri P. R. Das Gupta (*Asstt. Botanist*)
Shri C. Banerjee, M.Sc. (Tech) *Chemical Asstt.*

C. Indian Central Oilseeds Committee

Radiation mutation and improvement of groundnuts
Investigator-in-Charge : Dr. S. Bose
Dr. J. G. Bhowal, M.Sc., D.Phil., *Cytogeneticist* (up to 21.3.61)

D. Indian Central Jute Committee

Radiation mutation in jute
Investigator-in-Charge : Dr. S. Bose
Shri Asoke Das, M.Sc., *Cytologist*

E. Indian Central Oilseeds Committee and Indan Central Jute Committee

Installation of Cobalt 60 source
Shri D. N. Kumar, B.Sc., D.I.C., *Physicist*

F. Indian Lac Cess Committee

Constitution of lac resin
Investigator-in-Charge : Dr. P. K. Bose
Shri S. K. Chakraborty, M.Sc., *Senior Chemist*
Sm. Parul Chakraborty, M.Sc., *Junior Chemist*

*Died on 13-6-60.

G. Council of Scientific and Industrial Research Schemes

(*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
Shri Jyotirmoy Dutta, M.Sc., *Senior Research Fellow*
Md. Maminul Haque, M.Sc., *Junior Research Assistant*

(*ii*) Scheme on '*Chemistry of Indian plant resin*'

Investigator-in-Charge : Dr. P. K. Bose
Dr. S. P. Raman, M.Sc., D.Phil., *Senior Research Fellow* (up to 23.7.60)
Shri K. C. Padmanabhan, M.Sc. -do-

(*iii*) Scheme on '*Biosynthesis and Metabolism of Gibberellic acid*'

Investigator-in-Charge : Dr. S. P. Sen
Dr. A. N. Lahiri, M.Sc., Ph.D. *Senior Research Fellow* (up to 30.9.60)
Sm. Anima Datta, M.Sc. -do-

(*iv*) Scheme on '*Genetical and biochemical studies of induced mutants of Aspergillus niger*'

Investigator-in-Charge : Dr. P. Nandi
Sm. Arati Sarkar, M.Sc. *Junior Research Fellow*

(*v*) Scheme on '*Isolation in pure state of organic compound of biological importance including antibiotic and other drugs by countercurrent distribution technique and their characterisation*'

Investigator-in-Charge : Dr. P. Nandi
Shri Saroj Bandhu Ghosh, M.Sc. *Senior Research Fellow*

(*vi*) Scheme on '*Mycology and plant pathology*'

Investigator-in-Charge : Dr. P. Nandi
Dr. A. C. Das, M.Sc., D.Phil. *Senior Research Fellow*

(*vii*) Scheme on '*Production of antibiotic substances by streptomyces spp.*'

Investigator-in-Charge : Dr. P. Nandi
Shri Rabindra K. Sinha, M.Sc. *Junior Research Assistant*
Shri Pritindra Nath Naha, M.Sc. -do-

(*viii*) Scheme on '*Exchange of genetic material in microorganisms by transformation and transduction*'

Investigator-in-Charge : Dr. P. Nandi
Sm. Kalyani Chatterjee, M.Sc. *Junior Research Fellow*

(*xi*) Scheme on '*Microbial synthesis of proteins in relation to the biogenesis of nucleic acids*'

Investigator-in-Charge : Dr. D. P. Burma
Sm. Maharani Chakraborty, M.Sc. *Senior Research Fellow* (up to 30.4.60 and the scheme is kept in abeyance)

LIST OF PUBLISHED PAPERS 1960-61

Title of papers	Authors	Where published	Reference
1. The effect of nucleases on photosynthetic Co_2 fixation	B. B. Biswas S. P. Sen	Experientia	16:135 (1960)
2. Inhibition of Rhizobium and Azotobacter by pure antibiotic substances	S. P. Sen P. Nandi	Trans. Bose Res. Inst.	24:Part I
3. Absolute measurement of thermal neutron absorption in chlorine	A. M. Ghose	Ibid	24:Part I
4. The ultraviolet absorption spectra of some natural coumarins.	D. P. Chakraborty S. K. Chakraborti	Ibid	24:Part I
5. Studies on the Nucleoproteins of *Nostoc Muscorum*	B. B. Biswas	Ibid	24:Part I
6. On the antibiotic properties of some natural coumarins	D. P. Chakraborty Mira Sen P. K. Bose	Ibid	24:Part I
7. Spasmodic evening fall of the leaf of *Mimosa pudica*	B. K. Dutta A. Guhathakurtha	Ibid	23:91 (1960)
8. The effect of unsaturated lactone (δ-Hexeno) on the esterfication of inorganic phosphate in the tissues of higher plants	S. K. Roy Madhuri Dutta	Ibid	23:85 (1960)
9. The effect of certain metabolic inhibitors on absorption and translocation of phospate in the seedlings of higher plants	S. K. Roy Madhuri Dutta	Ibid	23:81 (1960)
10. The effect of pH on absorption and translocation of phospate in seedlings of higher plants	S. K. Roy Madhuri Dutta	Ibid	23:75 (1960)
11. Utilization of carbon sources by some higher fungi	A. P. Dasgupta P. Nandi	Ibid	23:47 (1960)
12. The use of countercurrent distribution for the characterization of Streptomyces antibiotic	S. B. Ghosh G. P. Sen P. Nandi	Ibid	23:51 (1960)

Title of papers	Authors	Where published	Reference
13. Effects of gamma radiation on nitrogeneous end products in urine of albino rats	P. N. Das, J. Dutta K. Mukherjee C. Chanda	Proc. Nat. Inst. of Sciences	26:B:I (1960)
14. Antibiotic substances from a strain of *Bacillus Subtilis*	M. Purakayastha P. Nandi	Jour. Applied Bacteriology	23:I (1960)
15. Mechanism of action of Transketolase (*i*) Properties of the crystalline yeast enzyme	A. G. Dutta E. Racker	Jour. Biol. Chem.	236:617 (1961)
(*ii*) The substrate-enzyme intermediate	Ibid	Ibid	236:624 (1961)
16. Electrophorese sur papier de quelques ions de metaux dans des solutions de sulfates	H. C. Chakraborty	Jour. Chrom.	5:2:121 (1961)
17. Paper electrophoresis of metal ions in bivalent acids and their salts	H. C. Chakraborty	Jour. Elect. Chem.	(in press)
18. Comparison of the Lactoglobuins of Buffalo's milk and Cow's milk	N. K. Sinha A. Sen	Nature	190:4772:343
19. Recent physiological and cytological investigations on plants with motile organs	D. M. Bose	Proc. Nat. Inst. of Sciences	26:B: (1960)
20. Recent studies in India on the application of radiation and radioactive isotopes to mutation induction and to metabolic processes in plants and microorganisms	Report on Work done at Bose Inst.	Science and Culture	26:150 (1960)
21. On the constitution of Mesuol, the bitter antibiotic principle of *Mesua Ferrea Linn.* Part I	D. P. Chakraborty P. K. Bose	Proc. Nat. Inst. Sci.	26:I (1960)
22. Survey of Antibitotic producing bacteria from the soils of India and Indonesia	M. Purakayastha P. Nandi	Antibiotic Chemotheraphy	10:4:242-48
23. New triterpenoid sapogenins from the fruits of *Barringtonia acutangula* Gaertn	A. K. Barua P. C. Maiti S. K. Chakraborti	J. Pharm. Science	50:(1961) (in press)