REPORT

OF

THE BOSE INSTITUTE

FOR

1961–62

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

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REPORT OF THE BOSE INSTITUTE

1961-62

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 Institute 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.

Obituary

The Bose Institute suffered a grieveous loss by the passing away on February 27, 1962 of Shri S. M. Bose, one of the group of devoted helpers whose assistance enabled Acharya Jagadish Chandra Bose to found the Bose Institute and to carry on its administration. He was the Secretary to the Governing Body till the day of his passing away. After the death of the Founder in 1937 it become necessary to divide the responsibility which till then had devolved on the Governing Body, of being the Trustees as well the Executive body of the Bose Institute, by the creation of a new Council on which the day to day administration of the Council was vested. Shri S. M. Bose was largely responsible for framing the new set of Regulations and Bylaws on which the present administration of the Bose Institute is based. His dealth has been mourned by the members of the staff, the Governing Body and the Council of the Bose Institute.

Governing Body

Five meetings of the Governing Body took place during 1961-62. Besides dealing with routine matter, the Governing Body, on the request of the Collector, 24-Parganas approved

a draft agreement for the acquisition of about 41.62 acres of land at Dohania-Homaipur, Barasat for the erection of the Jagadish Chandra Centenary Memorial Laboratory and Experimental Station. It authorized the Director and Shri K. N. Chatterjee member of the Governing Body to execute and register the draft agreement on behalf of the Governing Body.

Council

Three meetings of the Council were held during the year.

Some of the principle items considered by the Council during the year are given below.

i) *Amendment of the existing Regulations and Bylaws of the Bose Institute*—The draft amendments prepared by the Council and sent to the Government of India for consideration in July 1956, were returned by the Ministry of Scientific Research and Cultural Affairs with suggestions for minor modifications. The Council at the meeting held on 29th April 1962 decided to postpone their consideration till the Reviewing Committee appointed by the Government of India had made their recommendations, which may involve some further changes in the amended Regulations and Bylaws.

ii) *Reviewing Committee*—The Ministry of Scientific Research and Cultural Affairs in a letter No. 13(3)/61-SR.II. dated 1st March 1962, have appointed a Reviewing Committee consisting of the following to review the work of the Bose Institute.

1. Dr. D. N. Wadia, F.R.S.
   Geological Adviser, Dept. of Atomic Energy,
   New Delhi .. .. *Chairman*
2. Prof. S. N. Bose, F.R.S.
   National Research Professor in Physics,
   Calcutta .. .. *Member*
3. Dr. H. Santapu,
   Chief Botanist,
   Botanical Survey of India,
   Calcutta .. .. *Member*
4. Dr. B. C. Guha,
   Professor and Head of the Dept. of Applied Chemistry,
   University College of Science and Technology,
   Calcutta .. .. *Member*
5. Dr. K. Mitra,
   Director, Jagadish Bose National Science Talent Search,
   Calcutta .. .. *Secretary*

The terms of reference for the Reviewing Committee are as follows:

(i) To review the work of the Bose Institute, taking into consideration the purpose for which it was founded and the expansion which has taken place since then specially after 1948, when the Vallarta Committee made its recommendations.

(ii) To recommend changes, if any, which may be considered desirable for better achievements of the purposes of the Institute. The question of expansion of activities of the Institute will also be considered by the Committee who will recommend the amount of financial assistance needed from the Central Government to continue existing services as well as to initiate fresh lines of investigations in the Institute.

The Director with the Heads of the Departments have prepared a Note for the consideration of the Reviewing Committee. It contains (i) a progress report on the administration of the Institute, (ii) research activities of the different departments of the Institute since 1948, after the visit of the Vallarta Reviewing Committee had made their recommendations, (iii) Proposal for future expansion of the research activities of the Institute with an estimate of the amount involved in non-recurring and recurring expenditures.

(iii) *Grants-in-aid to the Bose Institute during the Third Five Year Plan period*—The Ministry intimated that a total grant up to Rs. 72.00 lakhs has been allocated to the Bose Institute for the period 1961-62 to 1965-66.

(iv) *Revision of the scales of pay of the staff of the Bose Institute*—In April 1961, the Director was requested by the Council to draw the attention of the Ministry to the necessity of introducing revision of pay scales of the employees of the Bose Institute, taking into consideration the scales of pay in other All India Research Institutes of equal standing.

After some correspondence and personal discussions, the Ministry in their letter dated February 27, 1962 forwarded to the Director a revised pay scale for the employees of the Bose Institute and suggested the same may be placed before the Council for consideration and approval.

In a subsequent letter dated March 16, 1962 the Ministry intimated that the Government of India have decided to agree to the scale of pay for various posts under the control of the Institute being revised by the Council as indicated in an attached sheet.

The principle on which the pay scale was to be revised is as follows:

A. *Head of the Department, Senior Research Fellows, Research Fellows (Corresponding to Professors, Readers and Lecturers respectively in the Universities)*:

Revised U.G.C. pay and allowances, as adopted by the Calcutta University.
Date of effect: as adopted by the Calcutta University.

B. *All other staff:*

Scales of pay and allowances will be revised on the same lines on which the existing scales have been translated into revised scales under the Council of Scientific and Industrial Research. In effecting the revision, the revised scales in respect of comparable scales in the Council of Scientific and Industrial Research will be kept in view. C.A. and H.R.A. will be allowed as under the C.S.I.R.

Date of effect: 1-4-60, except for those posts agreed to be upgraded viz., Office Superintendent, Workshop Superintendent and Librarian which will take effect from date of order.

The proceedings of the Symposium has attracted a great deal of attention in India and abroad.

Nilratan Sircar Centenary Celebration

The scientific session in connection with the Centenary Celebration was held in the premises of the Bose Institute on October 3, & 4, 1962, its programme included addresses and scientific papers on many topics of scientific, medical and public health interest.

Study abroad and Deputation

1. Shri Chiranjib Banerjee M.Sc., Chemical Assistant, in a scheme financed by the Indian Central Oilseeds Committee, was awarded a scholarship to work in the Deutsche Institut fur Fettforschung, Munster, West Germany.

2. Shri Saroj Bandhu Ghosh M.Sc., Senior Research Fellow, in a C.S.I.R. scheme was awarded a fellowship offered by the Purdue University.

3. Dr. S. P. Sen, Research Fellow in the Department of Chemistry has been granted leave for one year with effect from 16th August 1961 to enable him to accept the post of Reader in Botany, Kalyani University and to keep a lien on his post.

4. Dr. S. K. Roy, Senior Research Fellow in Plant Physiology has been granted leave for one year with effect from 2nd April 1962 to enable him to accept an appointment as Senior Technical Officer—Grade I under C.S.I.R. and to keep a lien on his post.

Grants for study abroad

Grant for Rs. 2000.00 out of Sir J. C. Bose Trust Fund was made available to Shri Saroj Bandhu Ghosh in partial defrayal of his cost of passage abroad.

Degree awarded

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

1. Shri R. K. Basu—Department of Botany—D.Sc. degree.
2. Shri N. K. Sinha—Department of Chemistry—D.Phil degree.

Visitors

The following visited the Institute during 1961-62:

(1) Professor John Couch, University of North Carolina, U.S.A. (2) Dr. Ernest Ball, University of North Carolina State College, U.S.A. (3) Prof. Robert H. Burris, Dept. of Biochemistry, University of Wisconsin, U.S.A. (4) Prof. P. Z. Beztnkov, and Dr. Jaruv, Institute of Oceanology, U.S.S.R. (5) Prof. F. N. Russanov, Director and Prof. P. T. Lapin, Deputy Director, Botanical Gardens of U.S.S.R., Taschkent. (6) Prof. Jacques Benoit, Dept. of Histophysiologie of College de France, Paris. (7) Prof. F. Sandberg, Royal Pharmaceutical Institute, Stockholm. (8) Prof. P. P. Cohen, University of Wisconsin. (9) Prof. S. Chandrasekhar, University of Chicago. (10) Prof. Sajiro Makino, Dean of Faculty of Science, Hokkaido University, Japan. (11) Dr. Robert D. Osler, the

Rockefeller Foundations, New York. (12) Dr. J. W. Dumond, Professor, California Institute of Technology, Pasadena. (13) Prof. J. P. Nitsch, Associated Director, Phytotron, Gif-sur-Svettee, France. (14) Dr. F. Gros, Dept. of Cellular Biochemistry, Pasteur Institure, Paris. (15) Dr. J. Y. Wilson, Dept. of Botany, University of Ghana, Ghana. (16) Dr. Roland Legendre, Institute of Zoology, University of Madagascar. (17) Prof. E. Ruggles Gates, F.R.S. (18) Dr. Paul Oman, Director, Far Eastern Regional Research Office, U.S. Department of Agriculture. (19) Prof. N. Sabagearu, Institute de Biologie, Bucarest, Roumania. (20) Prof. Robert Havermann, Physio-Chemical Institute, Humbold University, Berlin.

Special Lectures and Seminars

(1) Dr. F. Gros, Department of Cellular Biochemistry, Pasteur Institute, Paris gave a talk on 'Role of Messenger RNA in protein synthesis'.

(2) Dr. Ernest Ball of the North Carolina State College, gave a talk on 'Sterile Culture in Experimental Morphology'.

(3) Prof. Jacques Benoit of the College of France, gave a talk on 'The action of light on the genital activities of birds'.

(4) Under the auspices of the National Institute of Sciences of India, a Symposium was held on 'The History of Sciences of India'.

(5) Prof. P. P. Cohen, University of Wisconsins, gave a talk on the 'Biochemical Aspects of Metamorphosis'.

(6) Prof. Sajiro Makino, Dean of the Faculty of Science, Hokkaido University, gave talks on (i) 'Radiation effects on chromosomes of Grasshopper' and (ii) 'A study of human chromosomes'.

(7) Dr. J. W. Dumond, Professor, California Institute of Technology, gave a talk on 'Spectroscopy of Nuclear gamma rays'.

(8) Prof. J. P. Nitsch, Associate Director, 'Phytotron' France, gave a talk on 'Naturally occurring growth substances'.

(9) Dr. G. P. Talwar, All India Institute of Medical Sciences, New Delhi gave a talk on 'Differential metabolism of brain'.

(10) Dr. Ramen Poddar, Dept. of Biophysics, Saha Institute of Nuclear Physics gave a talk on 'Molecular mechanism of mutation'.

New Appointments and Awards

The following appointments were made during the period under review.

A. *Research Fellow*

1. Dr. B. B. Biswas in the Department of Chemistry with effect from 23-9-61.

B. *Research Scholar*

1. Sm. Itu Sanyal, M.Sc. in the Department of Microbiology with effect from 1-2-62.
2. Sm. Roma Ghosh, M.Sc. in the Department of Physiology with effect from 15-6-61.
3. Sm. Susweta Sen, M.Sc. in the Department of Chemistry with effect from 3-4-61.

C. *Government of India Research Training Scholar*

1. Sm. Shila Sen, M.Sc. in the Department of Botany with effect from 3-4-61.
2. Sm. Kalyani Bhattacharjee, M.Sc. in the Department of Microbiology with effect from 14-6-61.
3. Shri Ranjit Roychowdhuri, M.Sc. in the Department of Chemistry with effect from 3-4-61.
4. Sm. Roma Barat, M.A. in the Department of Microbiology with effect from 1-2-62.

D. The following three scientists have been appointed by the C.S.I.R. to the Scientists Pool and under the administrative control of the Director, Bose Institute.

1. Dr. A. G. Dutta, M.Sc., Ph.D. in the Department of Chemistry.
2. Dr. A. C. Das, M.Sc., Ph.D. in the Department of Microbiology.
3. Dr. (Mrs.) Maharani Chakraborty, M.Sc., D.Phil. in the Department of Chemistry.

Resignations

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

1. Shri S. K. Dutta, M.Sc., Mary and Richard Keating Student from 31-7-61.
2. Shri Josy Joseph, M.Sc., Govt. of India Research Training Scholar from 12-10-61.
3. Shri S. D. Bhattacharjee, M.Sc., Govt. of India Research Training Scholar from 3-1-62.
4. Shri R. K. Mandal, M.Sc., Research Scholar from 31-12-61.
5. Shri V. S. Sharma, M.Sc., Research Assistant from 28-2-62.
7. Dr. D. P. Burma, M.Sc., D.Phil. Research Fellow from 8-1-62.
8. Shri A. K. Bose, M.Sc., Junior Research Assistant in D.A.E. Scheme from 17-1-62.

Seminars

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. Enzymatic mechanism of Ketol group transfer	Dr. A. G. Datta
2. Biosynthesis of RNA and its role in protein synthesis	Shri R. K. Mandal
3. Ribosomes (Microsomes) as seat of protein synthesis	Dr. J. J. Ghosh, Calcutta University
4. Enzymatic synthesis of nuclear RNA	Dr. B. B. Biswas
5. Some biochemical aspects of bacterial genetics	Dr. D. P. Burma
6. Some fundamental aspects of Bacteriophage genetics	Dr. M. Chakraborty

RESEARCH ACTIVITIES

(Non-technical Report)

Cosmic rays

The studies of mu-meson interaction commenced last year at the D.V.C. underground power station at Maithon were continued. A large multiplate cloud chamber of dimensions $(90\times80\times50)$ cm^3 was used to record mu-meson induced events. About 16,000 photographs were analysed for the following phenomena.

(i) production by high energy mu-mesons of (a) penetrating showers and (b) secondary electrons.

(ii) occurrence of parallel multiple penetrating particles underground.

After eliminating the interaction due to the presence of pi-mesons, the cross section for the production of penetrating showers by mu-mesons of average energy 32.6 Gev was found to be $\sigma_\mu = (3.1\pm1.4)\times10^{-30}$ cm^2/nucleon. This value agrees satisfactorily with the cross section calculated on the basis of interaction between the electromagnetic field of the mu-meson and the meson field of the nucleon.

The energy spectrum of the knock-on electrons upto 1.5 Gev agrees within statistical errors with the calculated spectrum on the basis of the well known electrodynamic theory of mu-meson electron collision.

By comparison of the present observations with those of other observers it appears almost certain that the penetrating particles underground are remnants of extensive air showers initiated by high energy cosmic ray particles near the top of the atmosphere.

Gamma rays

Experimental studies of small angle coherent scattering of cobalt-60 gamma rays (1.33 and 1.17 Mev) have been made with a modified arrangement of shadow cone technique. The object of the investigation has been a study (i) the distribution of coherent scattering cross section at small angle from 2° to about 10°, (ii) the energy dependence of the Rayleigh scattering cross sections and (iii) the Z dependence of (Z^n) of the Rayleigh scattering.

The coherent scattering cross section has been obtained by subtracting the Compton scattering cross section (determined theoretically from the Heisenberg Bewiloguea formula) from the experimentally determined total cross section. As the Rayleigh coherent scattering is predominant at these energies and angles of scattering (contributions of Thomson and Delbruck scattering being very negligible), the entire scattering cross section so obtained is attributed to Rayleigh scattering.

Neutron Physics

The positive ray neutron generator tube in which a deuteron flux impinges on a tritium target is now working on a regular operational schedule.

Using an accelerating potential of 150 kV it is now possible to obtain a steady neutron flux of 10^9 per second, of energy 14 Mev.

A directional detector of neutron flux has been devised, in which the cross ratio i.e., the ratio of the detector efficiences for neutrons incident parallel respectively perpendicular to the detection axis, is an increasing function of the bias used in the output discriminator. A spherically symmetrical neutron counter made by polishing scintillating plastic rod to spherical shape has been constructed.

Two sets of investigations have been taken up, (i) measurement of total cross section of nuclei for fast (14 Mev) neutron and (ii) measurement of the non-elastic cross section for fast neutrons.

Radioactive Fallout

Early this year a survey of the radio activity of dust particles deposited on plants with large leaves was carried out by the Bose Institute over an area extending from Falta to the south to Shamnagar to the north. A quite appreciable rise in the count of beta partilcles over the background count was recorded. Chemical separation of the radioactive elements present in the dust as well as the intensity of its beta radiation is being carried out.

With the commencement of rain showers, associated with the western disturbances which gradually have passed over to monsoon rains, a new series of measurements on radio activity in rain was commenced from mid-April and is being still continued.

Gas Chromatography

With the help of the gas chromatography apparatus designed and constructed in the Bose Institute workshop, it has become possible to separate the low boiling fractions of gasoline into eighteen finer fractions containing C_3 to C_6 components. Some of these components have also been identified.

With the same apparatus some gas samples supplied by the Assam Oil Co. Ltd., have also been analysed. The samples were found to contain C_1 to C_5 hydrocarbons along with CO_2 and water-vapour.

Paper Electrophoresis

Separation of fourteen different natural coumarins was achieved with the technique of paper electrophoresis using NaOH solutions of different dilutions as the electrolyte.

A new simple method has been developed for the detection of thyroxine and allied compounds in connexion with the study of metamorphosis of tadpoles. It is sufficietly sensitive for detection of thyroxine and di-iodotyrosine of the order of 0.05 ug to 0.5 ug. The method is based upon the decomposition of iodated compounds under ultraviolet irradiation. If on a paper sprayed with starch solution the iodated compounds is introduced then blue coloration will appear at these places when irradiated with ultraviolet light.

Milk Protein

The occurrence of genetically different types of β-lactoglobulin and α-lactalbumin in the milk of Indian Zebu cattle has been subjected to a detailed study. While genetic polymorphism of β-lactoglobulin is general among the cattle of different countries, that of α-lactalbumin has been observed in two breeds of African Zebus, the Nigerian White Fulani

and the Boran cattle of Kenya but not in Icelandic, British, Danish and other Western breeds. The investigations carried out here with the milk of different breeds of Indian Zebu cattle show that α-lactalbumin polymorphism is a general phenomenon in these animals and its incidence obeys the Hardy-Weinberg law of genetic equilibrium. Comparison of these results with those obtained by others for African and Western breeds lend support to the view that the African Zebu cattle probably originated from Indian Zebu taken to Africa by nomadic people.

Comparison of the non-casein proteins of zebu milk and buffalo milk in paper electrophoresis has shown that the patterns of zebu milk containing α-lactalbumin-A and β-lactoglobulin-B are almost identical to those of buffalo milk. Thus, α-lactalbumin polymorphism and existence of combinations such as (α-A, β-B) in Zebu milk rule out the possibility of differentiation between cow and buffalo milk in a mixture.

Studies on the physico-chemical properties of the cow α-lactalbumin-A and buffalo α-lactalbumin show that the two proteins, as in the case of cow β-lactoglobulin-B and buffalo β-lactoglobulin, are almost indistinguishable in crystalline form, and have similar properties in electrophoresis, sedimentation and ultraviolet absorption. The molecular weight of buffalo α-lactalbumin has been found to be 16,200 g/mol, almost the same as that of cow α-lactalbumin.

Continuing similar studies with non casein proteins of goat's milk, it has been shown that these proteins are not subject to any variation due to genetic factors. Goat milk β-lactoglobulin has been obtained in a new form as salt free monoclinic crystals and α-lactalbumin has been isolated and crystallized in rhombohedral form for the first time.

Plant Chemistry

Survey of saponin bearing plants with a view to finding out new starting materials for the preparation of hormones has led to the isolation of a number of new sapogenins. New types of nitrogenous compounds have been isolated from a plant.

Biochemistry

Phytin complex from coconut milk

While isolating RNA from coconut milk it was observed that another unknown substance instead of RNA was precipitated.

It was isolated and identified as phytin (hexa-phospho-inositol). With the help of radioactive phosphorus it was found that phytin plays an intermediary role in phosphate conservation in coconut milk.

Triose phosphate dehydrogenase activity in different organs revealed that in the root the specific activity was higher compared to that in the cotyledons and hypocotyls of mung bean seedlings in 27 hours growth, whereas phosphohexose isomerase activity was highest in the cotyledons.

The role nucleic acids in biosynthesis

Evidence on the possible participation of RNA or oligonucleotides in CO_2 fixation was indicated, when CO_2 was fed to the spinach leaves for a short period of two minutes in

light. DNAase and RNAase were effective in reducing CO_2 fixation capacity of isolated chloroplasts showing that the nucleic acids participated in the process of CO_2 fixation.

Ribonucleic acids (RNA) which are the counterparts of the cell's genetic material deoxyribonucleic acid (DNA), are of various types. All the mechanisms of synthesis of the ribonucleic acids are being studied in this laboratory. An enzyme or enzyme system capable of synthesizing soluble RNA has been found to be present in the cell free extracts of the microorganism *Azotobacter vinelandii*. Another enzyme which has already been reported to be present in the same microorganism is found to synthesize a class of RNA, tentatively known as "Messenger" RNA. This RNA is supposed to carry the genetic message from the DNA present in the nucleus to the ribosomes of the cytoplasm, which are the sites of protein synthesis. The enzyme has now been shown to catalyse the formation of artificial polyribonucleotides, the homo and copolymers. The genetic aspects of the enzymes formed due to adaptation on substrates are also being studied with another microorganism *Lactobacillus arabinosus*.

A number of viruses of *Azotobacter vinelandii* have been procured and are being propagated to study the effect of viral DNA on the protein synthesizing machinery of the host microorganism.

Plant neoplastic tissues

For a better understanding of the process of transformation from normal tissue to neoplastic one, it seems desirable to have three types of tissue strains growing in *in-vitro* culture under identical conditions. Habituated tissue strain represents a transitional stage between normal and neoplastic tissue. Normal and neoplastic tissues of two plants species, namely, Hollyhock and Coreopsis, were successfully isolated and grown in synthetic media. Repeated attempts to isolate habituated tissue strains of these species failed, indicating a difference between normal tissues of these species and tissues of plant species reported to be easily adapted to media which do not support the growth of normal tissue.

Normal tissue of *Vicia faba* was successfully isolated and maintained in culture using the modified synthetic medium. The optimum requirements of the tissues are under study.

Comparative study of normal and crown gall tissues of Coreopsis showed no qualitative difference in their response to different carbon and nitrogen sources.

Normal tissues maintained in culture were found to have a lower rate of growth than that of the corresponding abnormal tissues. The growth stimulatory substances used accelerated the growth rate of normal tissues but failed to bring out their level to that of the corresponding abnormal tissue.

Plant Physiology

The electrical pulsations accompanying the transmission of excitation in different parts of pulvinus petiole tissue of *M. pudica*, *B. sensitivum*, *D. gyrans* have been studied in detail, during the previous year. In Mimosa the transmission of excitation takes place in three velocity groups: (i) fast, (ii) medium, (iii) slow waves. It was reported before, that an excitation starting from a stimulated leaflet of *M. pudica* after tranversing the petiole when it reaches the pulvinus, causes (a) contraction of the pulvinus and (b) transmission of a

reflected wave from the pulvinus along the petiole. This reflected wave consists of a fast biphasic component which is transmitted with almost instantaneous velocity followed by a medium 'm' wave. This year a detailed examination of the transmission of the excitation from the petiole through different portion of the pulvinus has been made. When the excitation reaches the middle of the pulvinus a pulvinar contraction results which is accompanied by a sharp negative wave of excitation. It is supposed that the biphasic hydrostatic wave observed in the petiole following pulvinar contraction is due to the sharp negative pulse.

J. C. Bose had already noted that the leaflet of *Biophytum sensitivum* when stimulated gave rise to multiple responses both mechanical and electrical. Due to the limitation of his galvanometer, the multiple electrical impulses could not be satisfactorily recorded. Using an electrical pen recorder it has been observed that a single stimulus given on the top of a leaf gives rise to a series of multiple responses up to 20 in number; these electrical responses travel in pair along the line of the midrib; one of them with medium velocity was of the nature of the 'm' wave in the Mimosa, and the other wave was of the nature of an excitation wave due to hydrostatic transmission similar to what has been observed in Mimosa.

J. C. Bose had observed that rhythmic pulsatory activity was not confined to the pulvini of *Desmodium gyrans* and a few other plants similar to those described above; he was able to show their occurrence in the cells of some of the so called nonmotile plants. With the help of the pen recorder whose sensitivity was magnified 20 times by a mirror arrangement spontaneous pulsations in different plant organs such as stem, root, petiole etc., have been observed. Arrangement is being made to record them photographically. The correlation of such irregular cellular pulsations with other cellular activities of the plant is being investigated.

In earlier reports the occurrence of endogenous biological rhythm in the movement of the main pulvinus of *M. pudica* was reported. It has also been noticed that the pinnules of *M. pudica* leaflets show a nyctinastic movement, remaining open during the day and closing at night. Automatic arrangement has been devised for recording the movement of the pinnules during 24 hours. In these pinnules the presence of an endogenous rhythm has been observed; the pinnules remain closed at night, opening out once in the early morning followed by a rapid closure, after which the pinnules open out fully and remain so during the day. This endogenous rhythm takes place even when the plant is kept in constant darkness for 24 hours.

Radiation genetics:

As reported last year the yield behaviour and morphogenical characteristics of plants of economic importance grown from irradiated seeds are being studied in successive generations. The sources of radiation used were X-rays, gamma radiation from the isotope Cobalt-60, beta rays from the isotopes Phosphors-32 and Sulphur-35. In addition the effect of several chemical mutagens has been investigated.

The two oilseeds species Sesamum and Brassica have been under investigation since 1950. Both dominant and recessive mutants of economic importance like increase in number of fruits, weight of seeds, oil content, its keeping quality and early flowering.

Sesamum: Further studies have been made on the whiteflowered mutant (WFM_1) and the small seeded mutant (SSM_1 and SSM_2), on the progeneis obtained by treatment of the seeds of mutants WFM_1 and SSM_1 with β-rays from radioactive isotopes phosphorous-32 and sulphur-35. No significant inprovement in their economic character or flowering behaviour were observed. The behaviour of progenies from seeds of T-12 and T-16 exposed to gamma radiation in 1959 in the Nilgunge field were studied.

Brassica: Similar studies were made on selected strains of Brassica under irradiation. Cytological observations on progenies of treated seeds of meitotic irregularities such as multivalents, bridges and laggards have been continued. So far it has not been possible to correlate these irregularities with the morphological and yield characteristics of the plants raised from treated seeds.

Groundnuts: A number of previously isolated mutants like (i) bushy type mutant (var. Coimbatore), creeping and small leaf mutants (var. AK 10) and (ii) stunted and dwarf type mutant (var. Small Japan) were continued.

Oil content of some of the irradiated progenies as well as of some of the mutants have been carried out during the year. An interesting observation was made that the yield of oil from six replicated plots of dwarf mutant (var. Small Japan) fluctuated from replications to replication; the highest percentage being obtained from replications V and VI. This seems to suggest that environmental factors may play an important role in determining the oil content, though some variation due to segregation is to be expected and cannot be ruled out.

Gamma irradiation.—No abnormality was found in any of the plants in the G_1 and G_2 generations; there was some evidence, however, of a stimulating effect on plants (of all varieties) growing in bed VI (distance from source 27.5 m and dosages 36.52 r) as observed by the number of seeds per plants.

Jute.—The varieties Tall Mutant (T.M.), R-26, Chinsurah Green (C.G.) from *Corchorus olitorius* were selected for radiation mutation work in jute. Both X-rays and beta-rays from P-32 and S-35 were employed. The variety D.154 (Capsularis) was found to be more resistant to X-rays and beta-rays treatments.

The characteristics of the plants grown from treated seeds like early flowering, average heights and basal diameter in the varieties T.M., R-26, C.G. and D.154 decreased when treated with increasing doses of P.32. The same is true in general in treatment with S-35.

In varieties R-26 and D.154 treatment with 70,000 r of X-ray led to increase of average basal diameter and height. The average yield of wood and fibre per plant paralled the average height and basal diameter of the plants raised from treated seeds.

Certain morphological changes were observed in plants raised from seeds treated with P-32 and S-35. In the first generation plants with abnormal morphological characters such as stems with heavy branching, bifurcation of stem, fasciation of stem, double leaves,

forking of leaves, crinkling of leaves etc., were obtained but most of the characters do not persist in subsequent generations.

Mutants or aberrant plants with changed morphological characters like trailing plants of D-154 isolated after P-32 treatment are being studied in their third generation. Similar plants in the same variety have also been isolated by X-rays treatments. In another variety (R-26) some abberrant plants with twisted stem have been isolated in the second generation.

Chemical mutagenesis.—Studies on the effect of the chemical Dimethyl sulphate on T.M. jute plants was continued to the C_3 generation. A complicated segregation in stem colour mutant isolated in the C_2 generation was noted. Further selfing and crossing have been made on these plants in an effort to clarify the nature of the mutants involved.

Further observations were made on the T.M., D-154 in the C_2 (Ethylene immine treated) generation. A single D-154 plant with twisted stem isolated in C_1 generation gave rise to a (single) progeny having twisted stem. The nature and mode of inheritance of this aberrant is not clear.

Paddy: Comparative studies were made on an early flowering mutant of Rupsail (Aman) paddy isolated in 1959 from the second generation of treatment with radioactive phosphorous (P-32).

The control and the mutant were sown in beds on five different dates at intervals of fourteen days and given similar agronomical treatment. It was found that differences in the intervals between date of sowing and mean flowering dates between the control and the mutants was the greatest for the first date of sowing (May 23), and lowest for the last date of sowing (July 22). As the sowing time was delayed, the time taken for flowering diminished gradually, both in the control as well as in the mutant. In all dates of sowing the range of variance in the dates of flowering in the mutant was greater than that in the control.

Double kernel mutant.—Dry seeds of the variety Marichbati (Aus paddy) were treated with 75,000 r units of X-rays; in the second generation (X-2) a single plant (S.221) was isolated with abnormal morphology of flower and seed. The abnormal characters of the selected progeny has bred true for four generations. The number of kernels in each seed pod are two in the selection (S-221) against one in control. The mean length breadth and width of kernels were 5.40, 2.30 and 1.80 mm in the control as against 4.72, 1.60 and 1.60 mm in the mutants. Morphological abnormalities in the leaf characteristics were observed such as fused leaf mutant (S-275).

A selection S-1 was isolated in the year 1958 from the variety Marichbati after treatwent with P-32. The grains of the selection S-1 showed black coloured husk instead of golden yellow in the control. There was however no significant differences in height of plant, length of panicle, length of the flag leaf. There was however some difference in the number of the tillers and number of panicles between Marichbati and S-1.

Cytotaxonomical studies were continued on the somatic chromosomes in six species belonging to four genera, in the family of Malvaceae e.g., Gossypium, Sida, Malachra, Abutilon. Cytological studies were also carried out on 17 varieties of *Pisum Sativum*, to find out the

extent to which karotypic differences could account for their varietal dissimilarities. The somatic chromosome number of different varieties so far studied have been found to be fourteen. A detailed karotypic analysis of the different varieties reveal a basic similarity in the complements.

Microbiology

(1) *Production of antibiotic substances.* A broad-spectrum antibiotic substance was obtained from *Streptomyces sp.* strain $A_{11}475$, a gamma irradiated mutant. It showed a distinctly higher yield when fermented in suitable medium at 25°C. The antibiotic is basic in nature and reacts with acids to form salts. Salts of inorganic acids, e.g., HCl, H_2SO_4 etc., are highly soluble in water but are insoluble in organic solvents except methanol. The chemical analysis shows that it contains C, H and N and comparative assay of the compound showed that the antibiotic is probably a new one.

$Ac_2(435)$.—A broad spectrum antifungal antibiotic has been purified and characterised. The substance possibly belongs to the class of *tetraene* compounds and comparative biological assay shows that it is distinctly different from other known antifungal *tetraene* compounds. Chemical and physical properties are being studied and toxicity tests were undertaken.

(2) *Induced mutations in microorganisms.* Investigations on the effects of UV radiation on some species of *Aspergillus* were undertaken and the results indicate significant changes in mutant production under the influence of different dose levels: (a) *Aspergillus chevalieri.*—biochemical and morphological mutants were obtained of which there were 29 difficient mutants among the former and three morphological mutants extremely different from the parent among the latter. (b) *Aspergillus oryzae*—a strain producing diastase enzyme was subjected to UV irradiation and mutants with increased diastase production were isolated. (c) *Aspergillus regulosus*—mutants were produced by UV irradiation and studies on the genetics of the organism were undertaken. Nitrogen mustard, a chemical mutagen, was used for raising mutants of *A. chevalieri* and *Rhizopus oryzae.*

(3) *Microbial Genetics.* Response of a thiamineless strain of *Neurospora crassa* to the effect of bromo-uracil has been studied. Bromo-uracil at concentrations of 1,4,6,8 and 12 ug/ml of medium was used and spore suspensions plated out. Wild type reverse mutations having varying growth characteristics appeared. A new ascomycete, *Sordaria* sp. occurring in soil has been taken up for genetical studies. This strain grows well in minimal medium and forms eight linear ascospores.

(4) *Studies on Rhizobium bacteriophage.* Six strains of bacteriophage active against *Rhizobium* spp. were isolated, purified and tested. Induction of resistance was observed among the host bacteria and strains resistant to bacterophage were isolated. Leguminous seeds were innoculated with phage-resistant bacteria and field experiments were conducted. Resistant strains stimulated high degree of nodule formation and consequent yield of the crop.

(5) *Fungal species in air.* Air acts as a good dispersing agent for microbes which may be carried from one place to another. A study of the spore composition of air has been undertaken in order to make an assessment of the important species generally transmitted through air at three localities of West Bengal. Methods used consist in exposing 1.5% malt agar plates at the selected sites for one minute, followed by incubation in the laboratory at 30°C–32°C. Number of colonies per plate and percentage of occurrence of a particular fungal species are recorded.

(6) *Industrial Fermentation.* Development of improved citric acid producing strains of *Aspergillus* niger was attempted in this Laboratory. A high-yielding mutant strain of *Aspergillus niger* (C110) was obtained by irradiation with gamma rays and UV irradiation successively. Results indicate that out of a total of 500 isolates studied 12 mutants acquired the capacity to produce very high quantity of citric acid, in the laboratory media. A high-yielding mutant was recorded to produce 138% more citric acid over the original parent strain.

(7) *Transformation in microorganisms.* Transformation in fungi was studied with a wild strain of *Aspergillus niger* (V_{35}) as donor and a mutant strain H_{14} of the same organism as the recipient one. DNA of the fungal mycelium of the wild strain V_{35} was prepared according to Hotchkiss' method with slight modification. After treatment transformants were scored from zero to eight hours of contact with prepared DNA. The newly developed mutant strains behaved like the wild donor strain after transformation.

Morphogenesis in tadpoles: For several years studies have been undertaken in this Institute on the metamorphosis of tadpoles in two species of frogs *Rana tigrina* and *Bufo melanostictus*, and of the effect of chemicals which can either delay or accelerate the normal period of metamorphosis in the tadpoles, which is about four weeks. It was found that the two antibiotics penicillin and streptomycin are effective retardants. Penicillin being much less toxic has been used in subsequent investigations. In addition thyro-uracil has been found to be an effective retarder. Both vitamin B_{12} as well as thyroxine are found to hasten the metamorphosis of the retarded tadpoles. Cultures of microorganisms from the intestinal flora of normal and retarded tadpoles show that in the former a number of B_{12} producing microorganisms are found while in the latter most of them are absent.

During the year under report, a comparative study showed that the effect of B_{12} was more marked in *R. tigrina* than in *B. melanostictus.* A large number of comparative observations has been made on the combined effect of thyroxine with either penicillin or thyro-uracil. It was found that penicillin has a slight and thyro-uracil has a marked inhibitory effect on thyroxine treated tadpoles.

It is now generally accepted that thyroxine, formed in the thyroid glands of tadpoles is responsible for effecting metamorphosis. Further the investigations carried out in the Institute make it probable that B_{12} secreted by intestinal flora influences the production of thyroxine. A series of histochemical investigations have been carried out on the development of colloids in the cells of the thyroid gland from the early larval stage to metamorphosis. It was noticed when the metamorphosis is complete, the colloid area in the thyroid gland cells suddently decreased. Further studies have revealed that both cell area and the colloid area in it first decrease and later increase in penicillin and followed by vitamin treated tadpoles, as compared to those in control larva.

Another investigation has been started on the biochemistry of synthesis of thyroxine and the role of vitamin B_{12} in the control of thyroxine synthesis.

A. PHYSICS

A.4. Cosmic Rays

A.4.1. *Underground studies of mu-meson interactions*

The studies of mu-meson interactions started last year at the underground power station of the D.V.C. at Maithon have yielded a number of important results. In these studies a big multiplate cloud chamber of dimensions $(90\times80\times50)$ cm^3 was used to record mu-meson induced events. About 16,000 photographs obtained have been analysed for the following phenomena:

(i) the production of penetrating showers by high energy mu-mesons,

(ii) the production of secondary electrons by high energy mu-mesons,

and (iii) the occurrence of parallel multiple penetrating particles underground.

It has been pointed out by several investigators that, even at large depths underground, the mu-meson beam contains a very small percentage of π-mesons which arises from the production of penetrating showers by high energy mu-mesons in the rock above the equipment. In our experiment, this effect was avoided by placing an additional layer of lead on the top of the cloud chamber and accepting only those particles as mu-mesons which traversed this lead layer without producing a shower in it. The thickness of the lead layer was such that any π-meson entering will either be absorbed or will produce a shower in it. In five cases the mu-mesons thus selected were found to produce a penetrating shower in absorbing plates inside the cloud chamber. With these five events the cross section of the production of penetrating showers by mu-mesons of mean energy 32.6 Gev was found to be $\sigma_\mu=(3.1\pm1.4)\times10^{-30}$ cm^2/nucleon. A second set of observation was taken, removing the top lead layer so that the penetrating showers recorded in this case are due to both mu- and pi-mesons. With twelve showers recorded in the second arrangement the value of the shower production cross section was found to be $\sigma_{\pi,\mu}=(7.6\pm1.7)\times10^{-30}$ cm^2/nucleon.

The results of our measurements on the underground penetrating showers show:

(i) that the cross section of shower production by fast mu-mesons agrees satisfactorily with the cross section calculated on the basis of interaction between electromagnetic field of mu-mesons and the meson field of nucleons,

(ii) that the flux of pi-mesons relative to mu-mesons at the depth of the experimental station (148 m.w.e.) is $\sim$0.025%,

(iii) that the penetrating secondaries produced in μ-induced showers are distributed isotropically in the centre of mass system, and

(iv) that the degree of inelasticity of the mu-meson-nucleon interaction for a mean mu-mesons energy of 32.6 Gev is 0.3.

A study has been made of secondary electrons (δ-rays, knock-on electrons and direct electron pairs) produced by high energy mu-mesons in argon gas and with Fe-plates inside the cloud chamber. The results obtained from this study are:

(i) the measured energy spectrum of δ-rays in the interval 30–150 Kev agrees satisfactorily with that predicted from the classical theory of elastic mu-meson-electron collison,

(ii) the energy spectrum of the knock-on electrons upto 1.5 Gev agrees within statistical errors with the calculated spectrum on the basis of well known quantum electrodynamical theory of mu-meson-electron collison,

(iii) the angular distribution of knock-on electrons from high energy mu-mesons follows a cosine-cube law, and

(iv) the process of direct electron pairs by mu-mesons does not find a good theoretical explanation over the entire range of energy transfers. The cross section of the direct pair production is found to be only one half that predicted by the Murota-Ueda-Tanaka theory for energy transfers <100 Mev whereas the cross section agrees with the theoretical value for energy transfers >100 Mev.

In course of our underground experiment, we have obtained 27 cloud chamber photographs showing contemporary parallel penetrating particle tracks. In none of these cases was there any visible nuclear interaction or perceptible scattering in the plates of the cloud chamber observed. It appears, therefore, that these parallel penetrating particles are mu-mesons. It is found that these events are neither due to random coincidence of independent muons nor due to secondaries of penetrating showers produced in the rock above the equipment. From a comprison of our data with similar data obtained by several other investigators at various depths below ground, it seems almost certain that the parallel multiple penetrating particles underground are remnants of the extensive air showers initiated by high energy primary cosmic ray particles near the top of the atmosphere.

A.4.2. *Studies on mu-meson interactions at Darjeeling (altitude 2.2 km)*

The analysis of thirtysix thousand pictures taken with a multiplate cloud chamber triggered by a four-fold coincidence and anticoincidence geiger counter telescope is now complete. Two papers entitled:

(1) Decay asymetry of cosmic ray muons, and

(2) Scattering of mu-mesons by copper nuclei

have been accepted for publication in the journals—Proceedings of the Physical Society (London) and IL Nuovo Cimento (Italy).

In paper (1) an account is given of the investigations on decay asymmetry of cosmic ray muons carried out in two different energy bands with the help of a multiple cloud chamber. Although similar investigations have been made in different laboratories with scintillation counters, employing the delayed coincidence technique for the detection of

decay electrons, the cloud chamber has been chosen for some special advantages. The most important among these are:

(i) the decay electrons are detected from zero delay upto a delay of about 50 milliseconds which is never possible in delayed coincidence method

(ii) the change of direction of the decay electrons in the absorbing material due to coulomb scattering is avoided by dividing the whole absorber into thin plates separated by gaps in which the electrons are observed. Even if these electrons are subsequently scattered their original direction is clearly observed.

After careful scanning of thirty-six thousand cloud chamber photographs 1503 unambiguous μ-e-decay events were found. Of these, 1021 events correspond to a muon momentum interval $0.3 < p_\mu < 0.4$ Bev/c and 482 events to a momentum interval $1.3 < p_\mu < 1.4$ Bev/c at the experimental station which was situated at a height 2.2 km. The up-down asymmetry of the decay electrons from muons in the two momentum intervals were 1.17 ± 0.05 and 1.18 ± 0.08 respectively, which yield a partial longitudinal polarization of 0.34 ± 0.09 and 0.36 ± 0.11 after correcting for the depolarization effect of the atmosphere and moderator. Our results, therefore, show no evidence for the increase in the degree of polarization with increase of momentum of muons which some workers have observed. The absolute magnitude of polarization observed is consistent with a K/Π ratio of about 5% in the pion spectral region 0.2 to 5.6 Bev.

In paper (2) an account is given of our experimental study of the scattering of slow mu-mesons. The principal difficulties associated with the mu-meson scattering experiments performed so far lie in the following facts:

(1) In the measured scattering, the effects produced by particles other than mu-mesons (π, p, etc.) are some times, unwittingly included.

(2) The momenta of the particles investigated are known only within wide limits.

Both these uncertainties have been eliminated in our experiment where we have selected for scattering measurements only those mu-mesons which *stopped* and *decayed* inside the multiple cloud chamber. This method not only gives a correct identity of mu-mesons but also provides an accurate determination of their momenta.

Altogether 2606 scattering angles were measured to an accuracy of 0.2° directly on the film with a goniometer eyepiece fitted in a microscope. From these measurements a differential distribution of the quantity $p\beta\theta$, where $p\beta$ is the product of the momentum p and velocity β of the mu-meson at the point of scattering and θ is the projected scattering angle, was found. It was found that our experimental scattering distribution agrees well with the multiple scattering distribution calculated on the basis of a purely coulomb interaction between the mu-meson and the nucleus. Thus the present experiment does not indicate the existence of an anomalous scattering for mu-mesons in the investigated momentum region $40 < p\beta < 170$ Mev/c.

A.5. Nuclear Physics

A.5.1. *Measurement of The Small Angle Coherent Scattering of Gamma Rays*

Experimental studies of the small angle coherent scattering of cobalt-60 gamma rays (1.33 & 1.17 Mev) have been made with a modified arrangement of the usual shadow cone technique. Double cone multiple section of iron filters are used to define a narrow cone of the incident as well as of the scattered beam, while conical shields of lead are provided for the source and the detector. The detector used is a NaI(T) crystal, $2 \times 1\frac{7}{8}''$, mounted on a 6292 photomultiplier. After amplification the pulses are analysed with a single channel differential pulse height analyser. With this arrangement it has been possible to practically eliminate any correction due to scattering from air and the sample dependent background correction has been reduced to less than 3%.

Measurements reported here correspond to a source to detector distance of 3 meters. The source used is a 450 mc Co^{60} cylinder of effective dimension 6 mm.$\times$6 mm. The scatterers, made in the form of circular rings, are placed mid way between the source and the detector. Scattering angle was varied by using rings of different diameters. Elements investigated include Aluminium, Copper, Tin and Lead in the solid metallic form; Molybdenum and Tungsten in the metallic powder state; mercury in the liquid state and Uranium in the form of a compound (Uranium Acetate). Powders and liquid were enclosed in containers of thin perspex of appropriate dimensions.

To obtain the absolute differential cross-section, the scattered intensity was simultaneously compared with a second weaker source placed in the position of the scattering ring. The relative strength of this weaker source compared with the 450 mc source was previously measured in a separate procedure. Detailed investigations were made for the estimation of the correction arising from change in pulse height with the counting rate. It was observed that for the counting rate varying from 50 cpm to 20,000 cpm, this correction is of the order of 1%.

The object of the investigation has been to study:

(i) the distribution of the coherent scattering cross section at small angles (2° to about 10°),

(ii) the energy dependence of the Rayleigh scattering cross-section, and

(iii) the Z-dependence (Z^n) of the Rayleigh scattering cross-section.

Absolute differential cross-section for the total and coherent scattering processes obtained with Co^{60} γ-rays at angles 2.18°, 2.67° and 3.18° for the various elements have been determined. Towards studies (i) and (ii), measurements have been undertaken at larger angles and for C_s^{137} γ-rays.

Coherent scattering cross sections have been obtained by subtracting the compton scattering cross-section from the total scattering cross-section. Compton scattering cross section for each element for the corresponding energy and angle of scattering has been calculated on the basis of Heisenberg and Bewilogua taking into account the binding effect of the electron in the atom.

The Z-dependence of the Rayleigh scattering cross-section has been analysed for angles of scattering 2.18° and 2.67° for energies 1.33 Mev and 1.17 Mev. As the Rayleigh scattering is the predominant process for coherent scattering at these energies and angles of scattering (Thomson and Delbruck scattering processes contributing to less than 1%), the entire conherent scattering cross section has been attributed to the Rayleigh scattering. The values of the index n were obtained from the slope of the curve σ (Rayleigh) *vs* Z plotted on a double log graph paper.

With the exception of Uranium for which the effective scattering mass was small, all the total scattering cross-sections have been measured to an accuracy of 4%. The scattering cross sections for Aluminium are particularly significant for the present measurements. Because of low Z, the entire scattering may be attributed to the compton process. Secondly, compton cross-section is not complicated by the effect of binding (binding energy of the electron in Aluminium is very small). The cross-section for Aluminium may, therefore, be predicted very accurately. Measured values for Aluminium serve as a check to the accuracy of the present measurements. It was observed that in each case the measured values agree with the theoretical values within experimental errors.

The values of n slightly larger than 3 are, however, uncorrected. Correction has to be made for the sharp rise of the Rayleigh scattering cross-section with decrease of angle. Consequently, this correction factor is large for smaller angles. Kane *et al*, [Phys. Rev., **122,** 1579 (1961)] has estimated it to be 11% for 2.43° angle of scattering. For 2.18° angle of scattering, therefore, this correction factor will be larger than 11%, while for 2.67° it is expected to be smaller than this. The correction values of the index n in our measurements will come down to less than 3. The exact estimation for this correction is now being made.

A.5.2. *Theory of uniform spectral sensitivity photon counters.*

It has already been reported previously that a uniform spectral sensitivity photon counter has been constructed in our laboratory using aluminium filter before a conventional NaI/T1 scintillator. The mathematical theory of this type of counters has been developed, assuming that all the rays incident on the filter crystal assembly are paraxial. Detailed theoretical and experimental investigations for wide beam geometries have been undertaken.

A.6. Neutron Physics

A.6.1. *Modifications of the Neutron generator*

The *d–t* neutron generator is now on regular operational schedule. A new precision bleeder chain of 30,000 Megohms has been incorporated in the Cockroft-Walton type high voltage source of the generator. This has considerably improved the precision of the measurement of the energy of the accelerated deuteron beam, which is usually kept constant at 150 Kev. The steady neutron flux which is utilised in the experiments is 10^9 neutrons per second.

A.6.2. *Development of directional and spherically symmetric fast neutron counters*

In many investigations like the measurement of total cross section, polarisation etc. it is desirable to have a neutron counter, the sensitivity of which shows a sharp maximum for neutrons traversing the detector along a preferential direction. Such a counter has been developed in our laboratory simply by arranging that the maximum range of recoil protons generated by fast neutrons in a filamentary plastic scintillator is larger than the diameter of the filament. In order to increase the net efficiency of the detector without unduly sacrificing its directional response, an array of filament is arranged in such a manner that not more than two of them are coplanar. The cross ratio i.e., the ratio of efficiencies for neutrons incident parallel and perpendicular respectively to the detection axis of the system, is an increasing function of the bias used in the output discriminator.

In direct contrast to what has been described just now, many experimental situations like the measurement of non-elastic cross sections of nuclei, demand the construction of a spherically symmetric neutron counter. A few such counters have been prepared in the laboratory by polishing plastic scintillator cylinders into spherical shape. The scientillators have been coupled to the photomultipliers by means of perspex light guides which unfortunately causes deviations from spherically symmetric response, especially for lower energy neutrons. Glass couplers to remove this difficulty are under construction.

A.6.3. *Measurement of the total cross sections of nuclei for fast neutrons*

Total cross sections of a few nuclei have been measured, more with a view of standardising and calibrating the various components used in the investigation than with the idea of establishing any new cross section value. A new procedure has been developed during the course of these experiments for making corrections for in-scattered neutrons. Contrary to the usual methods adopted in practice, this procedure does not require any optical model parameter but depends entirely on the experimentally determined transmissions under different geometrical conditions.

A.6.4. *Measurements of the non-elastic cross sections of nuclei for fast neutrons*

Sphere transmission technique is practically the only method at present available for the measurement of non-elastic cross-sections of nuclei. The conventional method for correlating transmission with cross section using Bethe's analysis for multiple scattering, suffers from the defect that it requires the values of transport and other cross sections for each nucleus to be investigated. Also the usual procedure for correcting the apparent absorption of elastically scattered neutrons presupposes an accurate knowledge of their angular distribution.

In our method the multiple scattering correction is made by an extrapolation formula based only on the assumption that fast neutron scattering is peaked in the forward direction. Also a simple theory has been developed for the relative efficiency of recoil proton plastic scientillation detector. This theory enables us to correct for elastic scattering by merely easuring transmission at two different bias settings. This procedure is a perfectly general

(iv) *Sedimentation studies on cow α-lactalbumin*

Both α-lactalbumin-A and B showed the presence of a major peak together with a faster-sedimenting component and the nature of patterns and the sedimentation coefficients were practically identical with those of buffalo α-lactalbumin. The sample containing both α-A and α-B also gave a single symmetrical peaks both at room and low temperatures with high protein concentration indicating absence of any interaction or aggregation.

(c) *Investigation on the non-casein proteins of goat milk and their occurrence in the milk of individual goats of different breeds:* Preliminary results of these investigations reported in the previous Annual Report (p. 33) indicated the presence of three well separated bands at *p*H 4.6 in paper electrophoresis. Further typing experiments carried out at *p*H 4.9 in acetate buffer with milk from about 75 goats belonging to Jamnapari, Black Bengal, Barbari and a few crosses between Saanen and Indian breeds showed the presence of five components, of which the β-lactoglobulin, the α-lactalbumin and two other minor components migrated towards the cathode and the fifth one to the anode. All the individual samples appeared to give uniform results indicating the absence of any genetic factor in occurrence of non-casein protein constituents of goat's milk in the breeds so far studied.

(i) *Separation and crystallization of β-lactoglobulin and α-lactalbumin of goat's milk and studies on their physico-chemical properties.*

In previous investigations (Askonas, *Biochem. J.*, 1954, **58,** 332; Bain and Deutsch, *Arch. Biochem.*, 1948, **16,** 221) on non-casein proteins of goat's milk, attempts of preparing β-lactoglobulin as salt-free crystals and isolating and crystallizing lactalbumin were not successful. The presence of a component similar to α-lactalbumin of cow's milk remained undetected.

The separation and crystallization of β-lactoglobulin and α-lactalbumin have been carried out by the method used with cow's or buffalo's milk, suitably modified in the present work for goat's milk. According to this procedure a filtrate containing the non-casein proteins, obtained by addition of Na_2SO_4 (20 g./100 ml.) to the whole milk, is adjusted to *p*H 3 when all the protein constituents with the exception of lactoglobulin were precipitated. From the filtrate, β-lactoglobulin was precipitated according to the usual procedure. The final concentrated solution of β-lactoglobulin was adjusted to *p*H 5.9 and dialysed against distilled water. A heavy oily layer separated from which diamond shaped crystals of β-lactoglobulin appeared in 10–12 days. β-Lactoglobulin precipitate obtained by this method can also be crystallized in the form of hexagonal bipyramids, (*cf.* Askonas).

The acid precipitated proteins were dissolved in water by adding dilute ammonia and freed from any residual β-lactoglobulin by reprecipitating at *p*H 3 in presence of sodium sulphate (15 g./100 ml.). This precipitate was dissolved in dilute ammonia and the *p*H adjusted to 4 with hydrochloric acid. The solution was centrifuged and from the supernatant α-lactalbumin was precipitated with ammonium sulphate between 45 and 80% saturation. The precipitate was dissolved in water and the solution after being adjusted to *p*H 6 was rendered slightly turbid by adding ammonium sulphate. This solution when allowed to evaporate slowly gave diamond shaped crystals of α-lactalbumin. A salt free

concentrated solution of α-lactalbumin gave needle shaped crystals when left in the cold (2–4°C).

In preliminary free electrophoresis experiments α-lactalbumin showed single symmetrical peaks at *p*H 5.2 and its iso-electric point appeared to be close to this *p*H. Its sedimentation coefficient, $s_{20,w}$ measured at the same *p*H was 2.0×10^{-13} sec. which was close to the $s_{20,w}$ values for cow and buffalo lactalbumins. With β-lactoglobulin, the hypersharp ascending and skew descending electrophoretic patterns obtained at *p*H 5.5 and the $s_{20,w}$ value of 2.9×10^{-13} sec. at *p*H 5.7 were similar to those reported by Askonas.

(ii) *Ultraviolet absorption:* Extinction coefficient of goat β-lactoglobulin was found to be 8.9–9 at 278–279 *mμ* in the neutral *p*H range and that of β-lactalbumin 20.6 at 281–282 *mμ*.

(d) *Electrophoresis of buffalo β-lactoglobulin:* Physico-chemical properties of buffalo β-lactoglobulin have been reported (Annual Report, 1959-60, p. 30; 1960-61, p. 29). The comparison of the electrophoretic patterns of buffalo β-lactoglobulin in the *p*H range 4.0 to 7.76 with those of cow β-lactoglobulin-B showed some minor differences in the degree of heterogeneity and mobilities. It was pointed out that like cow β-B, buffalo β-lactoglobulin did not show hypersharp or sharp descending patterns above its isoelectric point. To confirm this point, electrophoresis experiments were carried out with buffalo β-lactoglobulin and cow β-B of both Indian and European breeds at *p*H's 5.3 and 5.6 employing a potential gradient of 7–10 volts/cm. at 0°C in a Perkin-Elmer electrophoresis apparatus, as well as in the Tiselius apparatus at the Institute. β-Lactoglobulins from all the three sources gave identical electrophoretic patterns at the same *p*H's and protein concentrations, the descending patterns being sharper than ascending ones at *p*H 5.3, as found in the case of cow β-B by Timasheff and Townend (*J. Am. Chem. Soc.*, 1960, **82,** 3161). The mobilities of buffalo β-lactoglobulin were found to be -0.07×10^{-5} cm².sec./volt at *p*H 5.3 and -0.97×10^{-5} cm².sec./volt at *p*H 5.6 and those of cow β-B to be -0.06×10^{-5} cm².sec./volt at *p*H 5.3 and -0.94×10^{-5} cm².sec./volt at *p*H 5.6 at the protein concentration of 1 g./100 ml. These values are somewhat lower than the values of -0.12×10^{-5} cm².sec./volt at *p*H 5.3 and -1.46×10^{-5} cm².sec./volt at *p*H 5.6 for cow β-lactoglobulin-B reported by Timasheff and Townend.

(e) *Protein titrations:* Investigation on the titration curves of buffalo β-lactoglobulin has been commenced. The results of titration in the acid range (*p*H 1.50–3.50) with cow β-lactoglobulin-B and buffalo β-lactoglobulin show that the titration curve of buffalo β-lactoglobulin runs parallel to but below the curve of cow β-lactoglobulin-B indicating that the number of carboxyl groups per mole in the former to be about one to two units less than those in cow β-lactoglobulin-B. The titration curve of cow β-B was similar to that obtained by other workers.

B.2. Organic and Plant Chemistry

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

(a) *Barringtonia acutangula* Geartn: Work on the elucidation of the structure of barringtogenol C has been continued (*vide* previous report). Barringtonegenol C formed a mono-

trityl derivative, $C_{49}H_{64}O_5$, m.p. 256–261° and this indicated the presence of one primary hydroxyl group. The pentaacetate of barringtongenol C on oxidation with perhydrol-acetic acid gave a colourless 12-keto compound, m.p. 244–246°, which is saturated to tetranitromethane. Further work is in progress.

(b) *Albizzia procera* Benth: The sapogenol (Fraction B, *vide* previous report), m.p. 198–199°, $[\alpha]_D^{31}$ +81°($CHCl_3$) obtained from the beans of *A. procera* has been proved to be a triterpene. Analytical data indicate either $C_{31}H_{50}O_4$ or $C_{32}H_{52}O_4$ as the molecular formula. Infra-red spectrum shows band for hydroxyl (3400 cm^{-1}), ester carbonyl (1700 cm^{-1}) and gem dimethyl groups (1365 cm^{-1}, 1370 cm^{-1}). It forms a diacetate, m.p. 223–224°. The ethylenic linkage, indicated by tetranitromethane colour, has been found to be resistant to catalytic hydrogenation though it consumed nearly one mole of perbenzoic acid slowly.

Fraction B must contain an ester group as its yielded an acid, $C_{30}H_{48}O_4$, m.p. 294–296° on alkaline hydrolysis. The above acid, on treatment with diazomethane, furnished a methyl ester, $C_{31}H_{50}O_4$, m.p. 232–234°. Fraction B appears to be a triterpene of the oleanen series containing two hydroxyl groups and an ester group. Further studies will be continued.

(c) *Albizzia lucida* Benth: From the alcoholic extract of the seeds of *A. lucida*, saponins have been isolated in good yield. On hydrolysis, acid and neutral sapogenins were obtained. The acid fraction was esterified with diazomethane and chromatographic resolution of the product over alumina furnished a crystalline methyl ester, $C_{31}H_{50}O_4$, m.p. 213°, $[\alpha]_D^{31}$ −33.5° (ethanol), which formed a diacetate, $C_{35}H_{54}O_6$, m.p. 198–200°. The above methyl ester furnished, on hydrolysis, an acid, $C_{30}H_{48}O_4$, m.p. 305–307°, $[\alpha]_D^{31}$+38° (ethanol), which was identified as echinocystic acid. The neutral sapogenin fraction is being studied.

(d) *Albizzia stipulata:* Crystalline acid and neutral sapogenins have been isolated from the seeds of *A. stipulata* and are being studied.

(e) *Samanea saman:* Alcoholic extract of the pods of *S. saman* yielded a crude saponin which on hydrolysis followed by chromatographic resolution over alumina, furnished two crystalline sapogenols, called provisionally, samagenol A, m.p. 251–254° and samagenol B m.p. 243–245°. Both responds to Liebermann-Burchard colour reaction.

B.2.2. *Chemical investigation of the bark of* Ougeinia dalbergioides *Benth*

The petroleum ether (b.p. 60–80°) extract of the bark of *O. dalbergioides* on chromatographic resolution over alumina furnished two crystalline compounds having melting points 206–210° and 244–246° respectively. Both responded to Liebermann-Burchard colour reactions. They furnished the corresponding acetates m.p. 214–216° and m.p. 218–221°. Further work on the constitution of the above compounds is in progress.

B.2.3. *Chemical investigation of Indian plant resins*

Chemical examination of Indian plant resins has been continued and the following plant resins are being investigated.

(a) *Canarium* spp.

The resins from *C. euphyllum* and *C. skimmase* were systematically extracted with different solvents and residues obtained from each solvent extract was separated into acid and neutral

fractions. The amount of acidic fractions was negligible but a good amount of neutral resinous matter could be isolated from petroleum ether, benzene and chloroform extracts. Attempts to isolate crystalline materials from the neutral resin fractions were unsuccessful. All the neutral fractions, however, responded to Liebermann-Burchard colour reactions indicating the presence of terpenoids.

The resins from *C. euphyllum* and *C. skimmase* showed inhibitory action against *Bacillus subtilis*, *Escherichia coli* and *Fusarium* spps.

(b) *Shorea tambuggia*

The resin after steam distillation, was extracted with petroleum ether (b.p. 60–80°) and benzene. The glassy mass obtained from the extracts was separated into acid and neutral fractions. The acid fraction was esterified and the product was chromatographed but only glassy products could be isolated which gave positive test with Liebermann-Burchard colour reactions.

B.2.4. *Chemical investigation of lac resins*

The light brown amorphous material obtained by temperature-phase separation of the palas seed lac (*vide*, previous report) after removal of the fat and soft resin, has the following characteristics: Melting point 90–105°, sap value 212–213, acid value 60.61–64.43, hydroxyl value, 8.27–12.57%. Preliminary combustion analysis of the Fraction I leads to the empirical formula C_4H_6O. It shows no characteristic absorption in the visible and ultraviolet region except an absorption of low intensity at 228 $m\mu$ in ethanol. The molecular weight of Fraction I could not be determined by Rast's method as it was insoluble in camphor. Crysocopic method of molecular weight determination gave incompatible results. Fraction I in glacial acetic acid is converted into a straw coloured fibrous material which did not melt up to 320°.

Owing to the unfavourable solubility of the Fraction I in organic solvents, it was found difficult to study its homogeneity by counter-current technique. Paper chromatograhphic study reveals that the constituents of Fraction I are either very closely related substances having almost the same R_f values or they are associated in such a manner as to behave as a single compound. It contains no carbonyl group as it failed to react with dimedone and 2:4-dinitrophenyl hydrazine. Attempts to prepare crystalline derivatives by acetylation or amide formation were not fruitful. Methylation of the Fraction I with diazomethane gave an ether-insoluble amorphous methyl ester, m.p. 70–80° and a small amount of viscous, ether-soluble ester. The acid values of the above two esters were found to be nil and their R_f values were different. Column chromatography over alumina, cellulose powder-charcoal and subsequent attempts for crystallization failed to provide any crystalline products from the above two methylated products. The molecular weight of the purified ether-insoluble methyl ester (methoxyl value 2.94%) has been found to be approximately 1200.

Fraction I was methylated by Purdus' method (methyl iodide-silver oxide) and a brown resinous solid, m.p. 85–110° was obtained. Its methoxyl value was found to be 6.1% and molecular weight 1200–1250. Further work is in progress.

B.2.5. *Chemical investigations of* Murraya koenigii

Chromatographic separation of the benzene extract of the stem bark of *M. koenigii* yielded three crystalline materials, two of which have been reported earlier (*vide* previous report). All of them contain nitrogen but show no basic properties.

The first compound, m.p. 176–7° seems to have the molecular formula $C_{18}H_{17}ON$. The UV spectrum showed absorption maxima at 238 *mμ* (log ε 4.60) and 288 *mμ* (log ε 4.56). IR peaks were at 3450 cm^{-1} (—NH or —OH), 1644 cm^{-1}, 1610 cm^{-1} and 1492 cm^{-1} (aromatic nucleus). From the nuclear magnetic resonance spectrum the total proton integral confirmed the presence of 17 hydrogens, as indicated by the elementary analysis. The compound forms no acetate but gives a crystalline dark red picrate m.p. 198–200°. All attempts to hydrolyse the compound were futile. With concentrated acids the compound got resinified. The second compound, m.p. 167–8°, conforms to the formula $C_{13}H_8ON(OCH_3)$ and contains 2 active hydrogen atoms. The UV spectrum showed maximum absorption at 238 *mμ* (log ε 4.48), 274 *mμ* (log ε 4.56) and 330 *mμ* (log ε 4.16). IR peaks were at 3420, 1625, 1620, 1598, 1497 cm^{-1}.

The compound forms no acetate but a DNP and an oxime.

Another nitrogenous crystalline compound, m.p. 94–95°, has also been isolated. Further work is in progress.

B.3. Radiochemistry

B.3.1. *Effect of RNase and DNase on CO_2 fixation by chloroplasts*

That RNase and DNase can reduce the overall photosynthetic CO_2 fixation in algal cells had been studied and reported earlier. To eliminate the possible hindrance of cell wall to the entry of comparatively large molecules of RNase and DNase, similar studies were made on chloroplasts isolated and suspended in 0.35 *M* NaCl. Such experiments revealed that half hour pre-incubation with DNase (1 mg./ml of suspension) at 25°C reduces $C^{14}O_2$-fixing capacity of the chloroplasts to the extent of about 65%. Similar treatment with RNase, however, seemed to be much less effective in reducing CO_2-fixing capacity. Chromatography and radioautography of the alcohol soluble fraction indicated that the RNase and DNase, however, do not alter the usual primary pattern of photosynthetic CO_2 fixation. The results stand in close agreement with those observed earlier with intact cells indicating a partial inhibition of an early step in CO_2 fixation.

B.3.2. *Binding of CO_2 to RNA*

The inhibition of CO_2 fixation with nucleases apparently indicated the possible participation of nucleic acids in this process. Though the reductive pentose cycle was well established in the case of photosynthesis yet the CO_2 transfer to ribulose diphosphate is not clearly understood. To demonstrate whether there is any activation of CO_2 prior to this transfer some experiments were designed. The possible participation of RNA in CO_2 fixation has been indicated when CO_2 was fed to spinach leaves for a short period of 2 min. in light. RNA extracted from these leaves in 0.14 *M* NaCl was found to bind

$C^{14}O_2$ which could be released from RNA by treating it with dilute KOH or HCl. RNA was found to bind as much $C^{14}O_2$ as was incorporated in its molecule over the said period of time when compared on the basis of C^{14} activity. The characterization of RNA-CO_2 complex is now in progress. With DNA such type of binding has not yet been observed.

B.3.3. *The role of iron-containing compounds in photosynthesis*

In continuation of the previous work, the possibility of the occurrence of an iron-containing compound in the primary steps of CO_2 fixation has been studied in greater details using Fe^{59} and $C^{14}O_2$ as tracers. *Xanthium* twigs were allowed to take up Fe^{59} (15°C) for 10 hour after which $C^{14}O_2$ was fed for 2 min. in light and then the leaves were extracted in 0.5 *M* sucrose. 66% acetone precipitate from the extract could be partly solubilized in 0.002 *M* phosphate buffer (*p*H 7) and in 1% and 5% concentrations of sodium deoxychlolate. The radioactivity in phosphate buffer-soluble fraction was mainly found to be due to C^{14} and the activity in the insoluble fraction was mainly due to Fe^{59}, the ratio of Fe^{59} to C^{14} in the insoluble fraction being about 3:1. Ammonium sulphate fractionation of the phosphate soluble portion revealed most of the activity in the fraction not precipitable by $(NH_4)_2SO_4$.

B.3.4. *Phosphate metabolism in different spectrum of light*

To study the action spectrum of ATP synthesis in green leaves, *Antigonon* leaves were allowed to take up $P^{32}O_4$ for 1 hour in the dark and then separate leaves were exposed to similar intensities of red and blue light for 15 mins. while control leaves were kept in the dark for the same period. Leaves from each treatment were separately fixed in (i) 6% $HClO_4$ and (ii) 80% ethanol (both in the cold). The $HClO_4$ soluble fractions, after neutralisation with KOH, were absorbed on activated charcoal and subsequently eluted with ammonia ethanol (0.5% NH_3). The eluate on chromatography and radio-autography revealed in all cases the presence of 4 compounds one of which was identified to be ATP. Though stimulation of P^{32} incorporation into these compounds was recorded in both red and blue light, yet red light was found to be much effective. Analysis of the alcohol-soluble fractions showed the presence of similar esterified compounds with greater incorporation of P^{32} in red light.

B.3.5. *Tracer studies in the post-harvest physiology and biochemistry of mango*

Mature green mango fruits were fed $C^{14}O_2$ both in intact condition as well as in the peel and the pulp. Pulp and peel of the intact fruits were separately studied throughout ripening period. Such studies showed typical photosynthetic CO_2-fixation in the green peel and very small amount of $C^{14}O_2$ was fixed by pulp in malic acid, sugar phosphates and few amino acids. With ripening PGA and malic acid increasingly became radioactive in the pulp but C^{14}-activity in sugar phosphates gradually decreased. This decrease in C^{14}-activity may be accounted for in the gradual increase in C^{14}-activity in the alcohol-insoluble fraction with storage and ripening. Results do not indicate any direct conversion of organic acids to sugar in the pulp of mango during ripening.

B.3.6. *RNA and protein synthesis by chloroplasts*

Extracts from spinach leaves were fractionated by differential centrifugation. Amino acid activating enzymes were found to be present in the chloroplasts. When chloroplasts were further fractionated into *p*H 5 and grana, each fraction was found to have the amino acid activating system. When both the fractions were combined, further stimulation of amino acid incorporation was noticed. From the experimental data it may be at present said that chloroplast has the general characteristics of its protein synthesis. Among the amino acid tested (leucine, valine, aspartic acid, glycine) leucine showed maximum incorporation.

Experiments were done to test the presence of RNA-polymerase in the chloroplast extract. As the RNA-polymerase is dependent on the presence of DNA for its activity it was the purpose of the experiments to demonstrate whether chloroplast could synthesise its own RNA independent of nuclei. Experimental results so far obtained did not indicate the presence of any RNA polymerase but ATP incorporation alone was found to be favoured. The purification of this ATP-polymerase is now in progress.

B.3.7. *Phytin complex from coconut milk*

While isolating RNA from coconut milk it was observed that another unknown substance was precipitated as contaminant. It was subsequently isolated and identified as phytin (hexaphospho-inositol containing Ca & Mg). As a matter of fact when $P^{32}O_4$ was injected to a coconut this substance became radioactive within a very short period. The point whether this substance has any role in PO_4 metabolism in the coconut should be looked for. Accordingly P^{32}-labeled phytin was isolated and purified. This was injected into a coconut and from the nuclei, nucleic acids were isolated; with time the activity in the nucleic acids increased. The possible mechanism by which the phosphate is being incorporated in nucleic acids might be through the release of inorganic phosphate from phytin. As a matter of fact it was observed that with time more and more P^{32} was released from tagged phytin indicating possibly the phytase activity. Phosphorus esterified in acid soluble fraction also increased appreciably with time when tagged phytin was injected. From the data so far obtained it was apparent that phytin plays an intermediary role in phosphate conservation in the coconut milk.

The probable role of phytin in growth-promoting mechanism has also been tested but without any positive result. Another possibility was explored in this connection that there might be a RNA-phytin complex as all attempts to isolate free RNA was futile. No pure RNA could be extracted from coconut milk by conventional methods. The possibility of phytin remaining as contaminant with RNA is remote due to the fact that phytin is soluble at *p*H 5.0 whereas RNA is not. So, if at all any RNA be present in coconut milk it might be present as RNA-phytin complex because when suspected RNA was isolated and purified from any contaminating phytin and subjected to paper chromatographic analysis after hydrolysis, spots of bases could be detected though this RNA did not record typical absorption spectrum. This is now being further studied.

B.3.8. *Effect of indole acetic acid on protein synthesis*

A special feature of the growth induction with coconut milk involves protein synthesis. Not only is protein synthesized in the stimulated cells, but a particular kind of protein, rich in hydroxyproline is formed. Since this type of protein seems to occur in other rapidly proliferating cells it may prove to be characteristic of cells in this state. Keeping this in view experiments were designed to see the amino acid incorporation as an index of protein synthesis by different cellular particles under the influence of auxin in growth-stimulating concentration (10^{-5} *M* final concentration).

Pea internodes were treated with IAA-solution for 20 hr. in darkness and then homogenized. The homogenized suspension was incubated with labeled leucine along with other amino acids for 1 hr. at 37°C and then subjected to differential centrifugation. It has been observed that the specific activity of mitochondrial protein increased about 10–15 fold under the influence of IAA. In nuclear and ribosomal fractions there was no stimulation of leucine incorporation. Characterization of the mitochondrial protein is now in progress.

B.3.9. *Effect of auxin on the enzymes of CO_2 fixation*

It has previously been reported that there was an enhancement of CO_2 fixation after auxin treatment and the major portion of fixed CO_2 accumulated in malate. As a matter of fact CO_2 may be fixed in a variety of ways and the different enzyme systems were tested.

It was expected that under the influence of IAA the level of some of the CO_2 fixing enzymes might be increased or there was an induction of some of the enzymes which normally were not present. G-6-P dehydrogenase, malic dehydrogenase, transketolase, malic enzymes, PEP carboxykinase, PEP carboxylase and PEP carboxytransphosphorylase were assayed. From the preliminary observations it seemed probable that auxin stimulated G-6-P dehydrogenase and malic enzyme to some extent. Other enzymes were not affected much.

B.3.10. *Effect of plant hormones on RNA metabolism*

Last year the process of binding of auxin to protein was reported. It has been proposed that auxin may induce separation of RNA from protein moiety and bind itself to it. The experimental results showed that there was a release of bound RNA as the time of incubation of pea internode sections (previously labeled with P^{32}) increased. Soluble RNA has been designated to those species which could be extracted with buffer and the bound RNA to ones extracted with 90% phenol. Within two hours the total activity from bound RNA decreased showing a corresponding increase in the soluble RNA. It seemed probable that at least some of the bound RNA became solubilized under the influence of IAA, whereas there was no release in the control. This is in good agreement with the previous observation on auxin-protein binding. $P^{32}O_4$ incorporation in RNA with IAA treated tissues was found to be increased 2–3 fold over the control. Isolated nuclear and mitochondrial fractions showed higher incorporation of P^{32} in *in vitro* system.

Practically nothing is known as to the mechanism of kinetin action on plant tissues. For this purpose, tritium labelled kinetin was synthesized according to the method of Wilzbach. Pith tissue (found to be most sensitive to kinetin) was incubated with tritium labelled kinetin to see whether kinetin gets itself bound with any compound before manifesting its effect. Of all the fractions RNA recorded some radioactivity and even after rigorous purification it showed the same activity. Only 2% of the activity of kinetin originally applied in the experiment was detected in RNA. The possibility of exchange of tritium has been found to be remote in this case, however, for confirming this result the synthesis of C^{14}-labelled kinetin is now in progress.

It has been observed that overall phosphorylation reaction was increased due to the application of kinetin. After kinetin treatment RNA, DNA and acid soluble fraction were isolated and found that in both RNA and DNA specific activity (measured from P^{32} activity) increased. From acid soluble fraction ATP recorded much higher activity in the experimental than that in control.

B.3.11. *Biosynthesis and metabolism of gibberellic acid*

In the last report it was mentioned that on irradiating *Gibberella fujikuroi*, with ultraviolet light, some mutants were obtained. It was the purpose of the study whether these mutants could produce gibberellic acid like the wild one and if not then where was the block in the chain of reactions leading to the production of gibberellic acid. It has been observed that by feeding C^{14} labelled acetate to the actively growing normal fungus gibberellic acid became tagged. But fungal homogenate was incapable of incorporating C^{14}-labelled acetate to gibberellic acid indicating that some factor was destroyed during the process. Identification of that factor has not yet been done. Instead of gibberellic acid C^{14}-acetate was incorporated in another compound which was tentatively identified as mevalonic acid. But when a mutant (white) homogenate was tested instead of mevalonic acid another compound was detected on the chromatogram. Characterization of this compound is now in progress.

B.4. Enzyme Chemistry and Biochemistry

B.4.1. *Biosynthesis of ribonucleic acid (RNA) in* Azotobacter vinelandii

With the detection of soluble, ribosomal and recently of messenger RNA and the recognition of their varied biological functions, research programmes have been initiated to study the modes of their syntheses. In connection with the study of the microbial protein synthesis in relation to the biogenesis of nucleic acids investigations were undertaken to find out the mechanism of syntheses of soluble and ribosomal RNA. A cell-free system from *A. vinelandii* capable of synthesising polyribonucleotide material, like soluble RNA, has already been reported. The properties of the enzyme system are now being investigated in detail. The P^{32}-labelled RNA synthesised *in vitro* by the cell-free system has been isolated from the incubation mixture and degraded by alkali. The 2′: 3′-nucleotides so formed have been separated by paper electrophoresis. Radioactivity associated with all the four nucleotides reveals that phosphate is incorporated into the intranucleotide chain

and not into the terminal nucleotide only. P^{32}-incorporation experiments with the ribosomes and the $105{,}000 \times g$ supernatant fractions clearly indicate that both the fractions are essential for the incorporation of the radioactivity into the soluble RNA-like material. P^{32}-labeled adenosine monophosphate (AMP^{32}) has been prepared chemically using the dicyclohexyl carbodiimide method and is being employed in the studies of RNA synthesis. Attempt is also being made to purify the enzyme(s) responsible for the synthesis of soluble RNA using C^{14}-ATP as the marker nucleotide.

B.4.2. *Studies with RNA polymerase*

Some preliminary investigations have been carried out with the partially purified fraction of RNA-polymerase isolated from *A. vinelandii* which indicate the formation of homo- and co-polymers. RNA-polymerase is known to mediate a deoxyribonucleic acid (DNA)-dependent synthesis of RNA. Using C^{14}-ATP as the single radioactive nucleotide one could detect a DNA-dependent incorporation even in the absence of other nucleoside triphosphates. That this type of unnatural synthesis is not due to polynucleotide phosphorylase has been ruled out. This type of DNA-dependent incorporation of a single nucleotide is stimulated by the addition of any one of the three other nucleoside triphosphates or more by a combination of two. The use of ATP-α-P^{32} as tracer and the characterisation of the polymers so formed are being planned. Incorporation of a single nucleotide like ATP can be conceived if one assumed that the thymine clusters of the DNA chain are brought close together by the looping out of the separating regions. Presence of other nucleoside triphosphate(s) helps to stimulate the incorporation by making the looping out processes more probable. Similar studies with the RNA-polymerase from *L. arabinosus* are in progress.

B.4.3. *Bacterial genetics and virology*

A programme of work has been initiated in these laboratories to study the genetic aspects of the enzymes formed due to the adaptation on substrates. *Lactobacillus plantarum* has been adapted on a number of pentoses and the characterisation of the isomerases so induced is in progress. Coupled to the study of the genetic aspects of bacteria the biochemical aspects of the virus multiplication in bacteria is also being investigated. A series of phages of *A. vinelandii* have been procured from abroad and the optimum conditions of their cultures are being studied with the object of extending the investigations to protein synthesis in cell-free preparations of *A. vinelandii* as already reported. It is expected that the particulate preparations from *A. vinelandii* when supplemented with phage DNA will initiate phage protein synthesis which should be detectable by seroloigical tests. The coding aspects of DNA in *E. coli* system which are just coming into light and our preliminary studies with *A. vinelandii* indicate that the coded information is not universal in nature.

B.4.4. *The biochemistry of rice plant*

Studies have been undertaken in an attempt to find the controlling mechanism of alternative pathways of carbohydrate metabolism in plants, specially in early stages of germination. Rice seedling (*Oryza sativa* L.) has been chosen as the plant material for this

purpose. As a prelude to the work, the known enzymes of the pentose phosphate pathway and those involved in terminal respiration have been checked. The following enzyme activities have been found in cell-free extracts of germinating rice seedling: glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, pentose phosphate isomerase, pentose phosphate epimerase, transketolase, phosphohexose isomerase, DPNH oxidase, TPNH oxidase, DPNH-cytochrome *c* reductase, TPNH-cytochrome *c* reductase, cytochrome oxidase, diaphorase, glutathione reductase and ascrobic acid oxidase.

B.4.5. *Studies on the biosynthesis of thyroxine in relation to the metamorphosis of tadpoles*

A new and simple method has been developed for the detection of thyroxine and allied iodinated compounds on paper chromatograms in connection with the study of metamorphosis of tadpoles. Thyroxine and other iodinated compounds were placed on Whatman No. 1 filter paper and the papers were developed with different solvents. The papers were sprayed with 1% starch solution and then with 1% potassium iodate solution and exposed to ultraviolet light. Within three minutes blue spots, typical of starch-iodine reaction, appeared on the chromatogram. The R_f values determined by this method corresponded with the R_f values determined by other procedures. This method is sensitive enough to detect thyroxine and diiodotyrosine up to 0.5 μg. and 0.05 μg. respectively.

B.5. Analytical Chemistry and Instrumentation

B.5.1. *Vapour phase chromatography*

(a) *Analysis of low-boiling components of gasoline up to C_6 components*

(i) The broad fractions of C_3 to C_6 hydrocarbons previously obtained from the low-boiling fractions of gasoline were further fractionated into a large number of finer fractions. For that purpose the gasoline was first fractionated in a column and fractions up to hexane were taken. These fractions were chromatogramed with a tricresyl phosphate-silver nitrate column and a dinonyl phthalate column in series. Both the columns had 40–80 mesh C-22 fire-brick powder as the solid support. Eighteen distinct peaks were obtained. The tricresyl phosphate-silver nitrate column retarded the unsaturated hydrocarbons more than the saturated hydrocarbons. As such, those saturated and unsaturated hydrocarbons which otherwise would produce overlapping peaks were separated. The dinonyl phthalate column with a higher resolving power for the saturated hydrocarbons then performed the fine separations.

(ii) *Identification of the peaks.* The different peaks obtained can be classified as follows:

C_3-hydrocarbon: propane

C_4-hydrocarbons: *iso*-butane, *n*-butane, butene, trans-2-butene, *cis*-2-butene

C_5-hydrocarbons: *iso*-pentane, 3-methyl-1-butene, *n*-pentane, cyclopentane

C_6-hydrocarbons: 2: 2-dimethyl butane, 2: 3-dimethyl butane, 2-methyl pentane, 3-methyl pentane, *n*-hexane.

In a few cases, such as *n*-pentane, cyclopentane and *n*-hexane, available pure samples obtained from the market were used as standards for identification. In most cases the individual hydrocarbons were synthesized in the laboratory and were purified by means of the fractionating column. In cases of those isomers which were synthesised together in any single synthetic process and were found not separable by fractionating column were separated and identified by the gas chromatograph from their retention volumes using a squalane column. This column separates hydrocarbons according to their boiling points only. A few of the peaks have been identified from published retention volume data. The amounts of some of the components corresponding to the major peaks were estimated from the areas under the respective peaks.

(b) *Analysis of some gas samples*

Some of the gas samples supplied by the Assam Oil Co. Ltd., have been analysed by using squalane column. The gas samples were found to contain mainly methane, ethane, propane, *iso*-butane, *n*-butane, pentane, carbon dioxide and water vapour.

B.5.2. *Paper electrophoresis*

The previous studies on the separation of natural coumarins by paper electrophoresis were further extended to include 14 different natural coumarins. The experimental arrangements were the same as described by Lederer and Ward. As coumarins are electrically neutral substances, they were dissolved in 10% alcoholic alkali to produce the sodium salts of the corresponding *ortho*-hydroxy-cinnamic acids which are ionizable in solution. 40 cm. × 14 cm. strips of Whatman No. 1 filter paper were used. The electrolyte used was *N*/10 NaOH solution in all the experiments. One per cent solutions of coumarins in alcoholic alkali were applied to the filter paper strips. Six spots, 2 cm. apart, of different coumarin solutions were applied along a line at the centre of the strip, and kept for 15 min. to attain equilibrium. A potential difference of 240 volts was then applied at the two platinum electrodes and the electrophoresis was carried for 3 hours. The strips were then taken out and dried at 75°C in an air-oven. The positions of the spots were then located by the fluorescence produced under ultraviolet light. The coumarins used were coumarin, psoralene, *iso*-psoralene, ayapin, murrayin, xanthotoxin, luvangetin, marmin, herniarin, wedelolactone, *ortho*-trimethyl-wedelolactone, marmesin, pimpinellin and nor-dalbergin. Each coumarin was found to have an anionic mobility under the experimental conditions. These mobility values were measured without any correction for electro-osmotic flow. Of these coumarins, pimpinellin and nor-dalbergin gave two fluorescent spots each, while the rest gave single spots. One of the two spots was suspected to be due to the *trans*-isomer of the corresponding *o*-hydroxycinnamic acid. To establish this, the spotted strips were irradiated with ultraviolet light in the moist condition, before the start of electrophoresis. This procedure converted the *trans*-isomers into the *cis*- and consequently only one spot,

namely that corresponding to the *cis*-isomer was observed after electrophoresis. The mobility values of the coumarins are given in the following table.

ELECTROPHORETIC MOBILITIES OF COUMARINS

Name of the coumarin	Approximate anionic mobility $cm^2 \times sec^{-1} \times volt^{-1}$
1. Coumarin	1.87×10^{-4}
2. Psoralene	1.90×10^{-4}
3. iso-Psoralene	1.74×10^{-4}
4. Ayapin	1.94×10^{-4}
5. Murrayin	1.31×10^{-4}
6. Xanthotoxin	1.80×10^{-4}
7. Luvangetin	1.36×10^{-4}
8. Marmin	1.11×10^{-4}
9. Herniarin	1.91×10^{-4}
10. Wedelolactone	1.99×10^{-4}
11. *O*-Trimethyl wedelolactone	1.13×10^{-4}
12. nor-Dalbergin	1.94×10^{-4}
13. Pimpinellin	1.80×10^{-4}
14. Marmesin	1.62×10^{-4}

B.6. Plant Biochemistry

B.6.1. *Glycolytic ezymes in higher plants*

B.6.1.1. *Purification of triosephosphate dehydrogenase*

Healthy germinating mung bean (*phaseolous aureus*, strain, N.P. 28) seedlings grown in the field and in the dark were collected at the end of every 24 hour from the time of soaking and weighed quantities of whole seedlings or different organs thereof were chilled for an hour before extraction. Chilled samples were macerated with 10 vol. of 0.1% potassium carbonate, strained through two layers of cheese cloth and the supernatant centrifuged at a high speed (15,000×g) for 20 mins. in the cold. The residue was discarded as it showed no enzyme activity. The *p*H of the supernatant was adjusted to 7.5 and treated with 0.05 vol. of 1*M* $MnCl_2$, solution to remove the nucleic acid. The resulting supernatant was subjected to the following fractionation with solid ammonium sulphate : 0–30%, 30%–45%, 45%–55%, 55%–65%, 65%–75%. Each fraction was collected and dissolved in Tris buffer *p*H 7.5. The *p*H of the solution was maintained at 7.5 throughout the whole procedure and all operations were carried out at 2°C in the cold room. All the fractions showed fair activity except the 0–30% precipitate and the supernatant of 75% saturation.

The maximum specific activity as well as total maximum activity was found in the fraction 55%–65% which was considerably free from aldolase activity.

B.6.1.2. *Triose phosphate dehydrogenase in whole seedlings and in different organs at different age of germination*

Experiments with age variation revealed that in the etiolated whole seedlings the activity reached an appreciable level in 24 hours. The maximum activity was found in 72 hours. Thereafter the activity gradually decreased with age to reach a maximum constant value by the 7th day of germination. The experiment with field grown samples also showed identical results and considerable activity on the 14th day of germination. Studies with different organs revealed that in 24 hrs. the root showed higher specific activity compared to that in the cotyledons and hypocotyls. The activity was higher in 48 hour and then the activity fell down with age reaching a minimum constant value by the 7th day. The activity in cotyledons gradually decreased with age while the activity in the hypocotyls was low up to 48 hours and thereafter increased gradually up to 7 days. Experiments with different root tissue showed that the activity was maximum in the elongation region e.g., 5 cm. above the root tip, while the basal root tissue showed less activity than the root tip region.

B.6.1.3. *Purification phosphohexoseisomerase*

The enzyme was isolated from etiolated whole mung bean seedlings or from different organs by macerating in glass mortar with 10 vol. of 0.03 *M* KOH solution. The solution was strained through two layers of cheese cloth and centrifuged at 15,000×g for 15 mins. The clear supernatant, adjusted to *p*H 7.5, was treated with 0.05 vol. of 1*M* $MnCl_2$ to precipitate the nucleate. The nucleic acid free supernatant was fractionated with solid ammonium sulphate. Only the fraction 45%–65% was collected and dissolved in minimum quantity of distilled water. This fraction showed the highest specific and total activity. This fraction was dialysed overnight against cold distilled water. The dialysed extract was refractionated with solid ammonium sulphate at 45%–55% and 55%–65%. Both fractions were collected, dissolved in minimum quantity of distilled water and dialysed for 3 hours against cold distilled water. The dialysed 55%–65% fraction showed the highest sp. activity and maximum total activity.

B.6.1.4. *Phosphohexoseisomerase activity in whole mung been seedlings and in different organs at different ages*

Experiments with whole seedlings showed that the activity is fairly high in 24 hours and increased with age reaching a maximum value on the 5th day and thereafter gradually decreased reaching a minimum constant value by the 7th day. In individual organs, the activity in 24 hours was highest in the cotyledons and gradually decreased with age. In the roots the activity was highest in 48 hours and then gradually diminished with age. In the hypocotyls the activity was rather low up to 48 hours but gradually rose reaching a maximum value on the 6th day. Thereafter it decreased with age. Experiments with

different tissues of the root showed that the activity was less in the elongation region than in the root tip.

B.6.1.5. *Tissue culture*

(a) *Habituation.* The experiments undertaken and reported in previous year to 'habituate' hollyhock and *Coreopsis* normal tissue were continued. The tissues could not be maintained for a long period on the basal medium devoid of any exogenous growth substance of the auxin type and repeated attempts to isolate habituated tissue strains were unsuccessful. This appears to indicate that hollyhock and *Coreopsis* tissue cultures do not belong to the same type as sunflower and carrot tissue which could be easily habituated.

(b) *Normal tissue culture of* Vicia faba. Preliminary experiments showed that satisfactory callus was formed when longer stem pieces (1 cm.) were used and the stem pieces were placed, on the Gautherets' basal medium with the basal end up. These conditions were maintained in the subsequent experiment to study the response of stem pieces to different growth substances used individually and, also, each in combination with coconut milk. Best callus formation was observed when the medium was supplemented with indole-acetic acid (10^{-6}). Other growth substances also produced callus varying from poor to moderate. However, in the presence of indole-acetic acid alone and also in combination with coconut milk extensive root development was noticed. In the presence of naphthalene-acetic acid in combination with coconut milk, root development was practically absent and callus formation was moderate. The callus tissue excised from the stem pieces were transferred to fresh medium containing the respective growth substances and 3–4 subsequent transfers at the interval of 30 days each would be necessary to confirm the growth substance necessary for maintaining the continued growth of the isolated callus tissue.

(c) *Comparative study.* Utilisation of different carbohydrates as sole source of carbon and of various amino-acids in presence and in absence of inorganic nitrogen by *Coreopsis* gall and normal tissue cultures was studied. No carbohydrate was found to replace sucrose as a sole source of carbon. Starch was not utilised by both gall and normal tissue. Inorganic nitrogen in the form of nitrate was found to be the best source of nitrogen. In presence of nitrate effect of amino-acids on tissue growth varied from slight inhibition to slight stimulation. When thiamine was present in the medium, glycine and cysteine had a marked stimulatory effect. No qualitative difference between the response of the gall and the normal tissue to the substances tested was observed.

The hollyhock normal tissue isolated previously and maintained in 'tobacco' medium was found to grow at a far slower rate than that of the corresponding crown gall tissue. In order to have a better comparison in biochemical studies it seems desirable to maintain the growth rate of the tissues at more or less the same level. Attempts made to increase the growth rate of normal tissue showed that the full complement of vitamin mixture stimulated tissue growth. However increase in the growth rate was not appreciable. This vitamin mixture had only a slight stimulatory effect on crown gall tissue. Other supplement such as adenine, guanine, glutamic acid, asparatic acid, aza-auxins etc., are being tried.

The main citerion of growth used mostly in plant tissue culture work is the total increment of weight in the growing culture. This suffices for a general purpose but for understanding the mechanism it has been recognised to take into account the growth as a result of increase in cell number and cell size and also to consider growth in terms of some important tissue constituent. It has been reported that tissue constituents such as total nitrogen level differ quantitatively in normal and gall tissue. The reports further show that there is variation in the differences reported by different authors. Discrepancies in the results based in terms of some tissue constituent levels appears to be due to differences in the incubation period of the tissues used. In order to study the progressive changes in the broad tissue constituents during the full period of growth curve of hollyhock gall and normal tissue, the methods for simultaneous estimation of total nitrogen, protein and non-protein nitrogen, total phosphorus, inorganic and organic phosphorus, RNA and DNA and total cell number and rate of cell division based on increase in non-vacuolated cells are being standardised.

C. BOTANY

C.2. Plant Physiology

C.2.1. *Electrical reaction of the pulvinus of Mimosa in a state of excitation*

In the last year's report (C2.2, 1960-61) some investigations undertaken for elucidation of the biphasic impulse appearing in the petiolar action wave with the fall of the pulvinus, were given. It was shown that the biphasic impulse preeedcs the fall of the leaf and the time lag is not due to the inertia of the leaf fall. It has also been shown how the biphasic impulse was probably due to a hydrostatic impact in the pulvinus.

It was next proposed to study the electrical variations of the different regions of the pulvinus in order to observe how excitation is transmitted through the organ.

From his studies of the electrical impulse J. C. Bose concluded that the excitatory reaction of the contractile tissue of the pulvinus is different from the conducting tissue of the petiole, as that of the muscle from the nerve. Bose's observation indicated that though the pulvinus has contractile tissue, it has also the conducting tissue through which excitation is transmitted to other regions of the plant. Recently Sibaoka in his studies of the action wave propagation in Mimosa has compared the pulvinus with a muscle strip attached to a nerve strand. According to him the action wave or the '*m*' wave as it is now called, propagated through the petiole along with the transmission of excitation, is not conducted through the pulvinus; the pulvinus is excited in a discontinuous way. In the pulvinus, the only electrical impulse he obtained by connecting at different regions, was a sharp negative one corresponding in time with the appearance of the biphasic impulse in the petiolar action wave. He further remarked that the propagation of excitation in the pulvinus is always unidirectional, from apex to base.

Eye observation indicates that excitation produced in a leaf by moderate stimulation passes to other leaves. Excitation can also be produced in a leaf by stimulation at the stem. This indicates that the propagation of excitation through the pulvinuscan occur in both directions.

Sibaoka has further shown that a slow wave is also propagated from the point of stimulation. He has assumed that this wave arises from the translocation of a chemical substance with the transpiration stream through the xylem. He has concluded that under certain conditions the '*s*' wave can freshly initiate '*m*' wave.

In the present study electrical impulses at different points in the pulvinus and the contiguous regions were recorded, with the idea of getting satisfactory answer to certain points mentioned above, which seem not to be fully explained.

The observations can be summarised as follows:

1) The petiolar action wave which gradually increases in amplitude as it is propagated towards the basal region, becomes reduced to some extent as it approaches near the pulvinar

junction. At the junctional point the duration of the action wave propagation is protracted and the impulse curve is a flattened one compared to that of the petiole. This indicates that the impulse encounters more resistance in passing through that region.

2) In the pulvinus within a millimeter of the apex the electrical impulse is composed of '*m*' wave, as well as, the characteristic sharp negative impulse of the pulvinus. The initial part can be distinctly recognised as the rising phase of '*m*' wave. But before the rising phase of '*m*' wave is completed the sharp negative impulse appears on it. In the closing part of the electrical impulse the falling phase of the '*m*' wave can be recognised.

3) In the mid region of the pulvinus the sharp negative impulse is largest in amplitude. The propagation of '*m*' wave can be discriminated in the falling phase of the impulse.

4) In the basal portion of the pulvinus the sharp negative impulse and the propagation of '*m*' wave can be clearly discriminated. In this case the sharp negative impulse appears first which is followed by the propagation of the '*m*' wave. In some cases the two peaks are quite distinct.

5) In the case of an insensitive pulvinus which shows neither the contractile response nor the sharp negative electrical impulse, the propagation of '*m*' wave is the only indication that excitation has been conducted there.

6) When excitation spreads beyond the leaf, the time of appearance of '*m*' wave in the stem after the fall of the pulvinus indicates that it encounters resistance to pass through the junction of the pulvinus and the stem.

7) When excitation enters the leaf from the stem side the electrical impulse of the pulvinus shows that it is transmitted from base to apex direction.

8) The '*s*' wave has been recorded in this apparatus by D.C. coupling arrangement of the amplifier. It has been found that the pulvinar reactions both mechanical and electrical are completed long before the '*s*' wave is propagated to the base of the petiole.

From the above observations the following conclusions may be drawn:

The '*m*' wave is propagated in the pulvinus in a continuous way with transmission of excitation from the petiole. The sharp negative impulse arises when the excitation is propagated in the mid region of the pulvinus. The sharp negative impulse may be assumed to be due to sudden contraction of some tissue and the biphasic impulse elsewhere is produced as a result of hydrostatic impact from that region. In different parts of the plant though there is continuity of conducting tissue there is difference in the velocity of conduction in different regions. Propagation of excitation through the junctional regions encounters resistance. Direction of the propagation of excitation through the pulvinus depends upon the source of stimulation. Transmission of '*s*' wave seems to have no relation with excitatory transmission in the pulvinus.

C.2.2. *Multiple response phenomenon in Biophytum*

J. C. Bose observed that the-leaflet of Biophytum executes multiple responses to a single stimulus. According to him when the energy of a stimulus is not wholely released in a single response, the residual energy is held latent in the tissue to be dissipated in

multiple responses. He studied the phenomenon by recording both mechanical and electrical responses. In the electrical records he obtained with the help of galvanometer, the nature of multiple responses is not well defined.

The present study of the electrical phenomenon of multiple response with pen recorder shows that each response is constituted of two impulses forming a pair. When a connection is made in the middle of the midrib and a stimulus is applied by a momentary touch of hot point at the tip of the leaf, responses one after another appear at the electrode for about 15 minutes. The number of such multiple responses to a single stimulus varies from 10 to 20 in different specimens. The time interval between responses is gradually prolonged. The amplitude of responses also gradually diminishes. Of the two impulses in each response the amplitude of the first is bigger than the second. The intervening time between the two impulses in the response also increases gradually.

By recording the electrical responses at two points of the petiole away from each other, it has been found that the second impulse in both appears quite simultaneously. The time difference in the appearance of the first impulse in the two responses indicates the velocity of transmission of excitation.

Simultaneous appearance of the second impulse at different points in the leaf indicates that it is reflected from the pulvinus like that of the biphasic impulse of Mimosa appearing in the petiolar action wave. The first impulse gradually increases in amplitude in its propagation from the apical towards the basal direction. This also happens in the propagation of action wave in the petiole of Mimosa. The second impulse of the paired response is bigger in amplitude in the basal region. The biphasic impulse in Mimosa has also been found to be bigger in amplitude in the basal portion of the petiole. When the excitation cannot reach the pulvinus as is indicated by the absence of response in the basal connection, the multiple responses in the apical connection are constituted of single impulse.

C.2.3. *Cellular pulsations in plant*

J. C. Bose showed that the rhythmic pulsatory activity is not limited to the pulvini of Desmodium and a few others similar plants with motile organs; it is present in all living cells of plants. This he showed by taking electrical records of non-motile plants. According to him this property of the living cells functions in the ascent of sap in plants.

Pen-oscillographic study also indicated minute traces of such pulsation but they lacked in definition. Addition of an improvised optical arrangement with the pen-recorder increased the efficiency of the system by about 20 times. With this arrangement it has been possible to obtain sizable pulses in ordinary plants.

A preliminary study has been made by following with a pencil on a moving drum, the spot of light reflected from the mirror attached to the pen axis. In this way electrical pulsations of different plant organs such as stem, root, petiole etc., of some plants have been recorded. The pulses are irregular and periodic in nature exhibiting a magnitude of 1 to 5 millivolts. Raising of temperature has been found to increase cellular activity as indicated by the enhancement of the amplitude and frequency of pulsation.

Cellular pulsations of the petiole and the midrib of the leaflet of Desmodium have been found to be periodically interrupted by the appearance of a large impulse correlated

with the contractile movement of the pulvinus. The impulse is constituted of a preliminary positive followed by a negative. It has been found from simultaneous recording of the electrical pulsations of the pulvinus and the petiole or the midrib that the preliminary positive in the impulse of the latter corresponds with the negative spike potential of the pulvinus. This indicates that during contraction of the pulvinus there is a hydrostatic impact from the pulvinus to other tissues, though it could not be detected in a previous study with the help of micropotometer.

Further studies will be undertaken by recording with the proposed photographic arrangement.

C.2.4. *Diurnal movement of the pinnule of Mimosa pudica*

The pinnules of Mimosa pudica exhibit a nyctinastic movement by remaining open during the day and closing at night. Correct recording of the diurnal movement can only show if its behaviour is as simple as that.

An automatic arrangement has been devised after many trials for correct recording of the diurnal movement. Automatic arrangements have been made for simultaneous recording of light and temperature variations.

Preliminary observations indicate that besides the influence of environmental variations on its movement, it has also an endogenous rhythm. It is however different from the one exhibited by the main petiole. In the main petiole the rhythm is executed in the evening; a thorough study of the latter has been published in a recent communication. In the pinnule the rhythm has been found to appear in the early morning. The pinnules open once and then close rapidly, after which the pinnules gradually attain the full opening position.

This primary opening and closing movement of the pinnule in the morning is executed even when the plant is kept in constant dark condition for twenty-four hours. Investigations are being continued to make a thorough study of this rhythm.

C.2.5. *Effect of electric current on the growth of coleoptile*

Various plant movements, such as geo, photo and other tropic responses have been found to be associated with the establishment of an electrical polarity. Different workers have suggested that production of an electrical polarity may be correlated with differential distribution of hormone, which is responsible for higher growth rate at one side of an organ. Indication of growth increase in coleoptile by application of a weak electric current, base to apex, has also been reported by some using reading microscope for the study.

It was proposed to undertake the investigation by employment of a self-recording apparatus constructed in the Institute. An automatic arrangement has been completed for simultaneous recording of the diurnal growth curves of two similar specimens at a time, so that the variations of the curve due to an applied electric current may be compared side by side with the control.

C.3. Cytology

C.3.1. *Cytology of some sensitive species of Mimosa*

The cytology of three species of sensitive Mimosa (i) M. pudica, (ii) M. pigra and (iii) M. invisa which were locally available was studied. The chromosome number of one of them (ii) is reported for the first time. The value of $2n$, the diploid chromosome number were found for (i) M. pudica ($2n = 48$), (ii) M. pigra ($2n = 48$) and (iii) M. invisa ($2n = 24$). Somatic chromosome numbers of the species which were reported previously appeared conflicting and for further confirmation both mitotic and meiotic behaviour were studied. Variations in chromosome numbers in some tissues were observed. Previous records and present observations indicate the existence of species with different chromosome numbers in different geographic region.

It appears probable from the present studies that polyploidy as well as structural alterations of chromosomes have played their part in the origin of new species.

C.4. Radiation mutations

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

This work has been carried out under a research project which is being financed by the Indian Central Oilseeds Committee since 1950. The aim of the investigation is to induce mutants with desirable economic characters in Til (*Sesamum*) and Rape—-Mustard (*Brassica*) by using ionizing radiations. Both the dominant and recessive mutants of economic importance viz., increased numbrer 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; fresh treatments were also given during the current year.

C.4.1. *Sesamum* (Til)

The season was favourable for the growth of *Sesamum* although some of the experiments were affected by a few unexpected rains at the time of maturity.

C.4.1.a. *Effects of X-rays*

During the year under report, observations were continued on the *Sesamum orientale* L. var. *T* 10, *T* 12, *T* 16, and *T* 20 of West Bengal and *TMV* 1 from Madras. Apart from these the further performance of the X-ray mutants viz., *White flowered mutant* (WFM), *Small seeded mutant* SSM_1 and SSM_2 (*Small seed mutant*) was also studied.

C.4.1.a.1. *Breeding behaviour of the recessive mutants*

The *White flowered mutant* (WFM) and the *Small seeded mutant* (SSM_1) isolated from *Sesamum* var. *T* 16 in the X_3 and X_4 generations after 20,000 r and 14,400 r X-ray treatments respectively were studied during the year under report with respect to their oil content and peroxide values and these are included in Tables 1 and 2.

TABLE 1

Control and mutants	Mean percent of oil ± S.E.
T 16 (W. Bengal)	44.66±0.31
White flowered mutant	45.26±0.31
Small seeded mutant	*54.34±0.31

* Significant at 1% level.

It will be observed from Table 1 that the mean oil yield in the *White flowered* and *Small seeded* mutants were 45.26 and 54.34 per cent respectively as against 44.66 per cent in the control (*T* 26), the difference, of 9.68 between the latter two being highly significant.

Table 2 gives the characteristics of the oil of the control and the mutants during the year under report.

TABLE 2

Control and mutants	Peroxide values of fresh oil	Colour	
		R	Y
T 16 (W. Bengal)	1.6	0.0	0.5
White flowered mutant	0.0	0.0	0.6
Small seeded mutant	0.0	0.0	1.2

It will be seen from table 2, that the peroxide value of fresh oil of *T* 16 was 1.6 while no peroxide value was noted in the *White flowered* and *Small seeded* mutants. But the oils of the mutants were slightly darker coloured than that of their control of the same year.

The other characteristics of the oils, such as refractive index, iodine value, sap value etc., of the mutants did not show marked variation when compared to the control.

The performance of the mutant viz., SSM_2 isolated in the X_3–1959 generation from *Sesamum* var. *T* 10 of West Bengal after 1,25,000 r X-ray treatment was studied and the mean yield of oil in the X_5–1961 generation is given in Table 3.

TABLE 3

Control and mutant	Mean percentage of oil ± S.E.
T 10 (W. Bengal)	47.85±0.43
SSM_2	*55.75±0.48

* Significant at 1% level.

It will be noted from Table 3 that SSM_2 continued to be higher yielding than the control (*T* 10) during the current year and gave about 8 per cent more oil agreeing with the previous year's finding.

The mean time of flowering in SSM_2 was 2.4 days earlier than the control during the year under report and this was found to be satistically significant.

C.4.1.a.2. *Breeding behaviour of higher yielding selections*

The yield performance of *Sesamum* var. *T* 10, *T* 12, *T* 16 and *T* 20 in different generations after X-radiation during the current year is given in the Table 4. These are the progenies of the best selections from the previous generation.

TABLE 4

Variety	Control and X-ray treatment	Generation	Selection No.	Yield	
				No. of fruits	Wt. of seeds (gm)
T 12	Control	X —1961	2/24	148	15.00
	14,500 r	,,	3/6	242	16.15
T 16	Control	,,	5/2	208	23.80
	14,500 r	,,	7/26	318	22.40
T 10	Control	X —1961	8/3	240	23.00
	56,000 r	,,	10/12	148	25.20
	80,000 r	,,	12/26	435	33.40
T 20	Control	X —1961	16/19	212	27.30
	80,000 r	,,	17/21	320	28.30

It may be noted also that selections in treatments made for higher yield in the previous year, continued to give higher yields with regard to the number of fruits and/or weight of seeds during the year under report in the different varieties, treatments and generations.

Some of selections in the treatments of *Sesamum* var. *T* 10, *T* 12, *T* 20 and *TMV* 1 after 75,000 r, 1,00,000 r and 1,25,000 r X-rays in the X_5 generation and var. *T* 10, *T* 16, *White flowered mutant* and *Small seeded mutant* after 10,000 r, 20,000 r, 30,000 r, and 50,000 r X-rays in the X_2 generation during the current year gave higher number of fruits and/or weight of seeds than their respective controls.

C.4.1.a.3. *Flowering behaviour*

The progenies of some of the treatments in the different generations during the year under report were early flowering upto 10 days as against their controls.

C.4.1.b. *Effects of radioactive phosphorus*—p^{32}.

C.4.1.b.1. *Breeding behaviour of higher yielding selections*

In the P_4–1961 generation, the best selections in 1830μc/48 and 72 hours in *T* 16; 1830μc/24 hours and 720 μ/48 hours in *White flowered* and *Small seeded* mutants gave higher yield in number of fruits and/or weight of seeds than their respective dry controls.

In the P_2 generation during the current year, the selections in 1740μc/24, 48 and 72 hours and 2610μc/24 hours in *T* 10, 1433μc and 2150μc/48 hours in *T* 16 and *T* 20 and 1433 and 2150μcs/96 hours in *T* 16 gave in most of the cases higher number of fruits and weight of seeds as compared to their corresponding dry controls.

C.4.1.b.2. *Flowering behaviour*

The progenies of most of the treatments in the P_4 and P_2 generations during the year under report were early flowering from 3 to 8 days as compared to their respective dry controls and the maximum earliness was noted in the P_2-generation of *Sesamum* var *T* 10 after 2610μc/24 hours treatment.

C.4.1.c. *Effects of radioactive sulphur*—S^{35}

C.4.1.c.1. *Breeding behaviour of higher yielding selections*

All the selections in the treatments of *T* 16 during S_4–1961 generation were lower yielding than the dry control. But the selections in 630μc/24 and 48 hours and 1960μc/72 hours in the *White flowered mutant* and 1960μc/48 hours and 72 hours treatments in the *Small seeded mutant* gave higher fruit number and seed yield than their respective dry controls.

1953μc/24, 48 and 72 hours in *T* 10; 1892 and 2837 *cs*/48 hours in *T* 16 and *T* 20 and 1892 and 2937μcs/96 hours in *T* 16 gave higher number of fruits and/or weight of seeds than their dry controls in the S_2 generation during the year under report.

C.4.1.c.2. *Flowering behaviour*

In the S_4 and S_2 generations during the current year, the plants in the treatments started flowering earlier in most cases than their respective dry controls, the difference being from 1 to 4 days.

C.4.1.d. *Effects of gamma rays*—Co^{60}

C.4.1.d.1. *Breeding behaviour of higher yielding selections*

Table 5 gives the yield performance of *Sesamum* var. *T* 12 and *T* 16 in the G_3–1961 generation where the seedlings were treated in the G_1–1959 generation.

TABLE 5

Variety	Control and gamma ray treatment*	Selection No.	Yield	
			No. of fruit	Wt. of seeds (gm)
T 12	Control	2/19	277	22.27
	16,524 r	5/3	178	17.83
	4,135 r	6/11	163	25.94
	1,034 r	9/41	303	31.75
	622 r	11/6	220	29.63
T 16	Control	13/29	193	28.29
	16,524 r	15/34	201	33.91
	4,135 r	16/6	184	32.20
	1,034 r	19/29	251	33.63
	662 r	20/19	225	35.60

* Seedlings treated in pots.

It will be noted from Table 5 that the selections in treatments of 1,034 r in *T* 12, and 16,524 r, 1,034 r and 662 r in *T* 16 had higher number of fruits and weight of seeds; other treatments, except 16,524 r in *T* 12 gave greater weight of seeds only than their controls.

Selections of the progenies of the chronically irradiated *Sesamum* var. *T* 12 in beds, V, VI and IX and var. *T* 16 in beds I, II, IV, V, VI, VII and IX gave increased weight of seeds, with lesser number of fruits in most cases, as compared to their controls in the G –1961 generation.

In the G_2 and G_1 generations of the *Sesamum* varieties some of the selections in the treatments, both acute and chronic, were higher yielding in number of fruits and/or weight of seeds as against their control.

C.4.1.d.2. *Flowering behaviour*

In the G_3 and G_2 generations during the current year, most of the treatments where the seeds, seedlings or plants from sowing till fruiting were treated, were earlier flowering by 1 to 6 days when compared to their respective controls. In the G_1–1961 generation of *Sesamum* varieties, no appreciable difference was noted in the time of flowering between the controls and the chronic treatments.

C.4.1.d.3. *Pollen sterility*

In the chronic exposure of *Sesamum* var. *T* 16, *White flowered mutant*, *Small seeded mutant* and *TMV* 1, the treatments in the G_1–1961 generation, in general showed higher sterility of pollen grains than their controls.

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

In continuation of the previous years investigation in *Brassica* and *B. campestries* further studies were made on the mutagenic effect of ionizing radiation with the following types: *Tori* 7 (W. Bengal), *Brown sarson* (E. Punjab), *B. juncea* var. *Rai* 5 (W. Bengal), *R.L.* 9 (E. Punjab), and the X-ray isolated *Appressed pod* mutant '(*APM*)' and *Thickened pod* pod mutant '(*TPM*)'.

Observations were made also during the year under report, on 23 new varieties brought from Regional Research Centre, Kanpur, which were put for comparative yield trial and experiment. Study was made of their genetical variability together with some stabilized mutants and some high yielding selections and their respective controls.

The season was favourable for the growth of *Brassica*; unfortunately plants under current beta rays did not survive.

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 of X_4 generation.

TABLE 6

Type and locality	Control and X-ray treatment	No. of days taken for first flowering	Selection No.	Yield		Height	Branch
				No. of fruits	Weight of seeds in gms.		
Tori 7 (W.Bengal)	Control	33	1/13	824	8.430	73.0	4
	40,000 r	38	3/41	478	9.450	88.2	5
	60,000 r	36	6/6	641	8.190	97.1	6
	80,000 r	37	11/37	819	14.910	95.0	5
	1,00,000 r	26	14/41	416	5.955	88.0	3

The treatment 80,000 r X-ray gave maximum yield with 819 fruits with 14.910 gms of seed weight respectively against the control with 824 fruits and 8.430 gms. None of the selections were early in flowering.

TABLE 7

Type and locality	Control and X-ray treatment	No. of days taken for first flowering	Selection No.	Yield		Height	Branch
				No. of fruits	Weight of seeds in gms.		
Rai 5 (W.Bengal)	Control	42	2/49	307	8.40	114.2	5
	10,000 r	56	3/3	1044	2.72	108.2	4
	20,000 r	41	4/20	555	5.50	113.2	4
	30,000 r	45	5/16	655	6..40	108.2	5
	40,000 r	41	6/7	1383	13.90	118.2	4
	50,000 r	39	7/43	618	3.80	93.2	5

It will be seen from the table (yield behaviour in X_3 generation) that the selection in 40,000 r X-ray treatment of Rai 5 gave higher number of fruits and weight of seeds than the control.

Flowering behaviour of the selections under treatments 20,000 r; 40,000 r and 50,000 r were earlier by 1 and 2 days as compared to the control.

TABLE 8

Type and locality	Control and X-ray treatment	No. of days taken for first flowering	Selection No.	Yield		Height	Branch
				No. of fruits	Weight of seeds in gms.		
Rai 5 (W.Bengal)	Control	53	2/5	1327	18.590	141.0	7
	26,000 r	63	3/10	937	24.770	141.2	7
	70,000 r	55	4/38	870	15.945	132.2	7
Brown sarson (E.Punjab)	Control	53	5/29	590	11.620	126.0	8
	26,000 r	44	6/23	448	20.240	109.2	9

Further selections made under treatment 26,000 r X-ray of *Rai* 5 and 26,000 r X-ray of *Brown Sarson* of X_{10} generation gave higher yield in seed weight compared with the controls.

C.4.2.a.2. *Flowering behaviour of selections*

It will be seen from the Table 6 that the selection in X_4 generation of *Tori* 7 under 1,00,000 r X-ray treatment is earlier in flowering than the control.

Table 7 shows that the selections in X_3 generation under treatments of 20,000 r, 40,000 r and 50,000 r are early in flowering in comparison with the control.

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

Two types, viz., *Code* 58 of *Brassica juncea* and *T* 42 of *Brassica campestris* were irradiated with P^{32} and S^{35} by soaking the seeds in isotope solutions for 24 hours. Seeds kept in

distilled water for the same period served as control. The activity and duration of the treatments were as follows:

P^{32} (i) 650.04μc, (ii) 975.06μc and (iii) 1625.10μc-24 hours. S^{35} (i) 931.42μc, (ii) 1397.13μc and (iii) 2328.55μc-24 hours. All plants under treatment failed to survive. This was due to the lethality of the dosages or heavy rains. However, germination behaviour has been observed.

C.4.2.d. *Effect chronic gamma rays* (Co^{60}) *Brassica*

C.4.2.d.1. *Yield behaviour of selections*

Table 9 presents the yield behaviour of the selections in the G_1 generation of the following varieties: (i) *Laha* 101, (ii) *Laha* 46, (iii) *R.L.* 9.

TABLE 9

Type and locality	Control and total gamma ray treatment in mr.	Selection No.	No. of fruits	No. of days taken for first flowering	No. of branches
Laha 46 (Kanpur)	Control	1/12	121	73	5
	20,13,263	I/8/1	230	60	8
	7,30,426	II/7/2	320	60	9
	2,62,903	III/2/3	278	60	8
	1,34,302	IV/4/5	279	61	8
	74,753	V/4/3	287	63	7
Laha 101 (Kanpur)	Control	5/17	200	62	9
	20,13,263	I/7/1	213	63	5
	7,30,426	II/7/1	320	60	9
	2,62,903	III/2/3	278	60	8
	1,34,302	IV/4/5	279	61	8
	74,753	V/4/13	287	63	7
R. L. 9 (Kanpur)	Control	4/10	662	48	10
	20,13,263	I/10/1	410	49	6
	7,30,426	II/4/3	487	47	7
	2,62,903	III/9/10	1042	53	9
	1,34,302	IV/8/9	942	53	9
	74,753	V/7/8	1320	67	12

It will be seen from the table that all the selections in the treatments of *Laha* 46, *Laha* 101 gave higher number of fruits than the control. In case of *R. L.* 9 higher yield compared with the control has been observed under treatments —262, 903 mr., 1,34,302 mr., and 74,753 mr. respectively.

C.4.2.d.2. *Flowering behaviour*

All the selections in the treatments of *Laha* 46 were earlier than the control. In case of *Laha* 101 and *R. L.* 9 some early flowering selections are mentioned here.

In continuation of the previous year's investigation further studies were made on the higher yielding and early flowering selections under chronic and acute gamma radiation (Co^{60}). The behaviour of these are under observation.

C.4.2.d.3. *Pollen grain sterility*

In the current treatment of gamma ray in *Laha* 46. *Laha* 101, *R.L.* 9, and *Tori* 7 no appreciable changes in the pollen sterility have been observed in the controls and 1st generation treated plants. The data are given in Table 10.

TABLE 10

Control and treatment	*R.L.*9	*Laha* 101	*Laha* 46	*Tori* 7
Control	2.11	2.91	1.12	3.85
20,13,263	6.43	2.01	1.37	4.67
7,30,426	6.63	1.41	1.07	4.12
26,02,903	1.36	0.87	0.88	5.80
1,34,302	3.24	1.41	1.04	4.20
74,753	5.53	1.99	1.29	2.11

C.4.2.e. *Cytological observations*

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

C.4.3. *Radiation mutation in Jute*

This scheme is financed by the Indian Central Jute Committee and is in the 6th year of investigation.

C.4.3.1. *Sowing season.*—The sowing of seeds of different varieties of jute was started at the end of April. Proper irrigation in the field was available.

C.4.3.2. *Treatment.*—The varieties T.M., R.26, C.G. from *Corchorus olitorius* were selected for the radiation mutation work in jute. The dry seeds of T.M., R.26 and C.G. varieties were treated with 2.0 mc and 3.0 mc of P^{32}; in D.154 (var. Capsularis) a dose of 5 mc and 10 mc of P^{32} were applied, as this type was found to be resistant to low doses. In treatments with S^{35}, however, the doses applied were 8 mc and 16 mc in all the above mentioned varieties. The doses mentioned so far indicate the initial activities of the isotopes used for the experiment. The treatments were continued for 12 hours and the treated seeds with their respective controls were sown in replicated plots at Falta Experimental Station. Seeds of the varieties T.M., R.26, C.G. and D.154 were also treated with 30,000 and 60,000 r of X-rays and these together with their respective controls were sown in replicated plots at Falta Experimental Station of the Bose Institute.

C.4.3.3. *Germination:* Radiation effects were first noted during the germination of seeds in the field. Altogether 600 seeds were treated in each treatment and were sown in the field; and out of these the percentage of germination was calculated. From Table 11a it can be seen that in all the varieties the percentage of germination after treatment with P^{32} falls gradually with the increase of doses; only one exception is noticed, i.e., in the variety R.26 with 2.0 mc treatment, where the value is lower than the next higher treatment. In Table 11b it can be seen that seeds after S^{35} treatment showed a stimulating effect in germination. In most cases the germination percentage increases with the increase of doses.

Thus the radioactive S^{35} increases the germination percentage whereas the P^{32} decreases the germination percentage of the seeds. In Table 11c the germination percentage of the seeds after X-ray treatment is shown where in each case 200 seeds were treated. It is seen from the Table 11c that the germination percentage falls gradually with the increase of doses.

TABLE 11a

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

Total no. of seeds sown—600/per treat.
Date of sowing—29-4-1961

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	42.33	30.33	33.00	35.00
2.0 mc	36.17	25.67	32.67	×
4.0 mc	22.17	28.00	29.50	×
5.0 mc	×	×	×	29.00
10.0 mc	×	×	×	27.17

TABLE 11b

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

Total no. of seeds sown—600/per treat.
Date of sowing—29-4-1961

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	36.50	29.50	32.50	31.30
8.0 mc	35.50	38.70	45.17	26.80
16.0 mc	46.50	41.50	45.17	38.50

TABLE 11c

GERMINATION PERCENTAGE OF SEEDS AFTER TREATMENT WITH X-RAYS

Total no. of seeds sown—200/per treat.
Date of sowing—11-5-1961

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	54.00	49.50	46.00	89.00
30,000 r	38.00	31.50	32.00	69.00
60,000 r	24.00	11.00	17.00	61.00

TABLE 12a

FLOWERING (IN DAYS) P_1—POPULATION

Variety T.M.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	111	140.95±0.58
2.0 mc	119	141.75±0.43
4.0 mc	140	144.27±0.72

Variety R.26

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	69	127.70±1.51
2.0 mc	73	105.25±2.12
4.0 mc	104	130.85±2.19

Variety C.G.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	80	103.35±0.79
2.0 mc	82	103.55±0.82
4.0 mc	95	108.33±1.17

Variety D.154

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	108	118.90±0.56
5.0 mc	110	121.55±0.74
10.0 mc	113	125.10±0.73

TABLE 12b

FLOWERING (IN DAYS) S_1—POPULATION

Variety T.M.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	114	138.05±0.78
8.0 mc	106	138.90±0.73
16.0 mc	114	138.00±0.75

TABLE 12b—*contd.*

Variety R.26

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	94	119.35±1.27
8.0 mc	77	105.65±1.51
16.0 mc	77	105.10±1.42

Variety C.G.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	88	102.80±0.76
8.0 mc	77	103.15±0.86
16.0 mc	92	105.60±0.61

Variety D.154

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	110	120.95±0.59
8.0 mc	108	120.49±0.52
16.0 mc	110	122.88±0.59

C.4.3.4. *Flowering:* The flowering behaviour of the P_1, S_1 and X_1 populations was observed in the field during the growth of individual plants. From Table 12a it has been found that almost all the varieties treated with P^{32} start flowering later than their respective controls, but as shown in Table 12b, the results are a bit different. Here in many cases the treated populations start flowering earlier than the control. Again from Table 12c, it is seen that the X-ray treated populations starts flowering later than their respective control; and with the gradual increase of doses the lateness of flowering in days also increases as well as the variability of flowering among the treated population.

TABLE 12c

FLOWERING (IN DAYS)—X_1 POPULATION

Plants studied—786
Date of sowing—11-5-1961

Variety T.M.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	97	115.95±1.04
30,000 r	97	118.90±1.15
60,000 r	102	121.70±1.43

TABLE 12c—*contd.*

Variety R.26

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	76	104.30±1.48
30,000 r	77	101.80±1.89
60,000 r	95	112.40±2.74

Variety C.G.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	77	91.70±1.09
30,000 r	85	96.90±1.55
60,000 r	95	104.10±2.12

Variety D.154

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	97	120.75±0.59
30,000 r	109	125.21±0.60
60,000 r	117	125.02±0.53

C.4.3.5. *Pollen sterility:* The pollen grains of control and treated populations were collected from the experimental field at random, mounted in iodine solutions and observed under the microscope; on the basis of these observations the percentage of pollen sterility was counted. From Table 13a it is seen that the percentage of pollen sterility increased with the increase of doses after P^{32} treatment in all the varieties. Similar results though are not observed after treatment with S^{35}. Thus in the Table 13b only in few cases increase of pollen sterility has been observed although the doses applied (with S^{35}) were much higher than the P^{32} treatments. In the Table 13c it is found that populations raised after X-ray irradiation in the varieties T.M., R.26, C.G. and D.154 also show a gradual increase of pollen sterility with the increase of doses irrespective of the varieties treated.

TABLE 13a

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

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	1.23	0.77	0.33	7.18
2.0 mc	1.67	2.19	0.39	—
4.0 mc	1.67	25.73	14.07	—
5.0 mc	—	—	—	9.20
10.0 mc	—	—	—	11.99

TABLE 13b

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

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	0.22	0.52	0.32	0.68
8.0 mc	0.46	0.44	0.38	0.36
16.0 mc	0.11	0.50	0.42	1.21

TABLE 13c

PERCENTAGE OF POLLEN STERILITY AFTER TREATMENT WITH X-RAY

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	0.90	0.30	0.21	1.11
30,000 r	1.73	7.32	0.35	2.77
60,000 r	5.23	23.05	18.48	8.93

TABLE 14a

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

(No. of plants—852)

Date of sowing—29-4-1961

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	407.69 (1.68)	400.60 (1.72)	421.02 (1.92)	313.94 (1.86)
2.0 mc	393.86 (1.75)	330.93 (1.47)	361.85 (1.67)	×
4.0 mc	378.95 (1.60)	299.90 (1.33)	374.89 (1.71)	×
5.0 mc	×	×	×	303.92 (1.85)
10.0 mc	×	×	×	270.26 (1.58)

TABLE 14b

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

(No. of plants—1149)

Date of sowing—29-4-1961

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	381.98 (1.53)	369.71 (1.46)	374.21 (1.49)	297.62 (1.55)
8.0 mc	380.41 (1.50)	346.30 (1.35)	360.00 (1.41)	297.48 (1.55)
16.0 mc	387.00 (1.59)	321.69 (1.27)	339.81 (1.36)	276.59 (1.32)

TABLE 14c

AVERAGE HEIGHT AND BASAL DIAMETER (IN BRACKET) IN CENTIMETRE IN CONTROL AND X_1 POFULATION

(No. of plants—786)

Date of sowing—11-5-1961

Control and treatment	Variety T.M.	R.26	C.G.	D.154	JRO-632
Control	417.46 (1.71)	292.61 (1.07)	364.20 (7.51)	245.40 (1.36)	338.89 (1.31)
30,000 r	395.51 (1.63)	292.61 (1.00)	273.10 (1.41)	233.33 (1.22)	308.33 (1.19)
60,000 r	365.14 (1.44)	283.08 (1.18)	293.57 (1.18)	266.34 (1.93)	306.28 (1.18)

C.4.3.6. *Average height and basal diameter of the control and treated populations in the first generations*

From Table 14a it is seen that the average height and the basal diameter in the varieties T.M., R.26, C.G. and D.154 after P^{32} treatment decreased with the gradual increase of doses; only one exception is noticed, i.e., C.G. with 2.0 mc treatment. It can be seen from Table 14b that after S^{35} treatment the average height and the basal diameter of the treated population in the above mentioned varieties decrease gradually with the increase of doses, with the exception of seeds of T.M. treated with 16.0 mc where the average height and the basal diameter can be seen to have increased. The average height and the basal diameter of the control as well as the treated populations after X-ray treatment are shown in Table 14c. From Table 14c also it can be seen that the average height & the basal diameter decreases

gradually with the increase of doses; but in varieties R.26 and D.154 with 60,000 r treatment an increase in average height and basal diameter was noticed. In general, it has been found that radiations from different sources has a tendency to cause retardation of growth in the treated plants.

C.4.3.7. *Weight of Fibre and Wood of the control and treated population*

The fibre and the wood weight of the control and the treated population were taken during the year under report and are shown in the following tables.

TABLE 15a

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

(No. of plants—852)

Date of sowing—29-4-1961

Control and treatment	Variety T.M.	R.26	C.G.	D.254
Control	59.86 (23.50)	73.23 (27.87)	87.68 (35.08)	34.94 (15.08)
2.0 mc	49.27 (22.49)	52.15 (18.84)	50.76 (19.50)	×
4.0 mc	43.40 (16.87)	37.92 (14.70)	39.47 (16.13)	×
5.0 mc	×	×	×	42.56 (17.44)
10.0 mc	×	×	×	28.73 (11.10)

TABLE 15b

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

(No. of plants—1149)

Date of sowing—29-4-1961

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	53.63 (19.57)	57.99 (18.92)	51.17 (18.86)	30.93 (15.21)
8.0 mc	56.24 (19.40)	43.63 (14.33)	49.74 (19.29)	29.71 (13.47)
16.0 mc	50.56 (17.40)	43.79 (13.81)	40.11 (15.30)	25.28 (11.54)

TABLE 15c

AVERAGE WEIGHT (IN GMS) OF FIBRE (IN BRACKET) AND WOOD OF X_1 POPULATION

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	56.91 (17.56)	35.22 (8.50)	58.83 (19.14)	10.59 (4.15)
30,000 r	43.91 (15.73)	26.09 (7.25)	41.14 (14.69)	8.75 (3.32)
60,000 r	27.70 (8.80)	28.89 (7.09)	22.59 (7.59)	10.88 (3.49)

From Table 15a it is seen that populations raised from P^{32} treatment produce less quantity of fibre and wood than their respective controls and the yield falls gradually with the increase of dose; only in D.154 and 5.0 mc treatment an increase in yield of fibre and wood over the control is noticed. In Table 15b it is seen that populations raised from S^{35} treatment exhibit similar results as populations raised after P^{32} treatment; an exception is found in the case of T.M. with 8.0 mc treatment. In Table 15c it can be seen that the yield of fibre and wood gradually fall with the increased doses of X-ray and this effect is shown in all the varieties so far irradiated with X-rays. In D.154 with 60,000 r, a slight increase of wood (weight) over the control was observed.

C.4.3.8. *Second generation:* Second generation plants of different varieties of jute were raised from the seeds collected from populations of the treated first generation. The seeds were sown in lines in the Experimental Station of the Bose Institute at Falta. In addition to the study of segregation, observations on flowering, yield and pollen sterility etc., were taken during the year under report.

C.4.3.9. *Flowering:* The flowering behaviour in the control and treated populations were observed in the field in the varieties T.M., R.26, C.G. and D.154 and the data are given in the following tables.

TABLE 16a

FLOWERING (IN DAYS) P_2—POPULATION

Date of sowing—19-5-1961

T.M.

Control and treatment	First flowering time	Mean flowering time ± S.E.
Control	106	123.02 ± 0.98
1.5 mc	98	121.50 ± 0.82
3.0 mc	106	119.54 ± 1.13

TABLE 16a—*contd.*

*R.*26

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	78	95.80±1.41
1.5 mc	84	96.74±1.58
3.0 mc	84	100.55±1.34

C.G.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	88	103.90±1.75
1.5 mc	90	102.45±2.49
3.0 mc	87	103.45±1.47

*D.*154

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	107	117.42±0.92
7.5 mc	102	117.73±0.94
9.0 mc	103	116.78±0.80

TABLE 16b

FLOWERING (IN DAYS)—S_2 POPULATION

Date of sowing—19-5-1961
T.M.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	108	122.26±1.00
3.0 mc	106	119.84±1.21
6.0 mc	95	120.00±1.40
9.0 mc	106	119.00±1.32

*R.*26

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	82	102.90±1.80
3.0 mc	79	97.50±1.91
6.0 mc	83	105.85±1.80
9.0 mc	80	96.70±2.08

C.G.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	90	103.65±2.10
3.0 mc	90	101.60±1.70
6.0 mc	90	101.35±1.25
9.0 mc	92	105.80±1.92

TABLE 16b—*contd.*

*D.*154

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	107	116.22±0.80
3.0 mc	107	117.09±0.64
6.0 mc	106	116.63±0.72
9.0 mc	106	115.64±0.75

TABLE 16c

FLOWERING (IN DAYS) X_2—POPULATION

Date of sowing—7-4-1961
T.M.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	146	160.45±0.64
20,000 r	139	161.45±0.46
40,000 r	127	162.80±0.87

*R.*26

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	131	144.60±0.82
20,000 r	130	153.65±0.99
40,000 r	107	140.15±0.94

C.G.

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	80	111.80±1.58
20,000 r	82	114.30±1.41
40,000 r	91	119.30±0.86

*D.*154

Control and treatment	First flowering time	Mean flowering time±S.E.
Control	126	142.00±0.78
20,000 r	116	136.60±1.04
40,000 r	122	138.25±0.74

From Table 16a it is seen that in the variety T.M., the population after 1.5 mc treatment started flowering 8 days earlier than the control; the mean time of flowering is early only by 2 days. In type R.26 the treated populations started flowering later than the control.

In types C.G. and D.154, irregular flowering behaviour is noticed among the treated populations in the P_2 generation. From the Table 16b it is seen that after S^{35} treatment, the treated populations in the variety T.M. start flowering earlier than the control whereas in the variety R.26 the population after 3.0 mc and 9.0 mc treatment start flowering earlier than the control. In the variety C.G. the mean time of flowering in populations raised after 3.0 mc and 6.0 mc treatments was 2 days earlier than the control; and in the variety D.154 treatments no significant result regarding flowering behaviour is observed after S^{35} treatment. The flowering behaviour of the X-ray treated population is shown in the Table 16c. The varieties T.M., R.26, C.G. and D.154 were treated with 20,000 and 40,000 r of X-rays and the flowering behaviour noted. From Table 16c it is seen in the variety T.M. that though the treated population start flowering earlier than the control their mean time of flowering is later. In R.26 the X-ray treated population starts flowering earlier than the control but only the 40,000 r treatment shows earliness in the mean time of flowering. In variety C.G. the treated populations exhibit lateness in flowering; in D.154 earliness in flowering is observed among the treated populations.

C.4.3.10. *Weight of fibre and wood in the second generation:* The average weight of 10 plants was taken from P_2 and S_2 populations for the examination of fibre and wood weight, and the data are shown in Table 17a and 17b. From Table 17a it is seen that the treated populations in all the varieties produce less quantity of fibre and wood than their respective controls; this behaviour is also noticed amongst populations raised after S^{35} treatment with the exception of treatments in the varieties T.M. and R.26 given 6.0 mc and 9.0 mc doses. In R.26 with 9.0 mc treatment fibre weight is less than the control although the wood weight is higher.

TABLE 17a

AVERAGE WEIGHT (IN GMS) OF FIBRE (IN BRACKET) AND WOOD OF P_2—POPULATION

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	11.34 (4.96)	9.92 (3.35)	9.21 (3.54)	7.09 (3.54)
1.5 mc	9.21 (4.25)	10.63 (4.25)	7.09 (2.13)	×
3.0 mc	10.63 (4.96)	8.50 (4.96)	7.80 (3.54)	×
7.5 mc	×	×	×	7.09 (4.25)
9.0 mc	×	×	×	6.38 (2.83)

TABLE 17b

AVERAGE WEIGHT (IN GMS) OF FIBRE (IN BRACKET) AND WOOD OF S_2—POPULATION

Control and treatment	Variety T.M.	R.26	C.G.	D.154
Control	9.92 (4.96)	7.80 (4.25)	12.76 (5.67)	7.80 (4.25)
3.0 mc	7.80 (2.83)	7.09 (2.83)	10.63 (4.25)	7.80 (4.25)
6.0 mc	11.34 (7.09)	7.09 (3.54)	7.80 (2.83)	7.09 (3.54)
9.0 mc	9.92 (4.96)	10.63 (3.54)	7.09 (4.25)	7.80 (3.54)

C.4.3.11. *Morphology:* It has been observed that radioactive P^{32} is much more effective than $S.^{35}$ This is shown by the fact that lethality of seedlings is obtained with very low dose of P^{32} in comparison to S^{35}. Treatments with P^{32} and S^{35} produce, in the first generation, plants with abnormal morphological characters such as stems with heavy branching, bifurcation of stem; fasciation of stem, double leaves, forking of leaves, crinkled leaves etc,. but most of these characters do not persist in subsequent generations. Treatment with gamma rays and X-rays produce also plants with similarly changed morphological characters. Mutants or plants with changed morphological characters persisting, have also been isolated and are being studied. Mention may be made that a few trailing plants in the variety D.154 have been isolated after P^{32} treatment and are being studied in their third generation. Similar plants in the same variety have also been isolated after X-ray treatment. In another variety (R.26) some aberrant plants with twisted stem have been isolated in the second generation. The above mentioned aberrant plants require further study for evaluating their usefulness.

C.4.4. *Radiation mutation on Groundnut*

This scheme is financed by the Indian Central Oilseeds Committee for inducing mutants with X-rays and radioactive isotopes. This is the 4th year of investigation.

C.4.4.1. The weather was suitable at the time of sowing. At the time of maturity and harvest, unfavourable rains occurred which damaged some of the experiments.

A number of previously isolated mutants were grown in the field for observation and for maintaining certain selections. These included (i) Bushy Type mutant (var. Coimbatore) (ii) Creeping and Small Leaf mutants (var. AK 10) and (iii) Stunted or Dwarf mutant (var. Small Japan).

C.4.4.2. *Result of oil analysis:* Oil analysis of seeds of some of the irradiated progenies as well as of some of the mutants have been carried out in the year under report.

TABLE 18

OIL ANALYSIS OF DWARF MUTANT (MAX. YIELDER) VAR. SMALL JAPAN

Variety Small Japan	Generation P_4	Rep.I	Rep.II	Rep.III	Rep.IV	Rep.V	Rep.VI	Rep.VI Control
1. Per cent oil		54.98	56.85	56.70	55.44	58.60	59.70	56.27
2. Relactive Index		1.4635	1.4652	1.4639	1.4628	1.4650	1.4642	1.4651
3. Colour by Livibond Scale		Y = 0.5 R = 0	Y = 0.6 R = 0	Y = 0.9 R = 0	Y = 0.7 R = 0	Y = 0.5 R = 0	Y = 0.6 R = 0	Y = 0.6 R = 0
4. Peroxide value (Fresh)		2.2	2.3	2.8	2.6	1.8	4.8	1.3

TABLE 19

OIL ANALYSIS OF SOME TMV 3 BULKED POPULATION (X_2 GENERATION)

Dose	60,000 r	70,000 r	80,000 r
1. Oil per cent	56.06	50.20	53.09
2. Refractive Index	1.4630	1.4620	1.4620
3. Colour by Lovibond Scale	Y = 1.7 R = 0	Y = 1.6 R = 0	Y = 1.2 R = 0
4. Fresh Peroxide Value	3.3	4.5	4.2

TABLE 20

OIL ANALYSIS OF TMV 3 BULKED POPULATION (CONTROL)

	Replication II	Replication V
1) Oil per cent	52.8	50.2
2) Refractive Index	1.4639	1.4640
3) Colour by Lovibond Scale	Y = 0.6 R = 0.0	Y = 0.75 R = 0.00
4) Iodine value	94.5	92.8
5) Fresh Peroxide value	3.90	3.17

Table 18 shows the result of one such analysis in the P_4 generation of the Dwarf mutant (variety Small Japan). It will be seen that the oil content (as expressed in percentage) fluctuates from replication to replication, the highest percentage being obtained in Replication V and VI. This seems to suggest that environmental factors may play an important role in determining the oil content, though some variation due to segregation is to be expected and cannot be ruled out. In Table 19 some data are given of the oil content of some TMV 3 bulked population (X_2-generation). Differences can be seen, in the colour of the oil in these population and that of control TMV 3 grown in a different plot (Table 20). Further selection is necessary to see if these changes are stable.

C.4.4.3. *New Morphological variants*

One aberrant plant with large pods containing three seeds per pod was isolated in the X_2-generation in the variety TMV 3 after 60,000 r treatment. Further observations are necessary to see if this character breeds true or not.

C.4.4.4. *New Treatment.*—Fresh irradiation with 70,000 r X-rays was given to seeds of variety TMV 3. No first generation aberrant plants were detected.

C.4.4.5. *Gamma irradiation.*—The following varieties of Groundnut were sown in the Cobalt 60 source under chronic irradiation :— AK 10, Small Japan and TMV 3, both in the G_1 and G_2 generation.

TABLE 21

Bed No.	Distance	Dose
I	2.5 m	4417.29 r
II	7.5 m	491.27 r
III	12.5 m	176.69 r
IV	17.5 m	90.17 r
V	22.5 m	54.51 r
VI	27.5 m	36.52 r

Table 21 gives the distance from the source at which they were grown and the doses received by the above varieties in the different beds (Bed Nos. 1–6). No abnormality was found in any of the plants in the G_1 and G_2 generation, though there was some evidence of a stimulating effect on the plants (all varieties) growing in bed No. VI, as observed by the number of seeds per plant.

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 radioactive isotopes, and is in the sixth year of investigation in the year under report.

C.4.5.1. *Studies in Aman paddy.*—No fresh treatments were made in the year under report. However investigation on the nature of the early flowering mutant was carried out. As reported earlier, this mutant was isolated in the P_2 generation in 1959, in the variety Rupsail, an aman (winter) paddy. The control and the mutant were sown in seed beds on five different dates, at intervals of fourteen days, and transplanted after one month in alternate rows. The number of days taken from the sowing date to the date of the first initiation of flowering was recorded for every plant under investigation. Data regarding height, number of tillers, number of panicles grain and straw yield for each individual plants were also noted at the time of harvest. Plants sown on the 4th and 5th sowings had to be discarded owing to loss caused by unavoidable circumstances.

As shown in Table 22, the difference in mean time of flowering between the control and the mutant was greatest (40 days) in the first date of sowing (i.e., 23rd May) and lowest (23 days) in the last date of sowing (22nd July). As the sowing time was delayed the time taken for flowering gradually diminished both in the control and the mutant population. The rate of fall varied from 13 to 15 days every fortnight in the control and from 6 to 11 days in the mutant. In all dates of sowing the range of flowering of the mutant was greater than that of the control as is evident from the fact that the variance of the mutant is significantly higher than that of the control.

TABLE 22

COMPARISON OF MEAN TIME (DAYS) OF FLOWERING AND VARIANCE BETWEEN CONTROL AND MUTANT

Date of sowing		Control	Mutant	Difference
23.5.61	Mean±S.E.	148.6±0.26	108.4±1.13	−40.2±1.16
	Variance	1.90	34.63	
	No. of plants studied	(29)	(27)	
7.6.61	Mean±S.E.	135.4±0.37	102.3±0.65	−33.1±0.75
	Variance	5.00	14.50	
	No. of plants studied	(36)	(34)	
22.6.61	Mean±S.E.	123.1±0.14	91.5±0.43	−31.6±0.46
	Variance	0.89	9.44	
	No. of plants studied	(49)	(50)	
7.7.61	Mean±S.E.	108.3±0.20	82.7±0.51	−25.7±0.55
	Variance	0.76	5.17	
	No. of plants studied	(19)	(20)	
22.7.61	Mean±S.E.	96.5±0.17	73.6±0.49	−22.9±0.52
	Variance	1.40	12.21	
	No. of plants studied	(50)	(50)	

The different characters that were examined in the mutant and the control on three different dates of sowing are shown in Table 23. Mutant plants were found to be significantly shorter in height and in length of panicle and poorer in grain and straw yields than the control plants. Number of tillers and panicles of mutant plants were less than those of control plants but no statistical difference was noticeable in first two dates of sowing.

In a second experiment, plants (mutant and control) flowering on different consecutive dates in the previous year were noted and their progenies sown separately in the following season. Thus in the previous year (1960) mutant plants flowered for twelve days and control plants for five days; so twelve selections each belonging to a different flowering date were made from mutant plants; similarily, five selections were made from control plants; all these selections were sown in randomised blocks on the same day.

TABLE 23

COMPARISON BETWEEN CONTROL AND MUTANT FOR DIFFERENT CHARACTERS

Date of sowing	23.5,61			7.6.61			22.6.61		
	Control	Mutant	Diff.	Control	Mutant	Diff.	Control	Mutant	Diff.
Height (cms)	154.0 ±0.44	133.3 ±2.51	20.7* ±2.54	148.7 ±2.18	133.6 ±2.51	15.1* ±3.33	150.4 ±2.13	125.4 ±1.25	25.0* ±2.47
No. of tillers	6.0 ±0.38	5.5 ±0.50	0.5 ±0.63	6.8 ±0.30	6.0 ±0.36	0.8 ±0.47	5.4 ±0.20	6.7 ±0.27	−1.3* ±0.34
No. of panicles	6.0 ±0.38	5.5 ±0.46	0.5 ±0.59	5.9 ±0.39	5.5 ±0.37	0.4 ±0.54	5.1 ±0.21	4.8 ±0.26	0.3 ±0.33
Length of panicle (cms)	30.6 ±0.22	26.9 ±0.59	3.7* ±0.63	30.0 ±0.36	27.5 ±0.55	2.5* ±0.66	26.5 0.30	26.2 ±0.32	0.3 ±0.44
Grain yield (gms/plant)	17.0 ±0.85	1.0 ±0.17	16.0* ±0.87	13.8 ±1.20	2.3 ±0.34	11.5* ±1.24	12.3 0.70	2.5 ±0.31	9.8* ±0.76
Straw yield (gms/plant)	32.5 ±1.90	9.2 ±0.86	23.3* ±2.09	34.3 ±1.27	9.9 ±0.71	24.4* ±1.46	29.3 ±1.24	10.6 ±0.47	18.7* ±1.32

* denotes significant at 1% level.

In Table 24 an analysis of variance is given which shows that there is no significant difference in the mean time of flowering between the selections in the control whereas there is a significant difference between selections in the mutant. The mean time of flowering in the control varied from 118.6 to 118.7 days, whereas it varied from 87.4 days to 89.3 days in the mutant (Table 25 & 26). The variation within mutant lines was also seven times higher than the variation within the control lines, indicating there-by that mutant lines had a more prolonged period of flowering.

TABLE 24

ANALYSIS OF VARIANCE

Source of variation	Control			Mutant		
	d.f.	m.s.	F	d.f.	m.s.	F
Between types	4	0.32	—	11	17.69	3.14*
Within types	242	0.79		584	5.64	

* denotes significant at 1% level.

TABLE 25

MEAN TIME (DAYS) OF FLOWERING (CONTROL)

C_1	C_2	C_3	C_4	C_5	G.M.
118.6	118.6	118.7	118.7	118.7	118.7

TABLE 26

MEAN TIME (DAYS OF FLOWERING) (MUTANT)

E_1	E	E_3	E_4	E_5	E_6	E_7	E_8	E_9	E_{10}	E_{11}	E_{12}	G.M.
88.9	88.5	88.9	89.0	88.7	89.3	88.5	88.6	88.6	87.8	87.6	87.4	88.5

C.4.5.2. *Studies on Aus Paddy*

C.4.5.2.1. *Double Kernel Mutant* (S. 221)

Dry seeds of variety *Marichbati* (Aus paddy) were treated with 75,000 r X-ray and a single plant was isolated (*S.* 221) in the X_2 generation, on account of abnormal morphology of flower and seed. The progeny of the selection was maintained for four successive generations for studying the behaviour of flower and seed. These abnormal characters bred true. The flowers of selection S. 221 showed addition and reduction in the number of anthers and ovaries.

The number of kernels are two in the selection (*S.* 221) against one in the control. The seeds (= caryopsis) of the selection *S* 221 is wider than the control. Each kernel of the selection S. 221 is narrower than the kernel of the control (*Marichbati* variety). A comparison of kernel size can be seen from Table 27.

TABLE 27

MEAN LENGTH, BREADTH AND WIDTH OF KERNELS OF *Marichbati* AND *S.*221

Character	Marichbati	S.221	Difference ±S.E.	*t*-value (d.f. 38)
Length	5.40	4.70	0.70±1.54	0.45
Breadth	2.80	1.60	1.20±0.66	1.81**
Width	1.80	1.60	0.20±0.25	0.80

C.4.5.2.2. *Fused leaf Mutant* (S. 275)

This mutant was isolated (*S.* 275) for its abnormal leaf shape in the X_2 generation after 75,000 r dry seed treatment of variety *Marichbati*. X_4 generation was grown in the year under report. The plants of the Selection *S.* 275 has strong vegetative growth; mean height of plants was lower, in Selection *S.* 275 (53.5 cm) than in the control (77.9), and the difference is significant at 1% level.

C.4.5.2.3. *Leaf breadth of Selection S.* 275. There was no difference in the length of the leaf between the control and *S.* 275 but a difference in breadth of leaf was noticed; measurement of breadth of leaf in control and selection *S.* 275 was 1.03 and 0.72 respectively. This difference was statistically significant at 1% level.

C.4.5.2.3a. *Morphological abnormality of S.* 275

Various types of leaf abnormality were noticed in plants of *S.* 275 but all these can be grouped under two chief types of abnormality, (i) simple leaf blade type and (ii) union of two un-equal leaf blade type.

In all these types of leaf blade abnormalities there are two mid-veins running, either parallel side by side close to each other, or through the middle region of the two different individual leaf blades.

C.4.5.2.4. *Studies on the Selection S.*1

This selection was isolated in the year 1958 from the variety Marichbati after treatment with 3 mc of P^{32}. The grains of selection *S.*1 showed black coloured husk instead of golden yellow colour of *Marichbati* and was maintained in the successive generations.

The differences in the agronomic behaviour during harvest between the selection S.1 and control (*Marichbati*) was observed in the 4th generation. The differences are shown in table 28.

TABLE 28

MEAN PERFORMANCE OF DIFFERENT AGRONOMIC CHARACTER FROM SELECTION S.1 AND *Marichbati* VARIETY

Character / Selection	Marichbati	S.1	Difference ±S.E.	*t*-value df = 22
Height of plant (cm)	79.5	78.8	1.97±9.49	0.18
No. of tillers	5.3	7.0	1.70±0.76	2.22*
No. of Panicles	3.6	5.3	1.70±0.52	3.26*
Panicle length (cm)	18.3	18.3	0	0
Flag leaf length (cm)	20.7	20.5	0.2±0.31	0.63

It is seen from the Table 28 that there is no difference in height of plant, length of panicle, length of flag leaf between *S.*1 and *Marichbati*. The number of tillers and number of panicles between *Marichbati* and *S.*1 show differences which are significant at 5% level.

C.4.5.3. *Studies on Boro Paddy*

C.4.5.3.1. Bulked seeds of P_2 and S_2 generation of *Chinsura Boro* I and *Chinsura Boro* II were sown in the field. A number of plants have been selected whose characters will be studied in the P_3 and S_3 generation.

C.4.5.3.2. *Studies in the Selection S.* 292

This plant was selected in the second generation after 6 mc P^{32} tratment. The progenies of the selection was grown and compared with the control *Chinsura Boro* II with

respect to certain agronomic characters in the 4th generation. The comparison is given in table. 29.

TABLE 29

MEAN TABLE FOR HEIGHT, NUMBER OF TILLERS, AND NUMBER OF PANICLES OF CONTROL AND *S*.292

Character	Treatment Chinsura Boro II control	S.292	Difference ±S.E.
Height	79.9 cm	78.5 cm	1.4±2.45
Tillers	8.9	7.0	1.9±0.64**
Panicles	7.1	5.5	1.6±1.60*

From the table it is clear that the height of the progenies selection S.292 is not significantly different but the number of tiller and number of panicle is significantly lower than the control at 1% and 5% level respectively.

C.4.5.3.3. *Selection S* 339

The progeny of the selection is in the 3rd generation during the period under report. One plant in the selection flowered earlier than the control. The first flowering time of the selection *S*.369 (i.e., the plant that flowered first) was noted, and the differences between the control and Selection S.369 can be seen from table 30.

TABLE 30

FIRST FLOWERING TIME IN SEELECTION *S*369 AND CONTROL *Chinsura Boro II*

Selection	Character First flowering	Earliness in days
Control	98 days	0
S. 369	88 days	10

A study of the behaviour of this selection will be continued in the next generation.

C.5. Chemical mutagenesis

C.5.1. Studies on the effect of the chemical Dimethyl sulphate on jute plants (T.M.) was continued in the C_3 generation. A complicated segregation in a stem colour mutant, isolated in the X_2 generation was noticed. Further selfing and crossing have been made in these plants in an effort to clarify the nature of the mutant involved.

C.5.2. Further observations were made on plants of T.M. and D.154 in the C_2 (Ethylene-imine-treated) generation. A single D.154 plant with twisted stem, isolated in the C_1 generation gave rise to a (single) progeny also having twisted stem. The nature and mode of inheritance of this aberration is not clear.

No other abnormalities were observed in the C_2 generation.

C.5.3. Fresh treatment with Ethylene-imine was made on seeds of T.M., R.26 and D.154. The effect of these treatments are being studied at present.

C.6. Cytological and Cytotaxonomical studies

C.6.1. *Cytotaxonomic studies in some genera of Malavaceae*

During the year under report (1961-62) cytotaxonomical studies in continuation of the work reported in previous years in order to investigate the somatic chromosome number and other details, have been carried out in six species under four genera in the family of Malavaceae.

Following is the list of types studied:

Genus

A. *Gossypium*
   1. G. arboreum L. var. Nanking, Meyen.
   2. G. aridum. (Rose and Standley)

B. *Sida*
   1. Sida spinosa L.

C. *Malachra*
   1. Malachra Capitata Linn.

D. *Abutilon*
   1. Abutilon avicenae. Gaevtn.
   2. Abutilon hirtum. Don.

For the study of the somatic divisions, root tips were tried with aesculin, paradichlorobenzene, 8-hydroxyquinoline, colchicine and 1-bromonapthalene as the prefixatives, out of which aesculin gave the better results. After over-night fixation in acetic alcohol (1 : 3) materials were squashed in 0.2% aceto-orcein. Number and origin of the nucleoli were studied by Feulgen Fast-green technique.

C.6.1.1. *Gossypium*

C.6.1.1.1 *G. arboreum. L. var. Nanking, Meyen.*

This variety was received from the Department of Agronomy, Cotton Section, Texas. It is a native of China and a lone representative of the country having a great similarity with var. typicum H & G. in vegetative characters, has a $2n$ complement of 26 medium sized chromossomes. Satellited chromosome number is 2 pairs of which one pair has bigger satellites connecting with shorter arm of the submedianly constricted chromosome with a short link, while the other pair has smaller satellites with longer connecting links. Feulgen Fast-green technique shows the existence of 4 small neucleoli at the early prophase assuring their origin from the satellited chromosomes.

Abnormalities like somatic bridges and polyploid cells were observed in this species.

C.6.1.1.2. *G. aridum. Skov* (Rose and Standley)

This is a wild species of New World origin, received from the Department of Agronomy, Cotton Section, Texas. It has a 2*n* chromosome complement 26 relatively small chromosomes having 2 pairs of satellites. One pair of satellites is comparatively bigger with shorter arms while the other pair is shorter with longer arms. Four nucleoli observed in early prophase with Feulgen Fast-green test confirms their origin from the satellited chromosomes.

No abnormalities like hyper-or hypoploid chromosome numbers were found.

C.6.1.2. *Sida*

C.6.1.2.1. *Sida Spinosa. L.*

This type commonly called 'Cuba jute' has been collected from different localities, of 24-Parganas and identified at Shibpur, Botanical Garden. It has 28 medium sized chromosome in its 2*n* complement having two pairs of satellites, one pair of which is connected with shorter arms of the sub-medianly constricted chromosomes while the other pair is terminal to the chromosomes with median constriction. Four small nucleoli observed in the early prophase stage with Feulgen Fast-green technique indicate their origin from the satellited chromosomes.

Haploid and polyploid cells and somatic bridges were noted as abnormalities.

C.6.1.3. *Malachra*

C.6.1.3.1. *Malachra Capitata. L.*

This is a wildly growing shrub collected from different localities around the suburbs of Calcutta and identified at Shibpur, Botanical Garden. It has a 2*n* complements of 56 comparatively small chromosomes, haiving 3 pairs of satellites. One pair of satellites small in size, is connected with the shorter arms of the sub-medianly constricted chromosomes. The second pair is connected with chromosomes having median constrictions while the third pair bigger in size as compared with the first pair and otherwise quite similar. Six nucleoli found in early prophase stage with Feulgen Fast-green technique indicate their origin from the satellited chromosomes.

Abnormalities like polyploid cells with 112 chromosomes and 12 nucleoli in the early prophase and somatic bridges of various types were noted.

C.6.1.4. *Abutilon*

C.6.1.4.1. *Abutilon avicenae. Gaevtn.*

This type was received from Shibpur, Botanical Garden. This is known as 'maba' commonly grown in China, has a 2*n* complement of 42 small chromosomes having 3 pairs of satellited chromosomes. One pair of satellites having small knobs is connected with the chromosomes with long arms while the other two pairs are bigger, out of which one pair has a longer arm than the other. Feulgen Fast-green technique shows the existence of 6 nucleoli in the early prophase stage, which confirms their origin from the satellited chromosomes.

Abnormalities like hyper- and hypo-ploid cells in metaphase stage and laggards and somatic bridges in anaphase stage were observed.

C.6.1.4.2. *Abutilon hirtum. Don.* (*Abutilon graveolens, W & A., Var. hirtum. G. Don.*)

This type has been received from Shibpur, Botanical Garden. It has a 2*n* complement of 42 chromosomes which are relatively small having 3 pairs of satellited chromosomes; one pair of satellites is small with long arms, while other two pairs are bigger, out of which one pair has longer arms than the other. Six nucleoli observed with Feulgen Fast-green technique suggest their origin from the satellited chromosomes.

Polyploid cells showing 84 chromosomes in metaphse stage was recorded as an abnormality in this type.

C.6.2. *Observation on the Soil and light conditions in the cultivation of Sea Island Cotton*

Eleven types of Sea Island Cotton, namely (1) Andrews, (2) West berry, (3) Sea Island White (4) Sea Brook, (5) Sea Brook, 191, (6) St. Kitts, (7) S.I. Nevis, (8) V 135 (9) Barbadose, (10) Mont. Serrat No. 1, and (11) Mont Serrat No. 2, which were received from Sea Island Cotton Research Station, Eastern Extension Road, Mandya (Mysore State), were cultivated at Shyamnagar Farm, with a view to find out their adaptibility there. It may be mentioned that Government of India has drawn up a scheme, for the cultivation of Sea Island Cotton, at their different experimental centres, in order to improve the yield and also to study the physiological conditions regulating the growth of the plants.

This type of cotton which is at present drawing great attentions of all the cotton growing regions, for its long staple is very difficult to grow in Bengal soil. Even if it is grown the yield is very low and much damage to the crop and the lint, is done due to various infections during the rainy season. Uptil now it has not been found to be profitable to grow these varieties of cotton in Bengal soil as an independent cash crop. As a counter step cultivation of another crop simultaneously in the same field is being tried at some places. But growing these varieties under shades in the orchard which, in general remain, as fallow will be a most welcome project. With a view to find out to what extent it may be possilble, a preliminary experiment was carried out at the mango-top of the Experimental Station of the Institute, at Shyamnagar.

These eleven types of Sea-Island Cotton which are famous for their long fibres were grown in two plots of land, one of which was an open land getting unrestricted sunshine and rains, while the other plot was selected in the mango-top of the farm where the plants did not receive direct sunshine, except for a short time at midday, and the rains were also obstructed due to the overhead branches. Both the plots being in sufficiently high lands, were not water logged at any time during the rainy season. No manuring was given in either of the plots but irrigation and interculturing were done when possible. Spacing between the rows was 3 ft. and plants 9 inches. All the sowings were done at a time in the last week of August 1961. The table below shows the records of harvesting for a period of two months (in March and April, 1962).

TABLE 31

AVERAGE NUMBER OF BOLLS HARVESTED FROM A SINGLE PLANT IN A PERIOD OF TWO MONTHS (March & April, 1962)

	Under normal field condition	Under shady condition (in the mango-top)	Difference of the yield from the normally grown condition
1. V_{135}	11.3	8.8	−2.5
2. Nevis	6.7	7.5	+0.8
3. Sea Island White	7.1	6.7	−0.4
4. Sea Brook	7.8	8.0	+0.2
5. Sea Brook No. 191	8.1	8.7	+.06
6. Mont Serrat No. 1	9.5	5.4	−4.1
7. Mont. Serrat No. 2	4.4	3.1	−1.3
8. West Berry	9.1	8.8	+0.3
9. Barbadose	5.5	6.4	+0.9
10. St. Kitts	11.0	4.0	−7.0
11. Andrews	7.0	7.5	+0.5

From table 31, it is observed that the types like Nevis, Sea Brook, Sea Brook No. 191, West Berry, Barbadose and Andrews give rather better yields in the shady conditions than those grown in normal field conditions. Although the differences are very small it can be inferred that these types can be taken up for the project. With the types St. Kitts, Mont Serrat No. 1, V 135, Mont Serrat No. 2 and Sea Island White the results are otherwise. In order to confirm these findings this experiment will be carried out in more details in future.

C.6.3. *Cytological studies on some varieties of Pisum Sativum L.*

C.6.3.1. During the year under report (1961-62) seventeen varieties of *Pisum sativum* L. were collected from different sources in order to find out the extent to which karyotypic differences could account for their varietal dissimilarities. The following were the varieties studied :—

(1) Kelvendon Wonder, (2) Show Perfection, (3) Laxton Progress, (4) Thomas Laxton, (5) Acclimatised Round Seeded, (6) Alderman, (7) Little Marble, (8) Early Badger, (9) American wonder, (10) T-61, (11) T-56, (12) N.P.-29, (13) Suttons Phenomenon, (14) Early giant, (15) Bonnivilli, (16) Globe's Evergreen, and (17) Blue Bantam.

For studying somatic chromosomes, various pre-treating agents as Para-dichlorobenzene, Aesculin, Coumarin and 8-hydroxyquinoline were tried and the best result was obtained with the last chemical. Treatment with .003 M solution of 8-hydroxy-quinoline at a temperature of 14°–18°C for two and half hours produced good results.

The somatic chromosome number of the different varieties so far investigated has been found to be fourteen. A detailed karyotypic analysis of the different varieties reveal a basic similarity in the complements of the different varieties and on the basis of gross morphological features, the following chromosome types can be recognised:

Type A—One pair of long chromosomes with sub-median to nearly median primary constrictions.

Type B—One pair of medium long chromosome with sub-median primary constrictions and a pair of small satellites in the long arm.

Type C—One pair of medium long chromosome with sub-median primary constrictions and a pair of large satellites in the long arm.

Type D—One pair of medium sized chromosomes with sub-median primary contriction.

Type E—A pair of medium sized chromosome with sub-terminal primary constrictions.

Type F—Two pairs of small chromosomes with median to near median primary constrictions.

The varieties can be identified on the basis of their karyotypes. They can be distinguished from one another by minor differences in the details of chromosome structure including secondary constrictions, in the position of the different types of chromosomes and in the total amount of chromatin matter in the varieties. Three pairs of secondary constricted chromosomes were observed in Little Marble and Sutton's Phenomenon instead of two pairs observed in other varieties. Polyploid cells with chromosome numbers of twenty-eight were noted in a few cells of the varieties American Wonder and Sutton's Phenomenon.

A study of the idiogram in the different varieties show that if the chromosomes of the complement are arranged according to their size or length, there is a gradual decrease in the length and that all of the types could be identified.

Meiosis in all the varieties were studied in detail but no regularities were noticed. Pollen grains were highly fertile.

C.6.4. *Cytotaxonomical studies on Chillies (Capsicum sps.)*

C.6.4.1. In continuation of the line of work reported in the previous year, cytotaxonomical studies on Chillies were carried out during the current year (1961-62) in the following varieties.

(1) World Beater, (2) Fordhook, (3) Yolo wonder, (4) Oshkosh, (5) Hungarian Wax, (6) Sunny brook, (7) Long Red, (8) Bholar, (9) Capsicum frutescens—(China), (10) C. frutescens—(Turkey), (11) C. frutescens—(Georgia, U.S.A.), (12) C. frutescens—(Ethiopia), (13) C. frutescens—(Iran), (14) Chilli—Red, and (15) Chilli—Yellow.

Root tips were pretreated for karyotypic studies with saturated solution of Para-dichlorobenzene for 3 hours at 14°–18°C which gave much better results than that with Colchicine reported earlier. Roots were squashed in and stained in aceto-orcein.

The 2*n*—chromosome complement of the varieties studied were found to be twenty four. The chromosomes are relatively large. Study of idiograms shows that most of the chromosomes have either median or sub-median primary constrictions with the exception

of one pair which has subterminal primary constrictions. In most of the varieties there are two pairs of secondary constricted chromosomes, one pair of which has very prominent satellites connected with the small shorter arm of the subterminal primary constricted chromosomes (Type—A), while the other pair is observed only in carefully prepared and well scattered metaphase plates only. In the latter case, the satellites are connected with the large shorter arm of the sub-mediun primary constricted chromosomes (= Type—B). Exceptions are however found in varieties like Long Red, C. frutescens—(Iran), C. frutescens—(Georgia, U.S.A.), C. frutescens—(Ethiopia). In Long Red, there are three pairs of secondary constricted chromosomes, two of which are similar to Type A, and the other is similar to Type—B and in this variety, sub-terminal primary constricted chromosome pair is absent. In C. frutescens—(Iran), there are also 3 pairs of secondary constricted chromosomes one pair of them is similar to Type—A and 2 pairs are of Type—B, but in this case sub-terminal primary constricted chromosome pair is present. In C. frutescens (Georgia, U.S.A.) and C. frutescens (Ethiopia), sub-terminal primary constricted chromosome pair is found to be lacking and is replaced by a pair of sub-median primary constricted chromosome.

Abnormalities in chromosome numbers were found in Yolo Wonder, C. frutescens (Georgia, U.S.A.) and C. frutescens (Ethiopia) where it was found to be 26, 23 or 25 respectively in a few cells. Polyploid cells were also recorded in C. frutescenss (Ethiopia).

Meiotic studies in all the varieties mentioned above reveal that all the varieties of C. frutescens show high irregularities in the form of laggards, bridges, formation of micronuclei, and early separation. In varieties like World Beater, Fordhook, Yolo Wonder, Oshkosh, Hungarian Wax, Sunny brook, Long Red and Bholar such irregularities are present but are much less than the former group. Varieties like Chilli—Red and Chilli—Yellow no such irregularities are found. Study of pollen grain sterility correlate with the meiotic irregularities.

C.6.4.2. Hybridization experiments were tried but the crosses were not successful.

C.7. Plant Anatomy

C.7.1. *Effect of Irradiation on the Anatomy of Plants*

C.7.1.1. During the year under report, preliminary investigations on the effects of X-rays on the developmental anatomy with special reference to shoot apical organization in *Sesamum orientale*, var. *T*-16 and its different malformations due to irradiation were undertaken.

Dry, pure-line seeds of *Sesamum orientale*, var. *T* 16 were treated with 20,000, 40,000, 60,000 and 80,000 X-rays by means of a Philips CT apparatus running at 50 kV and 1.6 mA current with tungsten target; the materials being kept in a single layer, 10 cm away from the window provided with aluminium filter.

Compared to the control (non-treated), accelerated germination with quicker growth was observed in 20,000 r treated plants, but with the increase in dosage both these features decreased systematically. The treated populations manifested morphological abnormalities in the form of fission and/or fusion of leaves and change in phyllotaxy.

For the study of shoot apical organization, shoot apices of both control and treated plants were collected at fourth nodal stage and fixed in F.A.A. Microtome sections

8–12μ thick were cut and various staining combinations were tried. Of these, tannic acid proved satisfactory. In some cases, however, staining with pyronin gave better results.

In the control plants the shoot apex is flat with two-layered tunica. The corpus layers are well differentiated. At 20,000 r no appreciable change could be observed excepting that the shoot apex became somewhat narrower.

At 40,000 r the damage was somewhat severe. Some cells of the tunica and corpus layers collapsed. The shape of the shoot apex often changed to irregular or dome-shaped structure. Irregular cell wall thickenning was also observed.

At still higher doses, i.e., 60,000 and 80,000 r, plasmolysis occurred in both the tunica and corpus layer cells. The identity of the tunica and corpus layer cells was often lost. The shape of the shoot apex was frequently irregular and never flat as observed in the control plants.

Most of these observations corroborates the findings of Pratt (1959) and Pratt, John Einst and M. Jahur (1959) on the shoot apex of "Concord" grapes.

It was further observed that the extent of damage in the shoot apices was very variable between plants within any treatment. This may be due to the position of the embryo and the physiology of the seeds during irradiation as well as due to intrasomatic selections during the ontogeny of the treated plants.

Preliminary observations indicate that compared to the control, treated tissues respond differently to different stains. Further work for confirmation is under progress.

C.7.2. *Anatomical study on a 'flower like' structure of a Jute plant* (D 154).

C.7.2.1. A jute plant (Corchorus capsularis var. D.154) with some abnormal "flower" like structures was observed in the Experimental Field at Shyamnagar. In early stages it had the appearance of a normal plant. Later the appearance of these "flowers" revealed the abnormal nature of the plant. Morphologically the abnormal structures appeared to be modified flowers having sepaloid perianth which in turn enclosed the other undifferentiated parts. In order to get a clearer view, the whole structure was taken up for anatomical investigation. A comparative analysis of the internal parts of these abnormal structures and as well as the floral and foliar parts of normal plants were made on a developmental basis. The anatomical investigation of the abnormal structure led to the following conclusions:

(i) Though the abnormal structures appeared to be modified flowers, it possessed no floral parts at all, instead it proved to be a suppressed branch bearing leaves and vegetative buds at regular sequences.

(ii) Differentiation of the tissue into leaves and vegetative buds were more or less completed but somehow disturbance in growth occurred and the whole thing appeared in this abnormal form.

(iii) The leaves at lower nodes (upto 5th node) were rather large and appeared as the sepaloid perianth whereas the other remaining parts were too small and immature to enable one to identify them externally.

(iv) It seemed that these abnormal structures arose through disturbance in auxin metabolism of the plant.

D. 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.

Production of antibiotic. A broad-spectrum antibiotic was obtained from the *Streptomyces sp* strain $A_{14}475$, a gamma-irradiated mutant MT-1017. It was selected for production of antibiotic for its higher yield when fermented in suitable medium at 25°C, using shake culture method.

In semi-large scale production, medium was selected and modified designated as 'A_7' as reported by H. B. Warren *et al.* (Anti. & Chem. 5, 6, 1955). The composition of the medium is as follows:—$(NH_4)_2SO_4$–5.0 gm; KCl–4.0 gm; K_2HPO_2–0.4 gm; $CaCO_3$–0.5 gm; yeast extract–2.0 gm; soyapeptone–6.0 gm. glucose–15 gm; distilled water 500 ml.+tap water 500 ml. and *p*H maintained at 7.2 before sterilising which was done at 15 lbs. pressure for 15 mins.

In shake-culture technique, the production of the active material reached its maximum at 48 hours and continued for 5 days. After that there was a decrease in the antibiotic titre. Production of the antibiotic *in situ* varied to some extent on the concentration of phosphate-ion as well as on the fluctuation of temperature. Maximum antibiotic titre was observed at a temperature range 25–28°C.

Purification of antibiotic. In isolation and purification procedures previously described methods were modified. The first step in the isolation of the antibiotic from the harvested broth is to separate the insoluble solids composed primarily of mycelium which is extremely fine and gelatinuous in character. For efficient functioning of the process and minimising the loss of the harvested broth, separation of the solid matter was carried out in Sharple's Laboratory centrifuge in which complete centrifugation of 5 litres of fermented liquid was done in 15 to 20 minutes.

Filtered liquid was then passed over a column of Amberlite IRC-50 in the form of Na-cycle; adsorption of the active principle was carried out in two successive stages. Firsty, it was passed through 80 c.c. of Amberlite IRC-50 (Na) packed to a height of 15 cms and then through another 40 c.c. of same resin of Na-cycle packed to a height of 20 cms. Loss was negligible owing to two stage adsorptions.

Elution of the active material was carried out with $\frac{N}{10}$ HCl and collected in 200 ml. fractions. Fractions containing the active material was neutralised by passing through Amberlite IRA-400 (OH) which not only made the eluates neutral but also removed the colouring matter appreciably.

Neutral eluates were again passed through a second column of Amberlite IRC-50 (Na^{+4}) and elution was carried out with NH_2OH. Ammoniacal eluates containing the active material were concentrated to a small vol. *in vacuo*, neutralised with $\frac{N}{10}$ H_2SO_4 and poured into ethanol. A yellowish-white ppt. was obtained, centrifuged *in vacuo*.

A dried mass, containing very little yellow coloured impurities was chromatographed over a charcoal column of Darco G-60 and Celite 545 (1 : 1). Aqueous solution was used and elution was carried out with 10% acetone. Fractions containing the highly active material collected, concentrated *in vacuo*, poured into equal volume of methanol and kept at 4°C overnight. After centrifugation and drying a pure white substance was obtained.

Properties of the antibiotic—Physical and Chemical. It is a basic antibiotic as shown by its ability to interact with acids to form salts and by its adsorption on cationic exchangers but not on anionic exchangers. Salts obtained from inorganic acids, e.g., HCl, H_2SO_4 etc., are highly soluble in water but are insolulble in organic solvents except methanol where limited solubility is demonstrated for the hydrochloride.

Antibiotic in the form of sulphate salt did not show any sharp melting point. Shrinkage took place at 135C and decomposition with light brown colour started at 240°C. A study was made regarding the behaviour of the antbiotic towards a variety of colour reagents and the results are shown in Table I.

TABLE I

1. Fehling	.. Negative	6. Sakaguchi	.. Negative
2. Tollens	.. Negative	7. Ninhydrine	.. Positive
3. Benedict	.. Negative	8. Molisch	.. Positive
4. Ferric chloride	.. Negative	9. Elson-Morgan	.. Positive
5. Biuret	.. Negative	10. Anthrone	.. Positive

The compound itself did not exhibit any characteristic adsorption in the UV range but after acid hydrolysis with 40% H_2SO_4 for 1 hour the product showed an absorption maximum at 280°$m\mu$ resembling that of neomycin.

An aqueous solution of the antibiotic easily reacts with Benzoyl chloride in strongly alkaline solution producing a white ppt. insoluble in water and common organic solvents. (M.P. 104–6 C).

It belongs to the class of antibiotics which are primarily active upon bacteria and the antibacterial studies were carried out in nutrient broth.

TABLE II

ANTIBACTERIAL SPECTRUM OF THE ACTIVE SUBSTANCE

Culture	Min. inhibitory conc.
1. Staph. aureus (sensitive)	0.5 μg/ml.
2. Staph. aureus (resistant)	1.0 ,,
3. Staph. albus	0.5 ,,
4. B. subtilis	3.0 ,,
5. B. anthracis	8.0 ,,
6. B. megaterium	12.0 ,,
7. A. acrogenes	5.0 ,,
8. P. vulgaris	15.0 ,,
9. S. typhosa	6.0 ,,
10. E. coli	0.5 ,,
11. P. aeruginosa	35.0 ,,

D.1.2. *Studies on the production of Broad-spectrum antibiotic from a Pseudomnas spp.*

Antibiotic titre. A broad-spectrum antibacterial antibiotic was isolated from a strain of *Pseudomonas* spp. (P*x*) was found to be a variant strain of *Pseudomonas aeruginosa.*

On screening the antibiotic activity of the isolated culture against common test organisms it showed strong antibiotical character and a faint antifungal activity. The results are given in Table I.

TABLE III

CROSS-STEAK ASSAY OF THE CULTURE AGAINST TEST BACTERIA AND FUNGI

Test organism		Zone of inhibition (mm)
Bacteria		
1. Staphylococcus aureus	..	32
2. Bacillus subtilis	..	16
3. Escherichia coli	..	23
4. Vibrio cholerae	..	25
Fungi		
5. Curvularia spp.	..	12
6. Alternaria solani	..	±
7. Fusarium vasinfectum	..	15
8. Aspergillus niger	..	—

± Doubtful; — negative

Physiology and Biochemistry of strain Px. While studying the nutrition of the culture in relation to its utilization of various carbon and nitrogen sources, minerals and trace elements with the antibiotic pigment formation as well, it was found that glycerol was the best carbon source. As regards nitrogen sources are concerned, it was noted that glycine, l-leucine, alanine, either singly or in combinations of two, had profound influence upon antibiotic production. Although other amino acids increased the growth of the organism yet they had very little effect on the yield of antibiotic. Phosphate, salts of iron and magnesium brought about spectacular increase in the antibiotic production. Studies on the trace element, however, indicated the presence of iron which is important in the antibiotic production and cell synthesis while others, e.g., Cu, Zn, Ni, Co had practically no effect.

The factors for the optimum production of the antibiotic in culture medium were also studied and it was noted that a temperature of 25°C, *p*H 7.4 and a period of incubation for 7 days in stationery flasks were the optimum conditions needed. In shake flasks, however, the time factor was surprisingly reduced to three days with an increase in the yield by 2.5 times as compared to static conditions.

Physical and chemical character of the antibiotic. The isolated antibotic substance m.p. (dec) 118–120°C, decomposed easily in presence of light and air and alkali. The acidic solution of the antibiotic was, however, stable if kept at low temperature. The alkali-decomposed product when distilled with Zn dust in nitrogen atmosphere gave phenazine m.p. 171°C–172°C (Mixed m.p. showed no depression). Detailed chemical analysis of the antibiotic is in progress.

Toxicity tests. The antibiotic showed that it was fairly toxic when injected to mice and had severe action on their respiratory centres.

Investigations are now being carried out to reduce the toxic action of the antibiotic substance with a view to evaluate the chemotherapeutic activity of the antibiotic.

D.1.3. *Antifungal antibiotic from a Streptomyces sp.* Ac_2 (435).

The isolation of an antifungal antibiotic from a *Streptomyces sp.* having marked activity against a wide range of yeasts and filamentous fungi, including pathogenic and nonpathogenic forms has been studied. The nutritional requirement of the organism and the optimum conditions necessary for attaining higher yields of antibiotic has been carried out. The purification of the active principle by column chromotography, solvent extraction and countercurrent distribution technique was done and a light yellow-brown, amorphous form was obtained. The following properties of the antibiotic has been studied.

Physical and chemical properties of the antibiotic Ac_2 (435). The substance is readily soluble in the lower alcohols, pyridine, formamide; moderately soluble in water, acetone and glacial acetic acid, insoluble in benzene, ether, petroleum ether, chloroform and carbon tetrachloride. The antibiotic is sensitive to light and high temperature above 40°C. Complete inactivation takes place at 100°C when *n*-butanolic solution is exposed for 1 hour to strong sunlight. However, it retains its activity when kept in dark at low temperature (10°C) for several months.

TABLE IV

DEVELOPMENT OF PAPER CHROMATOGRAMS USING CURVULARIA SP., A FUNGUS, AS THE TEST ORGANISM SHOWING ONLY ONE ACTIVE SPOT

Solvent systems	*Rf*
Ethanol—H_2O (3: 1)	0.714
Isopropyal alcohol—H_2O (7: 3)	0.838
t-butyl alcohol—H_2O (7: 3)	0.812
Pyridine acetic acid—H_2O (50: 35: 15)	0.842
n-butanol ethanol—H_2O (1: 1: 1)	0.913
Methyl ethyl ketone—Propionic acid—H_2O (75: 25: 30)	0.84

Antimicrobial properties. Activity of the antibiotic, thus isolated, has been tested against a wide variety of fungi which include saprophytic plant and human pathogenic forms following the conventional agar cup method of assay and its minimum inhibitory concentration for these fungi has been determined.

TABLE V

IN VITRO ACTIVITY OF THE ANTIBIOTIC PRODUCED BY Ac_1435

Test fungus	Minimum inhibitory concentration (μg/ml)
Saprophytic	
Saccharomyces carevisae	6.0
Saccharomyces ellipsodus	6.5
Torula sp.	5.0
Penicillium notatum	8.2
Penicillium chrysogenum	7.0
Syncephalastrum sp.	9.0
Cunninghamella	8.0
Trichoderma viridae	10.4
Plant pathogens	
Aspergillus niger	10.0
Aspergillus oryzae	30.5
Alternaria solani	16.0
Curvularia lunata	4.5
Fusarium oxysporum	20.0
Helminthosporium sativum	8.0
Glomerella cingulata	8.0
Human pathogens	
Candida albicans	18.5
Candida tropicalis	17.0
Trichosporan cutancum	15.0
Sporotrichum schencki	20.2
Microsporum gypseum	12.0
Microsporum audouini	10.5
Microsporum canis	9.0
Trichophyton rubrum	10.0
Trichophyton sulfurcum	11.2
Trichophyton interdigitale	10.0
Epidermophyton floccosum	15.4

To determine the inhibitory concentration of the antibiotic, a comparative assay of the known tetranes along with it, has been carried out against the yeasts and some of the plants using the agar cup method of assay.

The ultraviolet absorption spectrum showed maxima at 288, 305 and 320 *mμ*, these being characteristic peaks typical of conjugated tetraene structure and as such has great similarity with the absorption spectra of *rimocidin*, *nystatin*, *pimiaricin* and *amphotericin* A. It can, however, be distinguished readily from these well known antibiotics by paper chromatographic technique. When the chromatograms are developed under the same conditions using *Curvularia sp.* as the test fungus, the following results are obtained.

TABLE VI

COMPARATIVE PAPER CHROMATOGRAPHIC STUDIES ON THE ANTIBIOTIC PRODUCED BY Ac_2435 WITH SOME OF THE KNOWN TETRAENE ANTIBIOTICS

Solvent systems	*Rf* values with				
	Ac 435	Nystatin	Pimaricin	Rimocidin sulfate	Amphotericin A
n-butanol-acetic acid—H_2O (2: 1: 1)	0.857	0.746	0.838	0.794	0.792
n-butanol saturated with H_2O	0.525	0.270	0.262	0.413	0.257
n-butanol-pyridine—H_2O (1: 0.6: 1)	0.791	0.774	0.776	0.814	0.772

The following chemical tests have been performed with the antibiotic.

TABLE VII

Reagent	Reaction
Anthrone	Positive
Fehling's	positive
Molisch	positive
Tollen's	positive
Benedict	negative
Iodoform	negative
Biuret	negative
Millon's	negative
Xanthoproteic	negative
Ehrlich's *p*-dimethyl amino benzaldehyde	negative
Schiff's aldehyde	negative
Lassaignes	negative
Ninhydrin	negative
Potassium permanganate	decolourization
Conc. H_2SO_4	brown colour
Carbazole test	negative

TABLE VIII

COMPARATIVE IN VITRO ACTIVITY OF THE ANTIBIOTIC PRODUCED BY Ac_2435 WITH SOME OF THE KNOWN TETRAENE ANTIBIOTICS

Test organism	Minimum inhibitory concentration (μg/ml) of				
	*Ac_2(435)	Nystatin	Pimaricin	Rimocidin sulfate	Amphotericin A
1. Candida albicans	18.5	35.0	34.0	18.0	16.0
2. Candida tropicalis	17.0	42.2	40.0	16.0	17.5
3. Saccharomyces cerevisae	6.0	18.5	16.0	15.0	6.0
4. S. ellipsoidus	6.5	18.0	20.0	8.0	15.0
5. Torula sp.	5.0	18.0	12.0	12.0	13.4
6. Aspergillus niger	10.0	45.0	17.0	25.0	10.0
7. A. oryzae	30.5	100.0	100.0	100.0	18.0
8. Alternaria solani	16.0	35.0	30.2	26.0	12.0
9. Curvularia lunata	4.5	8.0	10.0	15.0	4.0
10. Fusarium oxysporum	20.0	100.0	35.0	20.0	32.0
11. Glomerella cingulata	8.0	10.0	4.0	25.0	4.5

* Purified antibiotic for comparison

Experiments for the toxicity test of the antibiotic and its chemical analysis are in progress.

D.2. Induced mutation in microorganisms

D.2.1. *Mutations in Aspergillus chevalieri*

Production of Mutants. Ultraviolet irradiation experiments were performed on *Aspergillus chevalieri*. Various types of morphological mutants were obtained which differed remarkably from the parent by colour of conidia, texture of the colony and sporebearing organs. Out of a few mutants two strains were selected for further irradiation to develop strains having biochemical deficiency.

The selected morphological mutant strains were (I) $U_7Ac(35)$—yellow-coloured and (II) $U_7Ac(35)$—yellowshiwhite-coloured.

(I) *Strain U Ac*(35).—A rapidly growing morphological mutant with yellow-coloured conidia obtained from the parent strain *A. chevalieri* through UV irradiation.

Spore suspension of the above strain was irradiated with UV in lots for 1, 1.5, 2.5, 3.5, and 4.5 minutes and plated out on CM agar medium. Colonies were transferred to MM agar medium at the next step as usual for detecting the biochemical mutants. Count of spores in suspension was 2.3×10^5 per ml.

TABLE I

SPORE CONCENTRATION—2.3×10^5/ml ENERGY-100 ergs/mm²/sec

Irradiation time in mins.	Survival percentage	Total colonies isolated	Percentage of biochemical mutant	Percentage of morphological mutants
1.5	5.9%	112	3.5%	1.7%
2.5	3.01%	96	1.4%	4.1%
3.5	1.4%	112	5.3%	4.0%
4.5	0.18%	112	6.1%	2.6%

(II) *Strain U Ac*(25).—This one also is a rapidly growing morphological mutant with yellowish-white conidia obtained from the parent strain *A. chevalieri* by UV irradiation. Spore suspension was irradiated for 2,4,6 and 8 minutes. After irradiation the suspension was diluted and plated out on CM agar medium. Colonies were picked up on MM agar medium as usual for biochemical mitants. Table II gives the result of irradiation and survival percentage.

TABLE II

DOSE—PERCENTAGE OF SURVIVAL IN A. CHEVALIERI AFTER UV IRRADIATION

Spore concentration 4.2×10^5 UV energy 100 ergs/mm²/sec.

Time in minutes	Survival percentage	Total colonies isolated	Percentage of biochemical mutants	Percentage of morphological mutant
2.0	23.9%	32	6 2%	3.1%
4.0	5.9%	96	8.2%	4.1%
6.0	1.7	144	4.1	2.7
8.0	0.22	48	4.1%	4.1%

The type of biochemical mutants obtained from *A. chevalieri* and its two morphological mutants $U_7Ac(35)$ and U Ac(25) are given in Tables III, IV and V respectively.

TABLE III

BIOCHEMICAL MUTANTS OF A. CHEVALIERI

Growth factor requirements of mutants	No. of strains derived
1. Guanine/Hypoxanthine	1
2. Paraamino benzoic acid/Riboflavin	1
3. Adenine/Guanine	1
4. Choline choloride	2
5. Calcium pantothenate/choline chloride	3
6. Calcium pantothenate/Pyridoxine HCl	1
7. Cytosine	1
8. Uracil	1
9. Xanthine/Hypoxanthine	1
10. PABA/Aneurin HCl	1
11. Threonine/Asparginic acid	1
12. Arginine/Ornithine	1
Total	15

TABLE IV

BIOCHEMICAL MUTANTS OF U_7 Ac(35)—(MORPHOLOGICAL MUTANT A. CHEVALIERI)

Growth factor requirement	No. of strains derived
1. Guanine/Hypoxanthine	1
2. Adenine/Hypoxanthine	2
3. Cal. pantothenate Folic acid	1
4. Biotin	2
5. Adenine	1
6. Pyridoxine HCl	1
7. Adenine/Xanthine	1
Total	9

TABLE V

BIOCHEMICAL MUTANTS OF U_7 Ac(25) (MORPHOLOGICAL MUTANT OF A. CHEVALIERI)

Growth factor requirement	No. of strains derived
1. Methionine/Cystine/Cysteine	1
2. Aneurine/Pyridoxine/Choline chloride	1
3. Pyridoxine/Choline chloride	1
4. Nicotinic acid/Folic acid/Biotin/Riboflavin	1
5. Calcium pantothenate	1
6. Adenine/Hypoxanthine	1
7. Biotin	1
8. Folic acid	1
9. Serine/Tyrosine/Isoleucine	1
10. Tryptophane/Phenylalanine/Proline	1
Total	10

D.2.2. *Utilization of growth factors by different Biochemical mutants of A.chevalieri*

A number of biochemical mutants were selected and experiments were done to determine the optimum concentration of growth factors required by them. Minimal medium was prepared (*p*H 6.0) and distributed into 100 ml flasks, each containing 25 ml. of the medium. Growth factors at different concentrations were added to the flasks and for each concentration replicates were maintained.

All the flasks were incubated at 30°C for 10 days after which the mycelia were harvested on a previously dried filter paper. The materials were dried overnight at 80°C and weighed again. Tables VI, VII, VIII and IX show the amount of growth of the parent strains in MM and the deficient mutants in MM supplemented with different Amino acids, purines, pyrimidines and vitamins etc., for respective deficiencies.

TABLE VI

COMPARATIVE GROWTH OF A. CHEVALIERI AND MORPHOLOGICAL MUTANTS IN TERMS OF DRY WEIGHT OF MYCELIUM

Strain	*p*H	Dry weights in mgs
A. chevalieri	4.6	292 mgs
$U_7Ac(35)$	4.8	150 mgs
$U_7Ac(25)$	4.8	155 mgs

TABLE VII

COMPARATIVE GROWTH OF MUTANTS WITH VARIOUS AMINO ACIDS

Strain number	Amino acid	Concentration in mg/ml				
		0.01	0.02	0.03	0.04	0.05
		Dry wt. in mg	Dry wt. in mg	Dry wt. in mg	Dry wt. in mg	Dry wt. in mg
U_1yw	DL-Methionine	17	40	95	170	102
	Cystine	160	180	210	127	120
	Cysteine	105	130	181	127	120
U_7Ac_{12}	Arginine	38	42	65	72	95
U_7Ac_8	Threonine	35	40	58	65	85
U_1yw_{16}	Isolucine	165	185	199	208	230
U_7Ac_8	Aspartic acid	20	23	28	30	40
U_7Ac_{12}	Ornithine	78	80	94	99	105

TABLE VIII

GROWTH OF MUTANTS OF A. CHEVALIERI REQUIRING PURINES AND PYRIMIDINES

Strain	Nucleic acid	Concentration in mg/ml				
		0.01	0.02	0.03	0.04	0.05
		Dry weight in mgs				
U_2AcM1	Hypoxanthine	238	330	360	369	395
U_7Ac_6	Uracil	295	415	470	500	490
U_2AcM14	Adenine	125	165	185	210	240
U_5Ac_7	Guanine	40	52	127	180	125
U_7Ac6	Cytosine	330	340	335	348	365
U_7Ac10	Xanthine	245	325	395	415	580
U_2AcM1	Adenine	65	90	175	275	435

TABLE IX

DRY WEIGHTS OF MYCELIUM IN RELATION TO VITAMIN REQUIREMENTS

Strain No.	Vitamin	Concentration in mg/25 ml of medium	Dry wt. in mg
U_2AcM18	Folic acid	0.025	295
		0.050	370
		0.75	380
		0.100	410
		0.125	445
U_1yw27	Choline chloride	0.01	160
		0.02	162
		0.03	167
		0.04	190
		0.05	226
U_1AcM9	Biotin	0.0001	412
		0.00002	316
		0.00003	314
		0.00004	326
		0.00005	275
U_2AcM	Calcium pantothenate	0.025	108
		0.051	165
		0.075	167
		0.100	190
		0.125	277
U_1yw27	Pyridoxine HCl	0.025	340
		0.050	385
		0.075	470
		1.100	390
		0.125	375
U_1yw32	Folic acid	0.025	205
		0.050	270
		0.075	220
		0.100	230
		0.125	365
U_1yw16	Calcium pantothenate	0.025	255
		0.050	365
		0.075	380
		0.100	390
		0.125	420

D.2.3. *Effect of UV radiation on Aspergillus oryzae*

Spore suspension was prepared as usual in calsolene solution (1: 10,000). The spore suspension was irradiated with UV (wave length) of 2537 Å. The time of exposure was adjusted to 4, 8, 12 and 16 mins. and the dosage was 100 ergs/mm²/sec as calculated from the dosimeter readings. It was observed that killing of the spores was increased with the increasing time of exposure. Table X shows the results of the irradiation.

TABLE X

EFFECT OF DIFFERENT DOSAGES OF UV IRRADIATION ON ASPERGILLUS ORYZAE

Spore count 2.2×10^7

Time of irradiation in min.	Survival percentage	Percentage of biochemical mutants	Percentage of morphological mutants
		%	%
4.0	11.6	4.5	1.5
8.0	1.1	3.9	0.79
12.0	0.33	1.5	0.79
16.0	0.22	2.3	3.1

A number of biochemical and morphological mutants have been isolated and tested for increase in diastase production.

D.2.4. *UV irradiation of Aspergillus rugulosus*

Conidia of *Aspergillus rugulosus* have been irradiated for 2, 4, 6, 8 and 10 minutes with a view to produce morphological and biochemical mutants for studying the genetics of the organism. The results of percentage survival

TABLE XI

PERCENTAGE OF SURVIVAL OF A. RUGULOSUS

Expt.	Time of treatment with UV in minutes	Percent survival
1	0	100%
2	2	12.6
3	4	8.3
4	6	1.7
5	8	0.2
6	10	0.1

TABLE XII

PERCENTAGE OF BIOCHEMICAL AND MORPHOLOGICAL MUTATIONS IN A. RUGULOSUS

Expt.	Time of irradiation with UV in minutes	Biochemical mutants %	Morphological mutants %
1	0	Nil	Nil
2	2	12.3	3.1
3	4	5.5	5.5
4	6	1.5	3.0
5	8	0.5	3.8
6	10	0.5	2.5

and percentage mutation are given in Table I & II respectively. The number of biochemical mutants reached a peak at 2 minutes of irradiation and those of morphological mutants at 4 minutes. The characteristics of scored mutants are studied at the next stage.

D.2.5. *Effect of nitrogen mustard as chemical mutagen on Rhizopus oryzae and Aspergillus chevalieri*

To 8 ml. of water containing 0.63 gms. of $NaHCO_3$, 1 ml. of methyl Bis B-chloro ethylamine and 1 ml. of heavy spore suspension of the above two fungi were added in two separate tubes. The mixtures were shaken regularly and at intervals of 5,10, 20, 40 and 60 minutes. 1 ml. quantity samples were withdrawn and added to 9 ml. of the decontaminating solution ($NaHCO_3$—0.7 gm., glycine 0.6 gm., Dist. water 1000 ml.); this was further diluted and plated out in complete agar medium. Total transfers were made on minimal agar medium at the next stage. The results of the experiment are given in Tables III and IV.

TABLE XIII

MUTATIONAL EFFECT OF NITROGEN MUSTARD ON RHIZOPUS ORYZAE

Duration of treatment with Nitrogen mustard	Percent survival
5 minutes	26.1
10 ,,	19.2
15 ,,	15.7
20 ,,	12.6
40 ,,	9.8
60 ,,	8.0

TABLE XIV

MUTATIONAL EFFECT OF NITROGEN MUSTARD ON ASPERGILLUS CHEVALIERI

Duration of treatment with nitrogen mustard	Percent survival
5 minutes	51.7
10 ,,	51.0
15 ,,	15.5
20 ,,	15.5
30 ,,	12.0
40 ,,	10.8
60 ,,	7.1

It was interesting to note that both the morphological and biochemical mutants were produced in the case of *Aspergillus chevalieri* which were stable but in *Rhizopus oryzea* the mutants were less stable in successive generations.

D.2.6. *A comparative study of a yellow mutant MT-10 with its parent pink coloured Streptomyces strain* Ac_{21}(460)

UV mutants were isolated from the parent strain Ac_{21}(460) and a yellow-coloured *Streptomyces* strain Ac (460). The mutant was not nutritionally deficient but possessed

certain morphological characteristics differeing from the parent in many respects. The characteristic diffusion of the yellow pigment in the medium and its consistent behaviour throughout several successive generations suggested the study of its cultural and physiological behaviour in different media. Its antibiotic activity, remarkable nature of pigment formation and also the effect of UV radiation were studied.

Cultural study. The cultural study was done by growing the strain in different media (as suggested by Waksman) and incubated at 28°C for 14 days. The *p*H was maintained at 7.0 in each case. The following table represents the cultural difference of the mutant strain with that of the parent one.

TABLE XV

Medium	Characteristics of the strains	
	Parent Ac_{21}(460)	MT-10
1. C-D agar	good spreading growth, vegetative mycelium Rose Dorce, spore shrimp pink, pigment not diffused.	good speading growth, veg. mycelium ca[illegible]m yellow, spore Maize yellow, pigment diffused in the medium.
2. Nutrient agar	fair spreading growth, vegetative mycelium pale orange yellow no aerial spore, no pigment diffused.	fair spreading growth, vegetative mycelium pale orange yellow, spore white, no pigment diffused.
3 Potato Dextrose Agar	fair spreading growth, spore shrimp pink, vegetative mycelium white, no pigment diffused.	good spreading growth, vegetative mycelium Apricot yellow, spore maize yellow, Buff-yellow pigment diffused.
4. Oat meal Agar	good spreading growth, vegetative mycelium Rose Dorce, spore scanty, shrimp pink; no pigment diffused.	good spreading growth, vegetative mycelium colourless, spore pale orange yellow, no pigment diffused.
5. Egg-albumen Agar	good spreading growth, vegetative mycelium shrump pink, spore shrump pink, no pigment diffused.	good spreading growth, vegetative mycelium pale orange yellow; spore pale orange yellow; light orange yellow pigment diffused.
6. Yeast-peptone Agar	good spreading growth, vegetative mycelium Geranium pink spore shrimp pink, no pigment diffused.	good spreading growth, vegetative mycelium Light orange yellow; spore pale orange yellow; Raw sienna pigment diffused.
7. Ca-malate	fair spreading growth, spore shrimp pink, vegetative mycelium colourless; no pigment diffused.	good spreading, vegetative mycelium pale yellow orange, spore pale orange yellow, no pigment diffused.
8. Salt nutrient Agar	moderate spreading growth; vegetative mycelium Rose Dorce, spore shrimp pink, no pigment diffused.	good spreading growth, vegetative myceluium pale yellow orange, spore pale orange yellow; Raw sienna pigment diffused.
9. Glucose-Asparagine Agar	good spreading growth, vegetative mycelium Rose Dorce, spore shrimp pink, no diffusion of pigment.	good spreading, vegetative mycelium colourless; spore pale orange yellow; Buff-yellow pigment diffused.
10. Yeast-Glucose Agar	good spreading growth, vegetative mycelium Brazil red, spore shrimp pink, no pigment diffused.	good spreading growth vegetative mycelium Buff yellow, spore Maize yellow; Raw sienna pigment diffused.

TABLE XV (Contd.)

Medium	Characteristics of the strains	
	Parent Ac (460)	MT-10
11. Starch Agar	good spreading growth, vegetative mycelium scarlet, spore scanty, shrimp pink, no pigment diffused.	good spreading growth; vegetative mycelium colourless; spore Maize yellow, Raw sienna pigment diffused.
12. Carbon nutrition medium	good olonial growth, vegetative mycelium Nopal Red; spore Eosine pink, no pigment diffused.	good spreading growth; vegetative mycelium Buff yellow; spore Maize yellow, Rawsienna pigment diffused.
13. Cellulose medium	no growth.	no growth.
14. Milk	growth in a circular ring, peptonisation completed in 14 days, slight alkaline *p*H—7.5.	growth in a circular ring; peptonisation completed in 14 days, highly alkaline, *p*H—9.0.
15. Potato plug	good spreading, spore Thulite pink.	good colonial, spore Maize yellow, Antique Brown pigment diffused.
16. Carrot plug	good spreading, spore shrimp pink.	scanty growth, not spreading, spore Maize yellow, Antique Brown pigment.
17. Gelatin stab	moderate growth, weak proteolytic action, liquifaction from 7th day.	moderate growth, weak proteolytic action-liquifaction from 12th day.
18. Peptone gelatin	fair growth, weak proteolytic actional liquifaction from 7th day.	–do.–

D.2.7. *Effect of irradiation on mutant strain MT-10 and parent strain* Ac_{21}(460).

The spore suspension of MT-10 was irradiated by ultraviolet rays of wave length 2537Å at a dose level of 100 ergs/mm²/sec. for 20, 40, 60 and 80 seconds respectively. The isolation of mutants was done by means of filtration technique and by total isolation method of Fries. Results of the treatment both for parent and mutant strain (MT-10) are given below.

TABLE XVI

Time of irradiation	Strain	Survival %	Morphological mutant %	Biochemical mutant %
20 secs.	Parent	9.7	6.5	0.0
	MT-10	18.2	3.9	1.5
40 secs.	Parent	1.3	6.9	0.9
	MT-10	0.2	9.8	1.1
60 secs.	Parent	0.21	11.1	1.86
	MT-10	0.006	15.6	2.3
80 secs.	Parent	0.03	10.7	1.2
	MT-10	0.002	6.5	0.9

To determine the characteristics of the biochemical mutants auxanographic technique was adopted. The following table represents the number of colonies showing nutritional deficiency both for the parent and the mutant strain.

TABLE XVII

(Biochemical mutants) Growth requirements		Number of strains isolated
Ac$_{21}$(460)—*parent*		
Glycine/Histidine/Alanine/Ornithine/Methionine	..	1
Histidine/Valine/Ornithine	..	1
Tryptophane/Hydroxyproline	..	1
Valine/Methionine/Cystine/Cysteine	..	1
Glycine/D-L-Proline/Histidine/Asparagine 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
MT-10 (*Mutant*)		
Second step mutants)		
Tryptophane/Cystine	..	1
Riboflavin	..	1
Citrulline	..	1
Histidine	..	3
Arginine/Tyrosine	..	1
Cytosine	..	1
Glutamic Acid/Alanine/Histidine	..	1
Lysine/Tyrosine	..	1

D.3. Microbial Genetics

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

In view of the affinity of the acridine dyes to the DNA complex in presence of visible light, its probable mutagenic action on the Neurospora gene was tested in the laboratory. Results of some preliminary experiments on the action of Acridine orange on a methionine less strain were given in the previous year report. Success with the Acridine orange dye in inducing mutagenic changes on a particular gene of *Neurospora* initiated a search for other allied acridines to be tried; of these, Acridine and Acriflavine could be procured locally. Experiments are now being continued to test the mutagenic action of different acridines and acridine derivatives on various known biochemical genes of *Neurospora*. For this purpose a fresh collection of biochemical mutations and wild types of *Neurospora* have been obtained from the United States and these are being maintained for future studies.

D.3.2. *Response of a thiaminless strain of Neurospora crassa*

The action of Bromo-uracil on thi –5 strain of *Neurospora crassa* is being elaborately studied at present. An accidental replacement of the Thiamin component of DNA by uracil may lead to hitherto unknown genetic effects and mutants arising out of such experiments might prove to be interesting. Actually mutants having different characteristics have been obtained through experiments with Bromo-uracil.

Fries *minimal* medium with sorbose having concentrations of Bromo-uracil at 1, 4, 6, 8, and 12 ug/ml of medium taking 100 ml in each flask were prepared. Each lot of medium was divided into 10 tubes taking 10 ml of medium in each. These were used for plating. 0.5 ml. concentrated spores of Thi-5 were pipetted in 50 plates and media containing Bromo-uracil of varying strengths were poured in petridishes taking 10 for each concentration. Ten platings were also done with 0.5 ml. of conc. spores in Fries minimal medium with sorbose as control.

After 7 days incubation at room temperature (25 C–28 C) some growth on the surface of a few plates started appearing. The growth behaviour observed was varying as follows:—

Results

1. Mild surface growth—spreading like wild type
2. Porfuse surface growth—spreading like wild type
3. Minute colonial growth
4. Small fluffy colonial growth
5. Typical compact back mutation colonial growth

Some of the spreading growth types have been tested with the addition of a maximum proportion of 12 gm. of sorbose per litre. Even such a high proportion of sorbose in the medium is resisted by the wild type growth of the variants obtained.

A detailed analysis of the mutants is in progress. It is proposed that along with genetic and biochemical tests of the mutants their respiratory behaviour will be tested as soon as possible.

D.3.3. *Studies on Sordaria sp.*

A search for Ascomycetous fungi other than Neurospora which might be adapted to genetical studies taken up since 1958, is being continued. A new Ascomycete, *Sordaria sp.* occurring in soil has been selected for genetical studies. This strain grows well in minimal medium and forms uniform colonies. Eight ascopores are formed in linear order in each ascus. The physiology and sexuality of the *Sordaria sp.* is being studied.

D.4. Studies on Rhizobium Bacteriophage

D.4.1. *Purification and concentration of Bacteriophage*

Thirty soil samples collected from the surroundings of roots of different Leguminous plants were tested to examine the presence of bacteriophage in them. Some showed their presence others did not. Bacteriophage was present in the nodules of many legumes except *Cicer aeritinum* (gram) and also in the soil adhered to the nodules when they were collected.

The presence of bacteriophage was detected by cross-streak test; dissolution test (Table 1), colony count test and finally confirmed by plaque test (Table II). Out of the thirty strains of phages only eight phages were selected for further work and purified by single plaque isolation.

Interractions of bacteriophage with the Rhizobium spp.

(1) Plaque count test

(2) Effect of different dilutions of bacteriophage on *Rhizobium* (Table III)

(3) Rate of growth of bacteriophage at two hours interval.

Irradiation test was done with UV light on different *Rhizobium* species to develop phage-resistant mutants. As a result, 7 phage resistant mutants were obtained. But in case of *Lens esculentum* all strains treated with UV became phage susceptible. Phage-resistant mutants were developed later by phage treatment.. The following methods were tried.

(1) A small quantity of 2–3 drops of conc. phage was spread on agar with heavy bacterial inoculum. After 7–10 days phage-resistant bacterial cells would be likely to appear. In this method the number of resistant cells were higher. This method, however, was adopted by in cases of *Rhizobium sp.* for *Cicer arietiumum*, *Phaseolus radiatum* and *Glycine hispida.*

(2) 24 hours broth culture of a *Rhizobium* strain was inoculated with 1 ml. of phage suspension. After 10–15 days of growth 1 ml. of it was transferred to another 10 ml broth and the process was repeated for 5–6 generations. At this stage most of the susceptible cells settled down and only phage resistant mutant cells were found on plating along with some moderately phage susceptible cells.

The cross inoculation test was performed with the bacteriophage against different *Rhizobium species*. The reaction was specific only in case of *Rhizobium* sp. for Cicer arietinum.

Irradiation test with UV light was done on single bacteriophage suspension. The bacteriophage became inactive with increase in time, and the phage inactivity did not interfere with the multiplication of *Rhizobium sp.*

Multiplication of the phage was done and it was tested each time by placing a drop of the mixtures of bacteria and phage on the plate and this was accompanied by a control set. Complete lysis or clearance of the plate indicated higher titre of the multiplied phage.

Precipitation of the phage was done in case of the host *Rhizobium* sp. for *Phaseolus radiatus* and the corresponding phage P13 and P7 for *Glycine hispida.*

Bacteriophage was found strong in case of the host *Rhizobium sp.* for *Cicer arietinum*, *Phaseolus radiatus* and *Glycine hispida.* In case of *Trifolium alexandrianum* (Berseem) both nodules and bacteriophage were absent in the different field soil.

Precipitated phage was inactive in case of P13 but in case of P7 full activity was demonstrated.

TABLE I

DISSOLUTION TEST OF BACTERIOPHAGE

Phage index	Rhizobium species	Hours or days of clearance of culture fluid	Date of second turbidity
P1	Crotolaria juncea	3— 4 days	The clearance remains for only 24 hrs. after which it became turbid.
P6	Cicer arietinum	24 hrs.	After 24 hrs. the clear culture fluid became turbid.
P7	Glycine hyspida	24—36 hrs.	For 15–20 days the culture fluid remained clear after which it became turbid.
P12	Cajanus indicus	4— 6 days	Turbidity appeared after 24 hrs.
P13	Phaseolus radiatus	2— 3 days	The fluid remained clear for 1–2 months.
P19	Arachis hypogea	4— 6 days	Turbidity appeared after 24–48 hrs.

TABLE II

PLAQUE TEST OF RHIZOBIUM

Phage index	Host bacterium	Diameter or size of plaque	Nature of plaque
P1	Rh.sp. for Crotolaria juncea	1 0–2.0 mm	Clear
P6	Rh.sp. for Cicer arietinum	2.0–3.0 mm	sharp, clear
P7	Rh.sp. for Glycine hyspida	1.5–3.0 mm	shar p,clear
P12	Rh.sp. for Cajanus indicus	1.0–2.5 mm	clear
P13	Rh.sp. for Phaseolus radiatus	1.5–2.5 mm	clear
P19	Rh.sp. for Arachis hypogea	1.0–2.0 mm	clear

TABLE III

EFFECT OF DIFFERENT DILUTIONS OF PHAGE ON DIFFERENT RHIZOBIUM SPECIES

Rhizobium host cells corresponding phage	Percentage killing of host cells					
	Phage dilution conc.	Phage dilution 10^{-1}	Phage dilution 10^{-2}	Phage diluation 10^{-3}	Phage dilution 10^{-4}	Phage dilution 10^{-5}
Crotolaria+P1	70.9%	65.1%	59.7%	47.3%	34%	22.1%
Cicer+P6	95.6%	89.1%	71.4%	51.4%	38.2%	26.7%
Glycine+P7	81%	75.2%	68%	54%	26.9%	10%
Cajanus+P12	61.1%	45.6%	38.6%	29.3%	21%	16.6%
Phaseolus+P13	80.4%	68.1%	54.6%	33.8%	30.5%	27.9%
Arachis+P19	64%	51%	41%	28%	18%	12%

D.4.2. *Field Expt. at Falta Exptl. Station*

Field trial was done with sixteen different Leguminous plants. It was found that in the soils of Falta Experimental Station occurrence of *Rhizobium sp.* of *Cicer arietinum* and *Trifolium alexandrianum* was rather scarce. In those cases where the yield was poor, heavy seed inoculation resulted in proper nodulation and increase of yield.

Inoculation of the field soil with phage resistant Rhizobium strains results in a high degree of nodulation and in increase in dry weight of plants. The nodule-forming capacity of the strains was also determined.

D.5. Ecology of fungi

D.5.1. *Fungal species in soil*

In continuation of earlier research programme, field experiments were continued for the third year in succession. Aman paddy of the variety patnai-23 was transplanted in the first week of August in plots kept under three treatments (Fo, F and F_D) since May 1959. This being the year of natural drought, the crop suffered from acute shortage of water supply and as a result, yield was very low, (Table I) which when compared with those of earlier years, will give an idea about the extent of loss sustained in years of adverse climatic conditions.

Isolation of fungi and their systematic studies were continued and data collected over the last three years have been analysed and the followng conclusions may be drawn.

Out of many isolates, (Table II) only eight are of common occurrence, showing marked seasonal variations. The rest had been obtained only occasionally and if frequently, only at a very lower percentage (Table III). Fungi occurring from root surface, are distinct and follow a pattern of succession.

Of the 8 common isolates, *Trichoderma viride* and *Cephalosporium* sp. are more frequent in drier and winter months (November-March) while hot and wetter months are dominated by *Aspergillus niger*, *A. terreus*, *Helminthosporium sp.*, *Curvularia sp.*, *Arachniotus indicas* and *Penicillium sp.*

Deep-ploughing and application of fertilisers have affected the population of some fungi significantly but others seem to be unaffected (Table IV).

Succession of fungi on paddy roots presents an interesting feature. Specialised root inhabitants like *Monotospora sp.* and *Thielavia angulata*, are dominant on mature and living roots. However, they sharply decline in growth and finally disappear from the dead and decomposing paddy roots left after harvesting. The reverse was noted with *Trichoderma viride* and *Cephalosporium* sp. which were less frequent on living roots but became rather dominant on dead ones. Several well-known plant-parasites were also noted from roots but the significance of their presence is too early to assess. Amongst these *Sclerotium oryzae* had been noted from paddy as parasite causing stem roots.

TABLE I

YIELD OF PADDY (mds/acre)

	1959	1960	1961
F_0	9.8±0.73	27.4±1.47	13.9±1.3
F	9.5±0.75	25.9±1.47	13.6±1.3
F_D	12.4±1.02	25.1±2.11	12.9±1.9

TABLE II

SEASONAL VARIATIONS OF COMMON FUNGI ISOLATED

	November-March	April-October
Aspergillus niger	++	+++
A. terreus	++	+++
Helminthosporium sp.	+	++
Curvularia sp.	+	++
Arachniotus indicus	++	+++
Trichoderma viride	+++	+
Cephalosporium sp.	+++	+
Penicillium sp.	+	+

\+ indicates presence
+++ ,, present at a higher frequency.

TABLE III

RARER SPECIES OF FUNGI PRESENT IN PADDY SOIL

1. Mucor-1	13. Sclerotium oryzae
2. Mucor-2	14. Pestalosia sp.
3. Syncephalastrum sp.	15. Phoma sp.
4. Cunninghamella	16. Monotospora sp.
5. Rhizopus nitgricans	17. Asco-4
6. R. oryzae	18. Thielavia terricola
7. Fusarium sp.	19. Phaeo-cycostroma
8. Sordaria bosensis	20. Alternaria sp.
9. Thielavia angulata	21. Hydropus sp.
10. Beltrania rhombica	22. Oospora sulphuria
11. Rhizoctonia solani	23. Vermicularia sp.
12. Nigrospora sp.	24. Several sterile form

TABLE IV

SOIL TREATMENTS INFLUENCING FUNGAL POPULATION

	F_0	F	F_D	L.S.D.
Trichoderma	17.9	10.7	6.2*	6.02
Helminthosporium sp.	3.3	4.4	1.1*	1.79
Curvularia sp.	8.7	5.6	3.7*	3.83
Aspergillus niger	13.1	20.5	35.9*	8.22
Aspergillus terrus	13.2	11.7	9.9	5.50
Penicillium sp.	12.7	12.9	9.2	4.536
Ciphale sporium sp.	12.5	16.2	14.8	4.329
Arachniotus indicus	12.5	9.2	13.2	3.85

* significant at 5% level,

D.5.2. *Fungal species in air*

Air acts as a good dispersing agent from microbes which may be carried from one place to another. This is specially important if some pathogenic forms are concerned. Though suitable and well-founded controlling measures may be under taken against the endemic diseases, the invasion by new forms from some distant or unknown places is likely to occur. It is, therefore, essential to know and gather informations whether such invasion is likely to follow in a particular region. Apart from the above considerations, it has also been demonstrated that fungal spores in air may act as allergens and thus emphasising its importance in public health as well.

A study of the fungal composition of air has been undertaken from February (1962) in order to make an assessment of the important fungal species generally transmitted through air, their seasonal variations and correlations if any, with temperature, rainfall and humiduty at three localities of West Bengal, e.g., Bose Institute in Calcutta, Shyamnagar and Falta in the Dist. of 24-Parganas. It is also proposed to trace the source of occurrence of some fungal species, if possible, in the localities concerned.

Methods used, consist of exposing 1.5% malt agar plates at the selected sites for one minute, 'ollowed by incubation at the laboratory temperature (30 —32 C); number of colonies per plate, calculated from 15 replications and percentage occurrence of a particular fugal species are thus recorded.

It may be seen from table 1 that air sampled for one minuite in February and March in the year 1962, contained a good number of fungal units, but the highest counts have been recorded from Shyamnagar area. In general, counts taken from Calcutta proper have always been less than those taken from outside the city.

Another most important point emerging from Table II, is the highest percentage of occurrence of *Cladosporium* species, which is known to act as allergens. Next come, *Alternaria* and *Curvularia* species. Of the three fungi named above, *Cladosporium* and *Alternaria* were found to be growing and producing abundant spores on some mature and senescent seasonal crops, as is shown in Table II. Thus it is evident that they are produced locally and hence account for the highest counts already noted.

Other fungi e.g., (Table II) are of rare occurrence, and therefore prolonged and careful investigation might reveal the real significance of their occurrence.

TABLE V

COUNT OF FUNGAL COLONIES PER PETRIDISH EXPOSED FOR 1 MINUTE

Month of the year	Bose Institute (Calcutta)	Shyamnagar (24-Parganas)	Falta (24-Parganas)
February, 1962	57.9	148.8	149.0
March, 1962	53.0	208.5	63.0

TABLE VI

OCCURRENCE OF FUNGI ON EXPOSED MALT AGAR PLATES (%)

Names of fungi	Bose Institute		Shyamnagar		Falta	
	February	March	February	March	February	March
	Percentage of fungi out of total count					
Cladosporium spp.	41.5	36.5	44.6	75.3	64.7	53.6
Aspergillus spp.	0.3	35.8	2.2	1.9	0.5	0.0
Penicillium spp.	5.0	2.4	0.8	1.9	0.0	5.2
Curvularia spp.	11.5	11.3	3.2	16.0	4.1	4.2
Alternaria spp.	5.7	5.8	9.8	28.8	7.7	19.9
Helminthosporium spp.	1.3	0.0	1.6	0.3	0.5	5.2
Cephalosporium spp.	9.3	7.6	2.4	5.1	0.0	1.1
Phoma sp.	0.3	0.0	1.6	0,3	0.5	5.2

TABLE VII

FUNGI FOUND GROWING ON MATURE AND SENESCENT PLANT ORGANS

Plant species	Parts of the plants examined	Locality	Identity of the isolate
Solanum melongona L. (Bringal)	Leaf	Falta	Alternaria sp.
Solanum melongena L. (Brinjal)	Leaf	Falta	Cladosporium sp.
Brassica oleracea L. (Cabbage)	Leaf	Falta	Cladosporium sp.
Brassica oleracea L. (Cabbage)	Leaf	Falta	Alternaria sp.
Lycopersicum esculentum Mill (Tomato)	Leaf	Falta	Alternaria sp.
Lycopersicum esculentum Mill (Tomato)	Leaf	Falta	Cladosporium sp.
Rosa sp. (Rose)	Leaf	Falta	Alternaria sp.
Dianthus caryophyllus L. (Carnation)	Leaf	Falta	Alternaria sp.
Dianthus caryophyllus L. (Carnation)	Leaf	Shyamnagar	Alternaria sp.
Dahlia sp.	Leaf	Shyamnagar	Alternaria sp.
Althea rosea L. (Holly-hock)	Leaf	Shyamnagar	Alternaria sp.

D.6. Industrial Fermentation

D.6.1. *Development of improved Citric acid producing strains of Asp. niger Second step radiation with Uv*

The strain C 110, the high-yielding mutant of *Aspergillus niger*, obtained by treatment with gamma ray irradiation, was again subjected to UV-radiation and the surviving sub-strains were tested for the potency of citric acid production. Results indicate that out of a total of 500 isolates studied here, 12 mutants acquired the capacity to produce increased quantity of citric acid than the parent one. The high-yielding mutants CGV 163 and CGV 1227 were found to produce 138% and 120% more citric acid over the original parent strain C.

D.6.2. *Effects of the period of incubation on the production of citric acid*

The strains CGV 163 and CGV 1227 were grown in fermentation medium and results on the citric acid production were recorded from the 3rd to 12th day of incubation at 30°C. Original *p*H of the medium was adjusted to 2. It was observed that CGV 1227 produced a peak yield of 56 mgs of citric acid per ml on the 9th day of incubation, while CGV 163

produced a maximum of 73 mg/ml on the 10th day of incubation. Both the mutants produced citric acid in the same medium.

D.6.3. *Growth in mixed culture of improved strains*

To determine if further increase in the yield of citric acid could be attained, the selected mutant strains were used for fermentation in mixed culture. The two strains, CGV 1227 and CGV 163 were, therefore, grown together in fermentation medium and results were recorded on the 8th day of incubation at 30°C. Table I indicates that the experiment did not result in increased yield on the 10th day where the results are compared separately.

TABLE I

FERMENTATION IN MIXED CULTURE

Strain	*Citric acid mg/ml	Mycelial weight g/culture
CGV163	73.0	1.05
CGV1227	54.4	0.88
CGV163+CGV1228	70.4	0.917

*N/10 NaOH titre value

D.6.4. *Determination of the optimum condition for fermentation with strain CGV* 163

To determine the optimum condition of nutrition for citric acid production, the culture was grown for a period of 10 days at 30°C. A number of experiments were repeated with Doelger and Prescott's medium in which several variations were made in the composition and concentration of the constituents of the medium.

Effect of sucrose concentration.—Sucrose was given to the basal medium in varying concentrations and results obtained are presented in Table II.

TABLE II

EFFECT OF SUCROSE CONCENTRATION ON CITRIC ACID PRODUCTION

Sucrose g/100 ml	Citric acid mg/ml	Mycelial weight mg/culture
10	31.68	584
15	79.68	1035
20	95.52	1142
30	98.40	1460
40	91.20	1715

The above results indicate that highest yield of citric acid was obtained when the fermentation medium contained 30% sucrose.

Effect of nitrogen sources.—In the basal medium NH_4NO_3 was replaced by the addition of other nitrogen sources in the concentration of 805 mg of nitrogen per litre. $NANO_3$ $KNO_3(NH_4)_2$, SO_4, NH_4Cl, Asparagine, Glycine, Urea and Methionine were used as nitrogen sources. Results indicate that NH_4NO_3 was the most favourable for citric acid production.

Effects of different concentrations of NH_4NO_3.—Careful consideration of the effects of different concentrations of NH_4NO_3 revealed that 2.3 g of $(NH_4)NO_3$ per litre was the optimum concentration for production of citric acid by mutant strain CGV 163.

D.7. Transformation in microorganisms

D.7.1. *Bacterial Transformation*

The transformation reaction was done in the case of *Micrococcus pyogens* var *albus* by treating the competent cells with deoxyribonucleic acid (DNA), *Micrococcus pyogens* var *aureus*, a golden coloured coccus producing coagulase and phosphatase and is sensitive to penicillin and streptomycin (with respect to the recipient organism). The organism produced acid from carbohydrates e.g., glucose, fructose, sucrose and also glycerol and mannitol.

Characters of Transformations.—The coloured mutants were produced after transformation and the isolates were identified. The transformants were then tested biochemically regarding their response to various carbohydrates and sugar alcohols, as well as gelatin liquifaction etc., showing the change of characters developed in the coloured variants. It has been observed that the mutants behave differently towards various culture media used.

D.7.2. *Fungal Transformation*

Transformation in *Micrococcus pyogenes* var *albus*, stimulated the extension of the study of transformation in other micro-organisms, e.g., fungi. A wild strain of *Aspergillus niger* (designated as V_{35}) was chosen as the donor while a number of morphological and biochemical mutants have been selected as the recipient strains. The parent, *A. niger* (V_{35}) is a fast growing organism having dark coloured conidia. Deoxyribonucleic acid (DNA) of fungal mycelium was extracted using Hotchkiss' method with slight modifications. 50 gms. of fresh weight mycelium yielding 25 mg. of DNA approximately. The DNA from strain V_{35} was used for transforming a strain-H_{14} ; This was treated with 5 γ of DNA/ml. of Czapek-Dox liquid medium for 0,2,4,6,8 and 10 hours at 28°C. After treatment, the spores were plated out in MM agar medium. Colonies arising on MM were characterised as transformants. The results are given in Table I.

TABLE I

EFFECT OF TRANSFORMING DNA FROM V_{35} ON MUTANTS STRAIN H_{14}

Time of reaction of DNA in hours	Total no. of colonies treated with DNA	Total no. of transformants	% transformation
0	243×10^3	Nil	Nil
2	243×10^4	4	0.0001
4	243×10^3	17	0.006
6	243×10^3	18	0.007
8	243×10^3	11	0.004
10	243×10^3	1	0.0004

E. ANIMAL PHYSIOLOGY

.1. Studies on the Metamorphosis of tadpoles (Rana tigrina and Bufo-melanostictus)

E.1.1. *Antibiotic and Vitamin treatment and Vitamin B_{12} production by the bacteria of the alimentary tract of tadpoles*

Further investigations were carried out on the effects of penicillin on the metamorphosis of two species of tadpoles (Rana tigrina and Bufo melanostictus) in similar conditions in order to make a comparative study. The effect of penicillin on the inhibition of metamorphosis in Rana tigrina is more prominent than that in Bufo melanostictus.

The eggs of the same hatching were collected from the tanks of the Bose Institute and kept in earthen vessels containing hydrilla. When the tadpoles developed they were divided into batches and selected for experiments. Penicillin treatment was continued for a month and the change in metamorphosis observed. The period of metamorphosis was delayed to a considerable degree in both species of larvae, but the effect of the antibiotic on Rana tigrina was more marked than that on Bufo melanostictus. The results of some of the batches are given in the following tables (Table I and II).

TABLE I

EFFECTS OF PENICILLIN AND VITAMIN B_{12} ON THE METAMORPHOSIS OF TADOPOLES (*Rana tigrina*) TREATMENT CONTINUED FOR 30 DAYS

	Control grs.		Vitamin B_{12} grs.		Penicillin grs.	
Weeks	Percentage of meta-morphosis	Percentage of death	Percentage of meta-morphosis	Percentage of death	Percentage of meta-morphosis	Percentage of death
1	nil	nil	nil	nil	nil	nil
2	nil	nil	2	nil	nil	nil
3	8	nil	10	nil	nil	2
4	38	nil	46	nil	nil	2
30 days	61	2	80	nil	2	6
5	80	2	100	nil	4	6
6	96	4		nil	8	6
7				nil	20	6
8				nil	50	6
9				nil	60	6
10				nil	80	6
11				nil	82	6
12				nil	84	6
13				nil	88	6
14				nil	90	6
15				nil	92	6
16				nil	94	6

TABLE II

EFFECTS OF PENICILLIN AND VITAMIN B_{12} ON THE METAMORPHOSIS OF TADPOLES (*Bufo melanostictus*). TREATMENT CONTINUED FOR 30 DAYS

	Penicillin grs.		Vitamin B_{12} grs.		Control grs.	
Weeks	Percentage of meta-morphosis	Percentage of death	Percentage of meta-morphosis	Percentage of death	Percentage of meta-morphosis	Percentage of death
1	nil	nil	nil	nil	nil	nil
2	nil	2.14	1.42	nil	nil	nil
3	17.85	5	37.85	nil	42.3	nil
4	39.28	,,	98.57	nil	87.69	nil
30 days	44.28	,,	100	nil	92.3	nil
5	72.14	,,		nil	96.92	3.07
6	80.71	,,		nil		
7	90	,,		nil		
8	95	,,		nil		

It is now established that penicillin delays the period of metamorphosis in tadpoles. Some variations in the effects of penicillin, Vitamin B_{12} and thyroxine regarding the dose, inhibiting or stimulating the metamorphosis in different species of tadpoles have been observed. Detailed studies on this aspect may reveal some interesting phenomena as to the species variations in response to some chemicals or agents.

Additions of vitamin B_{12} along with penicillin reversed the effect of the antibiotic. In vitamin B_{12} plus penicillin mixture groups no inhibition of metamorphosis was observed, rather the tadpoles transformed into froglets a few days earlier than the controls.

In continuation of the work communicated in the report of the last year in connection with the microbiological production of vitamin B_{12} by the bacteria isolated from the alimentary tract of normal and penicillin treated tadpoles, it was further observed that a large number of vitamin B_{12} producing bacteria of several varieties were present in the alimentary tract of normal tadpoles. Such bacteria, though fewer in number, were also present in the tadpoles which were treated with penicillin a few months ago, but in the freshly treated larvae, two types of penicillin resistant rod-shaped bacteria were isolated and found to produce negligible amounts of vitamin B_{12}.

It was noticed that a very small percentage of tadpoles metamorphosed during the treatment of penicillin. The guts of these tadpoles were dissected out and cultured for the growth of bacteria in nutrient agar medium. These bacteria were all gram-negative bacillus (short rod) and produced vitamin B_{12} in the same medium as used for previous experiments.

E.1.2. *Inhibitory action of penicillin and thiouracil on the effect of thyroxine on the metamorphosis of tadpoles*

The inhibitory action of penicillin on the effect of thyroxine on the metamorphosis of tadpoles was studied. The tadpoles of same age were treated in different batches with thyroxine, thyroxine plus penicillin mixture and penicillin. No change was observed in the control and penicillin treated groups. The tadpoles metamorphosed in thyroxine and thyroxine plus penicillin mixture groups but the measurement of the length of hind limbs and tail and the determination of the ratio of the hind limb length to tail length showed the partial inhibition of the action of thyroxine by penicillin (Table III).

TABLE III

RESULTS SHOWING THE EFFECTS OF THYROXINE AND THYROXINE PLUS PENICILLIN MIXTURE ON THE METAMORPHOSIS OF TADPOLES (*Bufo melanostictus*). TREATMENT CONTINUED FOR 12 DAYS.

Measurement of hind limb and tail length started from the 6th day of the treatment. Average of 20 tadpoles taken.

Days	Average hind limb length in mm		Average tail length in mm		Ratio of hind limb length to tail length	
	Thyroxine group	Penicillin plus thyroxine mixture groups	Thyroxine group	Penicillin plus thyroxine mixture groups	Thyroxine group	Penicillin plus thyroxine mixture groups
6th	2.8	1.6	11.4	9.7	0.24	0.14
7th	4.9	3.2	11.2	11.0	0.43	0.29
8th	6.4	4.8	11.0	11.2	0.58	0.42
9th	7.5	5.6	10.7	11.4	0.7	0.49
10th	8.2	6.2	8.4	8.5	0.97	0.72
11th	8.6	7.0	5.7	6.7	1.5	1.0
12th	10.0	8.0	5.0	6.6	2.0	1.2
13th	10.0	8.0	3.3	5.5	3.0	1.5

Similarly the tadpoles were treated with thiouracil and thiouracil plus thyroxine mixture and thyroxine separately in different batches. Thiouracil was found to exert a marked inhibitory action on the thyroxine-induced tadpoles (Table IV).

TABLE IV

EFFECT OF THYROXINE AND/OR THIOURACIL ON THE METAMORPHOSIS OF TADPOLES (*Rana tigrina*)

Groups	No. of Tadpoles in each group	No. of tadpoles metamorphosed (stage XX) within seven days of treatment	No. of tadpoles with small hind limbs on the 8th day
Thyroxine groups			
No. 1	10	10	nil
No. 2	10	10	nil
No. 3	10	10	nil
No. 4	10	10	nil
No. 5	10	10	nil
No. 6	10	10	nil
Thyroxine-Thiouracil mixture groups			
No. 1	10	nil	10
No. 2	10	nil	10
No. 3	10	nil	10
No. 4	10	nil	10
No. 5	10	nil	10
No. 6	10	nil	10
Thiouracial groups			
No. 1	10	nil	nil
No. 2	10	nil	nil
No. 3	10	nil	nil
No. 4	10	nil	nil
No. 5	10	nil	nil
No. 6	10	nil	nil
Control groups			
No. 1	10	nil	nil
No. 2	10	nil	nil
No. 3	10	nil	nil
No. 4	10	nil	nil
No. 5	10	nil	nil
No. 6	10	nil	nil

The above table shows that penicillin (dose—80 units per ml) has a slight and the thiouracil (dose 0.5 gm. per litre) exerts a marked inhibitory action on the thyroxine induced tadpoles (dose of thyroxine 0.005 mg per litre). The average length of hind limbs in different groups of thyroxine treated tadpoles was from 15–20 mm whereas in thyroxine plus thiouracil mixture groups the average length in different groups was from 5–10 mm. Detailed studies are being made on this aspect on two species of tadpoles (Rana tigrana and Bufo melanostictus).

E.1.3. *Chemical thyroidectomy*

The treatment of tadpoles with anti-thyroid drugs, viz., thiourea, thiouracil, methyl-thiouracil etc., causes inhibition of metamorphosis probably by interferring the biosynthesis of thyroid hormone. The initiation of metamorphosis of the retarded (penicillin treated) tadpoles by Vitamin B_{12} indicates that this vitamin may be involved in the processes of the synthesis of thyroid hormone by the thyroid gland. Attempts are being made to reverse

the action of thiourea by Vitamin B_{12} treatment in tadpoles. It was found that this vitamin in doses of 50 μg per litre of water could not transform the thiourea-treated tadpoles into froglets (dose of thiourea—1 gm per litre). Application of 100 μg of vitamin B_{12} per litre of water increased the length of hind limbs of thiourea treated tadpoles. However, the results are not conclusive and it is being tried with various doses of vitamin B_{12} to reverse the effect of thiourea in two species of tadpoles.

E.2. Histological studies on the activity of thyroid glands of tadpoles

It has long been known that the thyroid hormone is solely responsible for the amphibian metamorphosis. Thyroidectomy causes complete inhibition of the metamorphosis of tadpoles and the treatment with the hormone induces the metamorphosis of thyroidectomized larvae.

In the early stage of the larval life of the amphibia the thyroid glands remain in rudimentary or inactive stage. As the metamorphosis gradually advances, it is probably preceded by a change in the activity of the thyroid glands. It has been studied that as the tadpoles (Rana tigrina) gradually approaches towards the adult form, the cell area and the colloid area gradually increase, but when complete transformation takes place the colloid area suddenly decreases though the cell area still becomes higher (Table V). This histological demonstration of the gradual increase or stimulation in the activity of the thyroid glands, when the metamorphosis is in progress, prompted further investigations on the activity of the thyroid glands of penicillin and Vitamin B_{12} treated tadpoles. Preliminary studies showed that the both cell area and the colloid area decrease and increase in penicillin and vitamin B_{12} treated tadpoles respectively than those in the control larvae.

TABLE V

Stage of tadpole	Cell area sq.mm.	Colloid area sq.mm.
N_1—Normal tadpole without any limb	788.1	1087.5
N_2—Normal tadpole with small hind limbs	839.6	1843.7
N_3—Normal tadpole with large hind limbs	1745.0	5013.7
N_4—Normal tadpole with four limbs and tail intact (not retracted)	2188.7	7390.6
N_5—Normal tadpole with four limbs and tail completely absorbed	2447.5	6061.8

E.3. Biochemistry of Metamorphosis (of tadpoles)

Histological studies demonstrate that the activity of the thyroid gland increases as the metamorphosis is in progress. It has been reported by others that biochemical differentiation preceeds morphological differentiation. Biochemical studies have been undertaken

to estimate the level of thyroixine in various stages of development of tadpoles. During this study a new method has been developed in collaboration with the Department of Chemistry of Bose Institute for the detection of thyroxine and other iodinated derivatives.

Thyroxine, iodotyrosines and iodothyronines give negative test with starch. A new method has been developed by which the iodine of the above mentioned compounds can be liberated on the paper chromatogram by ultraviolet irradiation and detected by starch and potassium iodate solution.

In view of the inhibition of metamorphosis of tadpoles by penicillin and the reversal of this effect by vitamin B_{12}, it has been planned to undertake the studies on the (a) biosynthesis of thyroid hormone, (b) iodine uptake by the thyroid glands of tadpoles using radioactive iodine and (c) the transport of thyroxine from the glands to the tissues and to find out the effect of penicillin and specially vitamin B_{12} on these processes. Preliminary studies are being made on the binding of iodine with albumin and the action of penicillin and vitamin B_{12} on it. Special emphasis is being given to find out whether vitamin B_{12} is involved as a coenzyme in the different stages of biosynthesis of thyroid hormone or any other mechanism of action of this vitamin on the formation and secretion of the hormone.

F. WORKSHOP

In the year 1961-62 the total number of requisitions received from the different departments were about 550. Some major works undertaken are mentioned below:

1. One complete 110KV X-ray unit.
2. Coherent gama-ray scattering apparatus with double cone, multiple section filter system, mounted on an optical bench.
3. One casing for G.M. counter with U.H.F. connector, complete with lead chamber, perspex shelf and a planchet holder for beta counting arrangement.
4. Source holder for one curie γ-ray source.
5. Optical couplers for photo multiplier tubes.
6. Accessories for the neutron generator.
7. Two thermal conductivity detectors of different designes.
8. Carrier gas by-pass system for multi column Vapour-phase chromatography apparatus.
9. Paper electrophoresis apparatus.
10. Double walled cell for scintillation counter, and cooling system for the same.

Besides these maintenance and installation of equipments, construction and remodelling of laboratories were carried.

Refrigeration Section:

The section deals with the maintenance of refrigerators, deep freeze chambers and air cooling systems in different laboratories. Besides general repairs and overhauling these machines, the section has completed the installations of air-conditioning and other refrigerated equipments in the following laboratories:

Air condition: 1. Plant Biochemistry new extension—4 rooms—3 ton water cooled plant. 2. Director's office—1 ton air cooling window model. 3. Enzyme laboratories—Air conditioning of 2 floors and one 0° cold room—near completion.

Arrangements are being made to air condition the control room of neutron generator laboratory, the electrophoresis and immunophoresis laboratories and two rooms in Microbiology Department.

Refrigerated chamber: The section has completed one deep freeze chamber which can be maintained at temperature at –22°F. The constructions of another refrigerated chamber $4' \times 2\frac{1}{2}' \times 3'$, with arrangements to vary temperature between 4°C to 20°C for immunophoresis investigations is nearing completion.

The electrical section has completed the following installations: Microbiology Department's new extension of fermentation laboratory; new extension of the Plant Biochemistry laboratory.

Water Supply:

A 6″ deep-well tubewell fitted with turbine pump powered by 10 H.P. motor has been installed; it has the lifting capacity of 12,000 gallons of water per hour.

Gas Supply:

Due to increased consumption of gas in the main building laboratories it has become necessary to lay a new 2″ gas main line to provide for the increased demand.

G. LIBRARY

The library contains 5780 volumes (2754 books and 3026 bound periodicals). During the year under report 416 volumes (113 books and 303 bound periodicals) were added to the stock as against 349 volumes during the previous year. The total number of periodicals received was 232 (inland 67 and foreign 165) out of which 126 were subscribed and the remaining 106 were received either in exchange of Institute publications or as presentations. The library also receives a large number of technical pamphlets, circulars, reprints, annual reports, etc. from various institutions and Governments, both Indian and foreign.

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 only.

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.

The library grant, for purchase of books and journals, from the Institute's limited resources is not sufficient to meet the pressing demands of readers for more books and Journals. After meeting the mounting cost of periodical subscriptions, specially of American origin, not much is left from the recurring grant of Rs. 22,000.00 for the purchase of books, annual reviews, etc. During the year under report a non-recurring grant of Rs. 6,000.00 was received for purchase of some important back volumes of "Chemical Abstracts" and back numbers of other periodicals. It is, however, felt that further addition of important books and periodicals with their back volumes is extremely desirable for building up an up-to-date research and reference library.

The library was experiencing great difficulty of storing the increasing number of books and periodicals. Last year a room on the ground floor of the library building, of almost the size as the present stack-room, was made available as additional stack-room for storing of less important periodicals, Institute publications, etc. Most of this space is being utilised for storing of Institute publications such as "Transactions of the Bose Research Institute", "Annual Reports of the Bose Institute", "Acharya Jagadis Chandra Bose Memorial Lecture", and the publications of the Acharya Jagadis Chandra Bose Birth Centenary Committee. While this has solved the problem of storing space for the time being, it will be necessary, in the near future, for further expansion of the stack-room for storing of increasing number of books and periodicals purchased for the library.

PUBLICATIONS

During the year under report Vol. 24, Nos. 2 and 3 (June and September, 1961 issues) of the Transactions of the Bose Research Institute were published and Vol. 24, No. 4 (December 1961, issue) is now in press and expected to be published shortly.

Several important books and monographs of Jagadis Chandra are out of print for some time. Following a plan of reprinting them, the popular account written by Jagadis Chandra of his investigations 'Plant Autographs and their Revelations' has already been published. The fourth impression of his first monograph 'Response in the Living and the Non-Living' will be published in September this year. It is proposed to take up next the reprint of the monograph 'Comparative Electrophysiology'. A collection of six lectures delivered under the auspices of Jagadis Chandra Endowment Lectures (1959-61) will soon be available for distribution.

APPENDIX A

I. Governing Body

Dr. Debendra Mohan Bose
Shri Sudhansu Mohon Bose*
Dr. Sunando Bose
Shri Kedar Nath Chatterjee
Shri Saibal Kumar Gupta, I.C.S. (Retd.)
Prof. Satis Ranjan Khastgir.
Shri Sudhir Kumar Mandal
Shri Amiya Nath Mitra
Prof. Sisir Kumar Mitra, F.R.S.
Shri Abani Nath Mitter
Shri S. C. Mukherjee, I.C.S. (*Retd.*)
Dr. Basanti Dulal 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

* Died on 27-2-62.

IV. Building Committee

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

APPENDIX B

Staff

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

Department of Physics

Head of the Dept.: The Director
Research Fellow: Dr. T. C. Bhadra, M.Sc., D.Phil. (on study leave)
Mary & Richard Keating Scholar: Shri S. K. Datta, M.Sc. (upto July 1961)
Govt. of India Scholar: Shri Arun Kumar Chatterjee, M.Sc.
D.A.E. Junior Fellow: Shri Amalendu Nath, M.Sc.
Attached Worker: Sm. Krishna Paul, M.Sc.

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. (up to 8-1-62)
Dr. S. P. Sen, M.Sc., D.Phil. (on leave)
Dr. A. K. Barua, M.Sc., D.Phil.
Dr. B. B. Biswas, M.Sc., D.Phil. (from 23-9-61)
C.S.I.R. Pool Officer: Dr. A. G. Dutta, M.Sc., D.Phil.
Dr. Maharani Chakraborty, M.Sc., D.Phil.
Research Assistant: Dr. D. P. Chakraborty, M.Sc., D.Phil. (on leave)
Dr. V. N. Gadgil, M.Sc., D.Phil. (from 1-5-61)
Research Scholar: Dr. Nirmal Kr. Sinha, M.Sc.
Shri B. K. Burman, M.Sc.
Shri Radhakanta Mandal, M.Sc. (up to 1-1-62)
Sm. Latika Bose, M.Sc.
Shri Kalachand Das, M.Sc.
Sm. Susweta Sen, M.Sc.
Govt. of India Scholar: Shri S. D. Bhattacharya, M.Sc. (up to 3-1-62)
Sm. Shila Chowdhuri, M.Sc.
Shri Deb Kumar Mukherjee, M.Sc.
Shri R. Roychowdhuri, M.Sc.

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 Workers: Dr. S. N. De, M.B., D.T.M., Ph.D. (Lond.)
Dr. P. N. Das, M.B.B.S.
Dr. Madhuri Roy, M.Sc., D.Phil.
Shri S. P. Saha, M.Sc.
Dr. D. K. Chattoraj, M.Sc., Ph.D.
Shri Sudhir Kr. Ghosh, M.Sc.
Shri Dulal Mukherjee, M.Sc.

Department of Botany

Head of the Dept.: Dr. Sunando Bose, M.Sc.(Cal.), Filosofic Doctor (Lund.)
Research Fellow: Dr. R. K. Basu, D.Sc.
Statistician: Shri A. K. Roychowdhuri, M.Sc.
Research Assistant: Shri B. K. Dutta
Shri A. T. Guhathakurta
Shri A. K. Adhikari, M.Sc.
Shri V. S. Sarma, M.Sc. (up to 23-1-62)
Research Scholar: Shri Biswaranjan Banerjee, M.Sc.
Shri S. S. Sarkar, M.Sc.
Govt. of India Scholar: Sm. Anima De, M.Sc.
Shri Josy Joseph, M.Sc. (upto 12-10-61).
Sm. Sheila Sen, M.Sc.
National Institute of Science
Senior Fellow: Dr. Smritimoy Bose, M.Sc., Ph.D.
Attached Worker: Shri D. N. Chakraborty, 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: Dr. A. K. Misra, M.Sc., D.Phil.
Shri P. P. Mukherjee, M.Sc.
Research Scholar: Sm. Ashalata Paul, M.Sc.
Shri S. L. Chakraborty, M.Sc.
Sm. Itu Sanyal, M.Sc. (from 1-1-62)
Birla Scholar: Sm. Gita Bhattacharjee, M.Sc.
Govt. of India Scholar: Sm. Itu Sanyal, M.Sc. (upto 31-12-61)
Sm. Kalyani Bhattacharjee, M.Sc.
Sm. Roma Barat, M.A. (from 1-2-62)
C.S.I.R Pool Officer: Dr. A. C. Das, M.Sc., Ph.D. (from Sept. 1961)
Attached Worker: Shri Narendra Nath Shee, M.Sc.

Department of Animal Physiology

Research Assistant: Shri G. C. Bhattacharjee.
Research Scholar: Sm. Roma Ghosh, M.Sc.
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, B.A.
Library Assistant: Sm. Pratima Chakaraborty.

STAFF APPOINTED UNDER GRANT-IN-AID SCHEMES

A. Department of Atomic Energy, Government of India

Scheme No. I. Cosmic ray investigation
Investigator-in-Charge: Dr. M. S. Sinha, D.Sc., F.N.I.
Junior Research Assistant: Shri S. N. Sen Gupta, M.Sc.
–do– Shri N. Chowdhuri, M.Sc.

Scheme No. II. Nuclear Physics investigations with Neutron Generator
Investigators-in-charge: Shri A. M. Ghose, M.Sc. & Shri B. Mitra, M.Sc.

Scheme No. III. Investigations on the induced mutagenesis in crop plants using X-rays and Radioactive isotope
Investigator-in-Charge: Dr. Sunando Bose
Junior Research Assistant: Shri Amal Kumar Bose, M.Sc. (up to 17-1-62)
–do– Shri Balaram Mazumdar, M.Sc.

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
Botanist: Shri G. Gopalan Nair, M.Sc.
Assistant Botanist: Shri P. R. Das Gupta
Chemical Assistant: Shri C. Bnaerjee, M.Sc. (Tech.) (up to 15-9-61)
–do– Shri Amalendu Gayen, M.Sc. (from 12-1-62)

C. Indian Central Jute Committee

Radiation mutation in jute.
Investigator-in-Charge: Dr. S. Bose
Cytologist: Shri Asoke Das, M.Sc.

D. Indian Central Oilseeds Committee and Indian Central Jute Committee

Installation of Cobalt 60 source.
Physicist: Shri D. N. Kumar

E. Indian Lac Cess Committee

Constitution of lac resin.
Investigator-in-Charge: Dr. P. K. Bose
Senior Chemist: Shri S. K. Chakraborty, M.Sc.
Junior Chemist: Sm. Parul Chakraborty, M.Sc.

F. Council of Scientific and Industrial Research

(i) Scheme on 'Application of vapour phase chromatography in the analysis of Indian natural oils and the essential oils present in Indian tea and studies on the possibilities of using this technique as a preparative tool'.
Investigator-in-Charge: Dr. D. M. Bose
Senior Research Fellow: Shri Jyotirmoy Dutta, M.Sc.
Junior Research Assistant: Md. Maminul Haque, M.Sc.

(ii) Scheme on 'Chemistry of Indian plant resin'.
Investigator-in-Charge: Dr. P. K. Bose
Senior Research Fellow: Shri K. C. Padmanabhan, M.Sc.

(iii) Scheme on 'Biosynthesis and Metabolism of Gibberellic acid'.
Investigator-in-Charge: Dr. S. P. Sen
Senior Research Fellow: Sm. Anima Dutta, M.Sc.

(iv) Scheme on 'Genetical and biochemical studies of induced mutants of Aspergillus niger'.
Investigator-in-Charge: Dr. P. Nandi
Junior Research Fellow: Sm. Arati Sarkar, M.Sc.

(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
Dr. G. G. Das, (up to 28-2-62).

(vi) Scheme on 'Mycology and plant pathology'.
Investigator-in-Charge: Dr. P. Nandi
Senior Research Fellow: Dr. A. C. Das, M.Sc., D.Phil.

(vii) Scheme on 'Production of antibiotic substances by streptomyces spp.'
Investigator-in-Charge: Dr. P. Nandi
Junior Research Fellow: Shri Rabindra K. Sinha, M.Sc.
–do– Shri Printindra Nath Naha, M.Sc. (upto)

(viii) Scheme on 'Exchange of genetic material in microorganisms by transformation and transduction'.
Investigator-in-Charge: Dr. P. Nandi
Junior Research Fellow: Sm. Kalyani Chatterjee, M.Sc.

(ix) Scheme on 'Microbial synthesis of proteins in relation to the biogenesis of nucleic acids'.
Investigator-in-Charge: Dr. D. P. Burma
Senior Research Fellow: Shri R. K. Mandal

LIST OF PUBLISHED PAPERS 1961-62

Title of papers	Authors	Where published	Reference
1. The constitution of Barringtogenol D.	S. K. Chakraborty A. K. Barua	Experientia	18,66 (1962)
2. Alkaloids of the root-bark of *Glycosmis pentaphylla* Retz (D.C.).	D. P. Chakraborty B. K. Barman	Trans. Bose Res. Inst.	24 (3) p. 121
3. The constitution of Albigenin– a new triterpene from *Albizzia lebbeck* benth.	A. K. Barua S. P. Raman	Tetrahedron	18,155(1962)
4. Chemistry of *Aegiceras majus* Gaertn. Part IV. Some minor constituents.	K. V. Rao P. K. Bose	Ann. Biochem. Exptl. Med.	31,355(1961)
5. A paper chromatographic study of some natural coumarins and related compounds.	D. P. Chakraborty K. R. Guha B. K. Barman	Trans. Bose Res. Inst.	24 (4), p. 211
6. Studies on a ribonucleoprotein isolated from *Azotobacter vinelandii.*	R. K. Mandal	Ibid.	24 (3), p. 113
7. Sites of protein and nucleic acid syntheses in *Azotobacter vinelandii.*	R. K. Mandal D. P. Burma M. Chakraborty	Proc. Fifth. Intern. Congr. Biochem.	10,288(1961)
8. Ribonucleic acid synthesis in a cell free system from *Azotobacter vinelandii.*	R. K. Mandal	Biochim.Biophys. Acta.	55,238(1962)
9. Studies on microbial protein synthesis with *Azotobacter vinelandii* as the test organism. I. Incorporation of radioactive phosphate and sulphate into the resting cells in the presence of antibiotics and antimetabolites.	M. Chakraborty D. P. Burma	Biochim. Biophys.Acta	55,110(1962)
10. Studies on microbial protein synthesis with *Azotobacter vinelandii* as the test organism. II. Incorporation of radioactive amino acids into protein of cell-free particulate preparation.	M. Chakraborty D. P. Burma	Biochim. Biophys.Acta	55,120(1962)
11. Chemistry of *Aegiceras Majus* Gaertn Part III. Structure of Aegiceradiol.	K. V. Rao P. K. Bose	Tetrahedron	18,461(1961)

Title of papers	Authors	Where published	Reference
12. Floral study of the sterile *Crotalaria striata* L. observed in the radioactive monazite sand.	G. G. Nair	Trans. Bose Res. Inst.	24 (2) p. 67
13. Electrical Correlate of the Rhythmic Pulsatory Movement of *Desmodium gyrans*.	A. Guhathakurta B. K. Datta	Ibid.	24 (2), p. 73
14. Chemical control of weeds in some important Dicotyledonous crops of West Bengal.	A. P. Mani	Ibid.	24 (2) p. 83
15. Localization of primary CO_2 fixation products in photosynthesis.	S. P. Sen D. K. Basu	Ibid.	24 (2) p. 105
16. Studies on Auxin-induced Metabolic Changes in Plant tissues: Effect of Indole Acetic Acid on the Metabolism of Pyruvate-3-C^{14} and Acetate-2-C^{14} in *Avena* Coleoptiles.	S. P. Sen A. Sengupta	Trans. Bose Res. Inst.	24 (2) p, 95
17. Photoelectric Cross-section of Lead for Co.60 Photons.	A. M. Ghose	Ibid.	24 (3) p, 125
18. Host susceptibility of the causal organism, *Agrobacterium tumefaciens* strain B-23.	V. N. Gadgil S. K. Roy	Ibid.	24 (3), p. 141
19. Isolation of bacteria-free crown gall tumour tissue.	V. N. Gadgil S. K. Roy	Ibid.	24 (3), p. 147
20. Liquid shake culture method for growing plant tissues.	V. N. Gadgil S. K. Roy	Ibid	24 (3) p, 155
21. Influence of hydrogen ion concentration and accessory growth factors on the growth of *Hollyhock* (*Althaea rosea*) crown gall tumour tissue and utilization of different carbon and nitrogen sources by the tissue.	V. N. Gadgil S. K. Roy	Ibid.	24 (3) p. 163
22. The cytology of some sensitive species of *Mimosa*.	Mridula Datta Anima De	Ibid.	24 (4), p. 197
23. Studies on the ovule sterility of irradiated population of *Brassica juncea* Coss.	A. K. Roychowdhuri P. R. Dasgupta	Ibid.	24 (4), p. 207

Title of papers	Authors	Where published	Reference
24. An expression for the evaluation of partition coefficient in single withdrawal procedure in countercurrent distribution.	S. B. Ghosh P. Nandi	Trans. Bose Res. Inst.	24(4), p. 215
25. Studies on the decomposition of Crag herbicide and its effect on the soil microflora.	M. Purakayastha P. Nandi	Ibid.	24(4), p. 219
26. Decay asymmetry of Cosmic ray *muons*.	S. N. Sengupta M. S. Sinha	Proc. Phys. Soc.	79,1183,(1962)
27. Energy dependence of the decay asymmetry of cosmic ray *muons*.	S. N. Sengupta M. S. Sinha	Proc. 7th Ann. cosmic ray Conf. Chandigarh	43
28. Some preliminary results on high energy-meson interactions underground.	N. Chowdhuri M. S. Sinha	Ibid.	52
29. Scattering of mu-mesons by copper nuclei.	S. N. Sengupta M. S. Sinha	Nuovo Cimento	(in press)
30. Isopsoralene and its use in karyotype analysis.	M. Chaudhuri D. P. Chakraborty A. K. Sharma	Stain Tech.	37,95 (1962)
31. The chemical basis of the radio -mimetic changes induced by coumarin derivatives on plants.	D. P. Chakraborty M. Chaudhuri A. K. Sharma	Acta Histo-Chemica	(in press)
32. Increased variability in first generation Tomato plants following beta ray $P.^{32}$.	V. S. Sharma A. K. Roychaudhuri	Trans. Bose Rest. Inst.	25(1) p. 17
33. Effect of radioactive isotope on the flowering behaviour of Jute (*Corchorus olitorius* Linn.).	A. Das A. K. Roychaudhuri	Ibid.	25(1) p. 21
34. Studies on the morphological mutants of *Streptomyces albosporus* strain $Ac_{21}(460)$.	S. L. Chakraborty P. Nandi	Ibid.	25(1) p. 29
35. The amino-acid composition of four species of *Azotobacter*.	M. Purkayastha P. Nandi	Ibid.	25(1) p. 25
36. Physico-chemical properties of crystalline-globulin of sesame seeds	N. K. Sinha A. Sen	Ibid.	25(1) p. 37
37. An expression for the evaluation of partition coefficient countercurrent distribution.	S. B. Ghosh R. K. Sinha P. Nandi	Iour. Ind. Chem. Soc,	38,10 (1961)

Title of Papers	Authors	Where published	Reference
38. Bacterial Transformation—Transformation reaction in *Micrococcus pyogenes* var *Qureus* (Staph. aureus).	K. Chatterjee P. Nandi	Bull. Nat. Inst. of Sciences	19: 1962
39. Total cross-section measurements with 14 Mev Neutrons.	B. Mitra	Proc. Nuc. Symp. Madras	pp. 61
40. Nonelastic scattering of 14 Mev Neutrons.	A. Chatterjee A. M. Ghose	Ibid.	pp. 66
41. Small angle coherent scattering of gamma rays.	A. Nath A. M. Ghose	Ibid.	pp. 158
42. A Directional neutron counter for fast neutrons.	B. Mitra A. Chatterjee A. M. Ghose	Ibid.	pp. 199
43. Diseases of Rajanigandha (*Polyanthes tuberosa* L.) and Larkspur (*Delphinium ajacis* L.) caused by *Sclerotium rolfsii Sacc.*	A. C. Das	Science and Culture	27:549 (1961)