# REPORT

OF

# THE BOSE INSTITUTE

FOR

# 1958–59

BOSE INSTITUTE
93/1 UPPER CIRCULAR ROAD CALCUTTA–9

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# REPORT OF THE BOSE INSTITUTE 1958-59

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**Governing Body.** During the year four meetings of the Governing Body were held. Under Regulation 11 VI(g) of the Regulations and Bylaws of the Bose Institute, the Governing Body nominated seven of its members to the Council of the Bose Institute, who will hold office for three years with effect from August 1958. Their names are given in Appendix A.

In connection with the Birth Centenary Celebration of the Founder of the Bose Institute the Governing Body at a meeting held on May 27, 1958, decided to approach the Ministry of Scientific Research and Cultural Affairs for a non-recurring grant of Rs. 10 lakhs to be utilised for the following purposes :

(i) To purchase a plot of land in the outskirts of Calcutta and to build on it a Centenary Memorial Laboratory .. Rs. 5 lakhs

(ii) To create a Jagadish Chandra Centenary Endowment Fund, out of whose income provisions to be made for Visiting Scientists, and for the popularisation of science .. .. Rs. 5 lakhs

At a meeting held on 10.6.58, the Governing Body considered a letter from the Secretary, Jagadish Chandra Bose Centenary Committee, requesting co-operation for implementation of certain items of the Centenary programme, and authorised its Secretary to sign a joint appeal with the Secretary, Centenary Committee for contributions to the Centenary Fund. It was also decided that all contributions received for the permanent programme of the Centenary Celebration were to be credited to a special Jagadish Chandra Centenary Endowment Fund of the Bose Institute.

**Council.** Three meetings of the Council were held during the year. The three years term of office of the Council having expired during the year;, a new Council has been constituted whose composition is given in Appendix A.

The draft revised Regulations and Bylaws of the Bose Institute which was sent to the then Ministry of Education and Scientific Research, Government of India, in July 1956 is still awaiting the approval of the Government of India.

A proposal for the amalgamation of the grades of the Readers and Research Fellows was considered by the Council. Revision of the salaries of some of the Research Fellows was given effect to on the recommendation of a Committee of Experts.

Shri S. K. Das, Ex-Director, Regional Meteorological Centre, Alipore, joined as Registrar on 3rd June, 1958; he was relieved on 31st December, 1958 to enable him to take up an appointment under the auspices of World Meteorological Organisation. Subsequently Shri Upendra Kumar Datta, former Principal, Cotton College, Gauhati, joined as Registrar, on 2nd Janauary, 1959.

The Government of India has approved the payment of an additional interim D.A. of Rs. 5/- to the members of the staff drawing salaries upto Rs. 300/- p.m.; this has been given effect to.

**Grants.** The following grants were received during the year 1958–59.

(a) *Non-recurring grants :*

(i) Government of India, Ministry of Scientific Research and Cultural Affairs: Rs. 1,47,000.00 from the Government of India which together with the unspent balance of Rs. 28,548.13 from the previous year, were spent on building construction, laboratory equipment, apparatus purchase, etc.

(ii) for erection of a 20 curie Cobalt 60 gamma field at Nilgunge :
from Indian Central Jute Committee .. .. Rs. 11,000.00
from Indian Central Oilseeds Committee .. .. Rs. 14,000.00

(iii) The Rockefeller Foundation, New York, grant of (for .. purchase of apparatus and chemicals for biochemical .. research) .. .. .. .. .. $ 10.000.00

(iv) Ministry of Scientific Research and Cultural Affairs, Government of India, grants to individual workers for fundamental research .. .. .. .. .. Rs. 29,179.00

(b) *Recurring grant :*

(i) Government of India (Ministry of SR and CA) .. .. Rs. 500,000.00
(ii) Government of India (for training in scientific research) .. Rs. 6,073.00
(iii) Governing Body, Bose Institute .. .. .. Rs. 44,000.00

(c) *Grants-in-aid schemes :*

The following grants-in-aid were received during the year :

| *Sponsoring Body* | *Schemes* | *Amounts received* |
|---|---|---|
| Indian Central Jute Committee | Radiation Mutation in Jute .. | Rs. 15,471.50 |
| Indian Central Oilseeds Committee | X-ray irradiation on Oilseeds | Rs. 28,680.00 |
| Indian Central Oilseeds Committee | Improvement of Groundnuts* | Rs. 18,670.00 |
| Indian Council of Agricultural Research | Weed Control .. .. | Rs. 3,694.52 |
| Council of Scientific & Industrial Research | Antibiotic production .. | Rs. 7,238.23 |
| ,, ,, ,, ,, | Isolation of macromolecules by counter current method* | Rs. 5,500.00 |
| ,, ,, ,, ,, | *Induced mutation in *Aspergillus niger* .. .. | Rs. 2,696.00 |
| ,, ,, ,, ,, | Mycology and Plant Pathology* .. .. | Rs. 3,675.00 |
| ,, ,, ,, ,, | Protein synthesis in Micro-organism* .. .. | Rs. 1,210.00 |



| *Sponsoring Body* | *Schemes* | *Amounts received* |
|---|---|---|
| Board of Research on Atomic Energy : | (i) Cosmic ray .. | |
| | (ii) Nuclear physics .. | |
| | (iii) Neutron generator .. | |
| | (iv) Induced mutagenesis in crop plants using x-ray and radio-active isotopes .. .. | Rs. 100,000.00 |
| **Sir J. C. Bose Trust Fund No. 1.** | Research scholarship, stipends and foreign scholarships. | 14,000,00 |

* New Schemes :

**Acharya Jagadish Chandra Centenary Celebrations.** . The Celebrations followed generally the plan outlined in the last year's Report. For this purpose a General Commitee and a Working Committee with Dr. B. C. Roy, the Chief Minister, West Bengal, as Chairman were formed.

The inaugural function, on the morning of November 30, 1958, took place in a specially erected pavillion in the quadrangle of the Bose Institute. Dr. B. C. Roy as Chairman of the Working Committee opened the proceedings with a welcome address which was followed by a short one by Dr. D. M. Bose, Director, Bose Institute. The Prime Minister Shri Jawaharlal Nehru who inaugurated the function gave a sensitively phrased appreciation of the contributions of Jagadish Chandra as a philosopher scientist, who forty years ago sensed the danger of suppression of humanistic values by the alarming growth of national egoism. The last address was by Shrimati Padmaja Naidu, Governor of West Bengal, who presided over the function. Many scientific societies from all over the world had sent congratulatory addresses on the occasion ; these were printed in a book form and circulated to the gathering. Representatives of some of the scientific bodies who were present, personally delivered the addresses entrusted to them.

The function opened by the singing of '*Matrimandir*', a song composed by Rabindranath Tagore on the occasion of the opening of the Bose Institute in 1917. Dr. S. K. Mitra, Secretary of the Centenary Celebration Committee, at the end thanked the distinguished guests who took part in the function.

The 20th Jagadish Chandra Memorial lecture was delivered in the afternoon by Dr. S. Radhakrishnan, Vice President, India. The meeting was presided over by Prof. Humayun Kabir, Minister for Scientific Research and Cultural Affairs, Government of India. He had on 29th November opened the Centenary Exhibition. The addresses delivered during the inauguration are being brought out in a book form.

The Centenary exhibition was arranged at two places—the scientific one in a specially erected building to the west of the Institute quadrangle. It contained a collection of chronologically arranged instruments, which were used by Jagadish Chandra for his micro-wave and plant physiological investigations. Another collection of appratus and diagrams were also exhibited, illustrating some of the recent investigations carried out in the Institute.

The second part of the exhibition was arranged mainly on the ground floor of Jagadish Chandra's house, with an overflow in the front hall of the Bose Institute. A well documented catalogue was printed free of cost by a well known printing house.

The inauguration function on November 30 was followed by a series of lectures extending from December 1–5, 1958, given by distinguished scientists and scholars dealing with the manysided contributions of Jagadish Chandra, to science and to humanities. These lectures were follwed every evening by a documentary film on the life and activities of Jagadish Chandra. The exhibition was kept open from December 1 to December 9, 1958 ; it was attended by about 30,000 persons, a large proportion being students of schools and colleges.

The Centenary Committee presented to each of about 1800 schools in West Bengal, a large halftone portrait and a short life sketch in Bengali of Jagadish Chandra. In addition a brochure in English on Jagadish Chandra and a special memorial volume of the Transactions of the Bose Research Institute were brought out. The Transaction contained 20 articles from some of the leading scientists from India and from abroad. The programme of publication will continue for a year or more.

A scheme called 'Jagadish Bose Science Talent Search" Scheme, restricted for the present to West Bengal, and with which the name of Acharya Jagadish Chandra will be associated, is being worked out. The Chief Minister Dr. B. C. Roy and Sir J. J. Gandhy of the TISCO are taking interest in the working out of the scheme. Through the courtesy of the Ford Foundation in India, Miss Margaret Patterson consultant was brought out from the USA to advise on the scheme.

**New Constructions.** A two storied building at the western boundary of the Institute grounds has been completed. With the exception of a room on the first floor the rest of this building is occupied by the workshop with its store rooms. The hall on the first floor is temporarily housing the neutron generator laboratory. To the east of it, adjoining the Institute quadrangle, the ground floor of a building about 120′×25′ has been erected, and used to house the scientific apparatus section of the Centenary exhibition. One of the two wings of this which will house chemical engineering units and the other wings being fitted up for refrigeration and electronic workshops. To the north of this annexe, is under construction a laboratory consisting of an underground room with a ground floor room over it, for housing the neutron generator. On the north side of the quadrangle a new glass house has been erected ; it has an underground dark room in which both temperature and illumination can be controlled.

At Nilgunge in the Experimental Farm of the Jute Agricultural Institute, under grants received from Indian Central Oilseeds Committee, and Indian Central Jute Committee, a 3 acre gamma field with a 20 curie Co 60 source has been erected. Except for the Cobalt source with its lead container supplied by Phillips and Co., Holland, all the other contrivances including an interlocking arrangement which keeps the gate (giving access to the field) closed so long the source is above ground, have been made locally by the work shop staff.



Another 2 curie Cobalt-60 source procured through the same firm is being erected in the Institute ground, according a blue print supplied by Phillips and Co. The arrangement, when completed, will incorporate all safety measures for housing the source under ground, and for raising it above ground for purposes of irradiating living and non-living targets under conditions which will keep the intensity of stray radiation well below the presently prescribed safety limits.

**Instruments received under the Wheat Loan grant.** A grant of $30,000 for purchasing scientific apparatus and $1000 for books was made available to the Institute for the year 1956–57. Out of this grant, the following equipments have been received :

| | | |
|---|---|---|
| (1) | Spinco model E Analytical Ultra centrifuge and accessories .. | $. 18410.00 |
| (2) | Beckman model DK-2 Spectrophotometer .. .. | $. 7800.00 |
| (3) | Books .. .. .. .. .. | $. 1000.00 |

**Study Abroad.**

Dr. B. B. Biswas, research scholar, in the Dept. of Botany,was awarded a post-doctorate Fellowship by the University of Texas, U.S.A.

Dr. A. K. Banerjee, research scholar in the Dept. of chemistry, was awarded a post-doctorate Fellowship by the Brandeis University, U.S.A.

**Grants for study abroad**

A grant of Rs. 2000/- each out of Sir J. C. Bose Trust Fund grant was made available to Dr. B. B. Biswas and Dr. A. K. Banerjee to defray their costs of passage to the U.S.A.

**Visitors**

The following visited the Institute during 1958–59.

(1) Prof. Humayun Kabir, Minister for Scientific Research and Cultural Affairs, Govt. of India. (2) Professor Deyson of the University of Paris. (3) Dr. Mono Mohan Das, Deputy Minister for Scientific Research and Cultural Affairs, Govt. of India. (4) Dr. B. N. Uppal, Agricultural Commissioner to the Govt. of India. (5) Dr. E. W. R. Steacie, President, National Research Council of Canada. (6) Dr. Ivan Malek, Director of Biological Institute of Czechoslovakian Academy of Science. (7) Dr. Ing. Anton Kuhelj, Professor of Mechanics, University of Lzubljana, Yugoslovakia . (8) Academician Otto Wichtrle, Head of the Institute of Macro-Molecular Chemistry of the Czechoslovakia. (9) Dr. A. S. Parkes, F.R.S., Medical Research Council, U.K. (10) Dr. Gunthar Rienacker, Secretary, German Academy of Science, Berlin. (11) Academician L. Janossy Secretary of Hungarian, Academy of Science, Budapest. (12) Prof. A. Tiselius, N.L., Institute of Physical Chemistry, Uppsala. (13) Prof. Yoshiki Ogawa, Faculty of Engineering, Tokyo University. (14) Dr. Hans Stubbe, Institute of Cultivated Plant Research, German Academy of Science, East Germany. (15) Prof. Axel Nygren, Institute of Genetics, Uppsala. (16)Prof. N. Khanh Toan, Vice-Minister for Education of the Democratic Republic of Vietnam. (17) Dr. J. B. D. Derksen, Director, International Statistical Education Centre, Calcutta. (18) Dr. (Miss)Easther Seiden, Visiting Professor, International Statistical Education Centre, Calcutta. (19) Soviet Goodwill Mission under the leadership of Mr. A. Andreev, Member of the Preasidium, Supreme Soviet Russia.

**New Appointments and Award**

(1) Dr. A. K. Barua, M.Sc., Ph.D., was appointed Research Fellow in the Dept. of Chemistry with effect from 1-6-58.

(2) Sri K. L. Choudhuri, M.Sc., was appointed Research Fellow in the Dept. of Microbiology with effect from 1-4-58.

(3) Dr. T. C. Bhadra, M.Sc., Ph.D., was appointed Research Fellow in the Dept. of Physics with effect from 1-4-58.

(4) Sri R. K. Basu, M.Sc., was appointed Research Fellow in the Dept. of Botany with effect from 1-4-58.

(5) Dr. Sunanda Bose, M.Sc., Filosofic Doctor (Lund) was appointed Research Fellow in the Dept. of Botany with effect from 6-11-58.

(6) Shri Ajit Kumar Haldar, M.Sc., was appointed Mary and Richard Keating Student in the Dept. of Physics with effect from 1-8-58.

(7) Sri Sankar Lal Chakraborty, M.Sc., was appointed Research Scholar in the Dept. of Microbiology with effect from 1-4-58.

(8) Sri Debdas Mukherjee, M.Sc., was appointed Research Scholar in the Dept. of Botany with effect from 2-6-58.

(9) Shri A. P. Mani, M.Sc., was appointed Research Scholar in the Dept. of Botany with effect from 30-10-58.

(10) Shri Mahendra N. Roychoudhuri, M.Sc., was appointed Research Scholar in the Dept. of Chemistry with effect from 11-3-59.

(11) Sri Tika Ram Sharma, M.Sc., was appointed Research Scholar in the Dept. of Microbiology with effect from 16-3-59.

(12) Shri Asoke Kumar Chatterjee, M.Sc. was appointed Senior Manpower Scholar with effect from 1-4-58.

(13) Sm. Archana Neogi, M.Sc., was appointed Senior Manpower Scholar with effect from 20-6-58.

(14) Sm. Itu Sanyal, M.Sc., was appointed Senior Manpower Scholar in the Dept. of Microbiology with effect from 2-1-59.

(15) Sri Amalendu Nath, M.Sc., was appointed Senior Manpower Scholar in the Dept. of Physics with effect from 6-3-59.

(16) Shri D. N. Kumar, B.Sc., D.I.C. was appointed Physicist in the I.C.J.C. and I.C.O.C. scheme "Installation of Cobalt -60 source" with effect from 1-9-58.

(17) Shri Asoke Das, M.Sc., was appointed Cytologist in the I.C.J.C. scheme "Radiation Mutation on Jute" with effect from 1-7-58.

(18) Shri Saroj Bandhu Ghosh, M.Sc., was awarded a Senior Research Fellowship in a C.S.I.R. scheme with effect from 1-5-58.

(19) Sm. Arati Sarkar, M.Sc., was awarded a Junior Research Fellowship in a C.S.I.R. Scheme with effect 17-7-58.

(20) Dr. Atul Chandra Das, M.Sc., Ph.D., was awarded a Senior Research Fellowship, C.S.I.R. with effect from 1-8-58.



(21) Shri Pritindra Mohan Naha, M.Sc., was appointed Junior Research Assistant in a C.S.I.R. scheme with effect from 14-6-58.

(22) Shri Rabindra Kumar Sinha was appointed Junior Research Assistant in a C.S.I.R. scheme with effect from 22-4-58.

(23) Dr. M. G. Srivastava, M.Sc., D.Phil., was appointed Cytogeneticist in the I.C.O.C. scheme with effect from 9-4-58.

(24) Dr. J. G. Bhowal, M.Sc., Ph.D., was appointed Cytogeneticist in the I.C.O.C. scheme with effect from 22-11-58 vice Dr. M. G. Srivastava resigned.

(25) Sm. Maharani Chakravorty, M.Sc., was awarded a Senior Research Fellowship, C.S.I.R. with effect from 8-1-59.

(26) Sri A. M. Ghosh, M.Sc., was reappointed in A.E.C. scheme as Research Assistant on his return from U.S.A.

**Resignations**

(1) Shri S. K. Das, M.Sc. (Lond) Registrar, resigned with effect from 31-12-58.

(2) Shri Asoke Kumar Chatterjee, M.Sc., Senior Manpower Scholar resigned with effect from 31-1-59.

(3) Dr. M. G. Srivastava, Cytogeneticist in I.C.O.C. scheme, resigned with effect from 31-10-58.

(4) Sm. Nilima Basu, M.Sc., A.E.C. Junior Fellowship resigned with effect from 1-8-58.

(5) Sm. Mira Purkayastha, M.Sc., research scholar in the Dept. of Microbiology, resigned with effect from 2-1-59.

(6) Sm. Maharani Chakraborty, M.Sc., research scholar in the Dept. of Botany was released from ther incumbency with effect from 7-1-59. to enable her to accept a C.S.I.R. Fellowship.

(7) Dr. A. K. Barua was permitted by the Govt. of India to resign his National Research Fellowship with effect from 1-6-58 to accept the appointment as Research Fellow in Chemistry in the Bose Institute.

**Degrees Awarded**

(1) Sm. Madhuri Dutta was awarded D.Phil degree of the Calcutta University.

(2) Sm. Mira Purkayastha was awarded D.Phil degree of the Calcutta University.

(3) Sri A. K. Banerjee was awarded D.Phil degree of the Calcutta University.

**Seminars**

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

| | *Subject* | *Speaker* |
|---|---|---|
| 1. | Design of Experiment in Agriculture .. | Sri A. K. Raychowdhuri |
| 2. | Sulphate reduction in higher Plants .. | Dr. B. B. Biswas |
| 3. | Constitution of clerodin, the bitter principle *Clerodendrum infortunatum* .. .. | Dr. A. K. Banerjee |

| *Subject* | *Speaker* |
|---|---|
| 4. Biochemistry of mental disorder .. | Dr. J. J. Ghosh of the Calcutta University |
| 5. Biology in Picture .. .. .. | Dr. S. C. Dutta of Calcutta University |
| 6. Scientific Research in Some U.S. laboratories and what we can learn from them .. | Sri A. M. Ghosh |
| 7. Plant Physiological research in the Universities of Europe, U.S.A. and Japan .. | Dr. S. M. Sircar of Calcutta Uiversity |
| 8. Sucrose Synthesis in Plants .. .. | Dr. N. C. Ganguli of Calcutta University |
| 9. Neurospora, a suitable system for genetical research .. .. .. | Sri K. L. Chowdhuri |
| 10. Certain biochemical aspects of cancer .. | Dr. N. K. Chowdhuri of the Calcutta University |
| 11. Genetics of *Aspergillus* spp. in the light of recent research .. .. .. | Sri A. K. Mishra |
| 12. Polarisation of Cosmic-ray $\mu$-mesons .. | Dr. M. S. Sinha |
| 13. Future Nuclear Physics investigation in the Bose Institute .. .. .. | Shri A. M. Ghose |
| 14. Measurements of cross-section of 14 Mev. Neutrons and improvement of the present neutron generator .. .. .. | Shri B. Mitra |

In addition each department had its own programme of seminars.

**Special Lecture and Seminars**

(1) Miss M. E. Patterson, one of the sponsors of National Science Talent Search for the Westinghouse Education Foundation, gave a talk entitled "Science talent search in the U.S.A."

(2) Dr. A. S. Parkes, F.R.S., British Medical Research Council, delivered a lecture on some recent investigations carried out in his research unit.

(3) Prof. L. Janossy, Secretary, Hungarian Academy of Science, gave a talk entitled "Statistical Evaluation of Measurements."

(4) Under joint auspices of Botanical Society of Bengal, Prof. Axel Nygren, Institute of Genetics, Uppsala, Sweden, spoke on the Phytotron Laboratory at Uppsala and the work on plant ecology carried on there. Dr. Hans Stubbe, German Academy of Science, gave a talk on his recent investigations on plant mutation.



## RESEARCH ACTIVITIES : NON-TECHNICAL REPORT

**Ultrasonic rediation.** Investigations are being continued on the absorption of ultrasonic radiation in a homologous series of organic compounds with a view to establishing a relation between structure and absorption coefficient. Energy distribution in the successive orders of diffraction of a monochromatic beam of light in an ultrasonic field is being measured.

**Microwave measurements.** A new laboratory for microwave measurements, which will partly continue the line of investigations commenced by the Founder of the Bose Institute, has been established. The programme of work envisages (i) the study of microwave dispersion in artificial dielectric. The aim will be to obtain from the study of behaviour artifical dielectic models under microwave radiation (wave length 3 cm—6 mm), giving information on analogous phenomena taking place in the submicroscopic region. This discipline named 'Analog Physics' was pioneered by J. C. Bose.

The other lines of investigations which it is proposed to be taken up include resonance absorption measurement: (i) in media containing paramagnetic ions and (ii) due to free radicals present in many organic compounds and living tissues.

**Cosmic Rays.** Last year, determination of residual range and ionisation of particles stopped in a Wilson chamber, provided evidence for the existence of an intermediate particle of mass $525m_e$. This year a systematic determination of masses of stopped particles has been commenced using the recently completed large Wilson Chamber ($36' \times 30'' \times 16''$) which will be filled with helium gas. By application of a microphotometer method, determination of the variation in ionisation density along tracts of particles, which stop in the Wilson chamber, will be made. The rectangular chamber in Darjeeling is being used to determine anisotropy (if any) in the direction of emission of decay electrons emitted from stopped muons.

**The I.G.Y. programme** is being continued at Mayapuri Darjeeling, with measurements carried out with two cubic meson telescopes and one neutron monitor pile. The tabulated observations are being sent to the World Data Centre at Mainneapolis, U.S.A.

Investigations on the nuclear active properties of $N$-radiation are being continued at Darjeeling and at Calcutta. For this purpose combinations of ionisation chamber (alternatively cylindrical plate phosphors) and GM counters are being used. The energy spectrum investigated has been divided into two groups: (i) $10^8$ $eV < E < 10^{10}$ $eV$ and (ii) $10^{10}$ $eV < E < 2.2 \times 10^{11}$ $eV$. The constants determined are (i) absorption mean free path $\lambda_a$ (ii) interaction man free path $\lambda_i$ and the size frequency distribution parameter $\gamma$.

**Nuclear Physics.** Interaction cross section of thermal neutrons with bound protons in $H_2SO_4$ at different dilutions as well as in benzene and its halogen compounds have been determined. These measurements supply information on the binding of H as function of dilution of $H_2SO_4$; it also enables the cross section of OH in chlorophenol being calculated from a knowledge of the cross section of flurobenzene.

The true photo absorption cross section in lead of Co-60 photons has been determined and was found to be $4.32 \pm 0.73$ barns, in general agreement with theory.

The apparatus for radio carbon dating is nearing completion. A research scholar has been appointed to work with this apparatus.

**Neutron generator.** Further measurements on the yield of 14.5 MeV neutrons with the recently assembled more powerful neutron generator, using the well known T-D. reaction has been made. Previously with a 1$\mu$A deuteron current accelerated through 52 KeV, a yield of $1 \times 10^7$ neutrons per cm$^2$ per sec. was obtained. With the new apparatus, by increasing the accelerating voltage from 62 to 100 KeV, the resulting neutron yield was plotted and found to agree with the results of other investigators. At 62 KeV and 50$\mu$ beam current, the neutron yield was found to be $2.4 \times 10^9$ per cm$^2$ per sec. The apparatus is at present housed in an air conditioned room on the first floor over the workshop. As it is not safe to work the apparatus with the maximum beam current at the present site, an underground chamber for housing the neutron generator is under construction.

## PHYSICAL AND BIOCHEMISTRY

**Vapour phase Chromatography.** A new apparatus was designed and its construction carried out in the workshop. A special feature of the arrangement is that a preliminary separation of the volatile liquid mixture to be analyzed is made by liquid phase chromatography columns. Any desired fraction of the complex mixture with a special functional group can be separated and then analyzed in detail by the vapour phase chromatography apparatus. It is proposed to analyze such complicated mixtures as mineral oils, essential oils including those obtained from tea samples procured from different localities.

**Metabolic effect of total body radiation in small animals.** Two groups of investigations have been undertaken (i) a preliminary one with a group of 6 male rats, of which 2 were kept as controls. The rest were subject to 200 KV X-radiation suitably filtered, with a dose of 600r. None of the rats died during the period of investigation. Excretion curves for urea, uric acid, creatinine, inorganic sulphates, were determined, (ii) a group of inbred strain of 30 female rats were taken and divided into five groups of six each; three groups of six each were exposed to gamma radiation from the 20 curie Cobalt 60 source at Nilgunge, of intensities 300 r (severe exposure), 600 r (semi lethal) and 1200 r (lethal); the other two groups were kept as controls. Excretion patterns with time, for urea, uric acid, creatinine, total inorganic sulphate, urinary amino acids, and ammonia were determined. The results obtained have been analyzed, and certain common patterns were observed for severe and semi lethal doses. How the pattern for lethal dose deviated from that for the other two were noted.

**Plant Chemistry.** Systematic surveys were conducted on the occurrence in India of plants containing coumarins, saponins and alkaloids; different species in the genus *Clerodendron* have been investigated for the occurrence of the bitter principle *clerodin*.

Eleven plants have been investigated for the presence of coumarins. The compounds obtained have been tested for their antibiotic properties on eight selected microorganisms. Other biochemical propeties of coumarins have been investigated. Twenty non-medicinal



plants have been investigated for the presence of alkaloids; five have been found to contain alkaloids.

Further studies on the constitution of *clerodin* has been made; a tentative structure of *mesuol* isolated from *Meseua ferra* Linn has been proposed.

Detailed study has been made of the chemical contents of two saponin bearing plants. *Albizzia lebbeck* Benth, and *Centella asiatica*. The former contains albigenic acid for which a structural formula has been suggested, and a neutral triterpene for which the name '*albigenin*' has been proposed. *Centella asiatica* contains a saponin (*asiaticoside*) and an alkaloid (*hydrocotyline*).

A new triterpene alkaloid has been isolated from *Aegiceras majus* Gaertn, and has been named *aegiceradienol*; it is believed to be the first $C_{29}$ triterpene occurring in nature, and is of biogenetic interest.

**Radiochemistry.** More than 35 years ago, J. C. Bose had made some observations on the utilization of malic acid by the aquatic plant *Hydrilla verticilleta* in the presence of light. The problem has been now studied in detail using malate compounds tagged with C-14. Present investigations indicate that (i) malic acid is decarboxylated and a part of $CO_2$ evolved is fixed back in the plant along the conventional path and (ii) malic acid is incorporated into starch through phosphophenol pyruvate and reversal of glycolysis. Russian investigators have claimed that one of the earliest products of photosynthesis is an iron compound precipitable with 60 per cent acetone. This observation has been partly confirmed in this Institute, with experiments carried on with leaves of *A. leptopus* in nutrients containing the C-14 and P-32; one of the proteins from leaf extracts precipitated by acetone, when separated by two dimensional chromatography, was found to contain iron. This was later confirmed by autoradiography when radioactive Fe-55 was introduced in the nutrient.

Detailed tracer studies have been made on the following aspects of auxin induced growth (i) $CO_2$ fixation, (ii) acetate metabolism, (iii) phosphorous metabolism, (iv) sulphur-metabolism. Evidence has been obtained supporting the hypothesis that auxins lead to metabolic changes resulting in synthesis of high energy phosphate bonds needed for growth processes, which included water uptake and energy consuming metabolic reactions.

Tracer studies have been made on metabolic changes induced by photoperiodic treatment of short day *Rupsal* rice plants.

**Enzyme Chemistry.** A large number of analytical enzymes have been prepared using as starting materials (i) rabbit muscle, (ii) dried baker yeast and (iii) plants like potato and *Spinacea oleracae*. From the acetone powder obtained from the last named plant, a new stereo specific dihydrolipoic dehydrogenase has been isolated. This enzyme has been found inactive with (+) dihydrolipoic acid and freely active with (−) dihydrolipoic acid. With this enzyme a new way has been opened for the investigation of the mechanism of $\alpha$–keto-acid oxidation.

Studies have been commenced on the synthesis of protein by the bacteria *Azotobacter vinelandii*. It is postulated that protein systhesis takes place in four steps, of which the last two, viz., the incorporation of activated aminoacids into ribonucleic acid, the formation of protein on the nucleic acid template and its subsequent release as fully formed protein, are not well understood. A project has been started to study in detail each individual steps, and to throw light specially on the two steps where ribonucleic acid is supposed to play a direct role.

**Plant Physiology.** With the recently acquired three channeled pen recorder, studies are being continued on the correlation between electrical and mechanical responses in *D. gyrans* and in *M. pudica*. It was mentioned in the last report that the electrical pulsation pattern in *D. gyrans* more or less corresponds to that of an animal heart with it P.Q R. S. T. structure; the latter is related to the initiation of an electrical potential from a region in the heart. Attempts are being made to measure the propagation of the potential along the pulsating pulvinus by inserting microelectrodes along it. So far the attempt has not been successful. Further studies have been made to record the electrical changes accompanying the successive steps in the mechanical responses in a Mimosa leaflet, when the tip of one of the leaflets is stimulated. At one stage after stimulation, the main pulvinuus falls down followed by the contraction in a time sequence of the pinnae in the remaining three leaflets. The electrical correlates of the mechanical reflex arc, starting from the main pulvinus to the leaflets pulvinules, has been found to be both fast moving and of low intensity, which makes it difficult to record.

The effect of different loads on the pulsatory movements of a *Desmodium* leaflet has been studied. When additional load (increasing from 10 mg to 100 mg), is added, and directed against the upward movement of the leaflet, the amplitude of pulsation decreases progressively but the frequency remains unaltered. When the added load is directed towards the downward movement of the leaflet, for small loads upto 10 mg., the amplitude of pulsation is increased from 60 to 80. With increasing load the amplitude is reduced but not totally obliterated, the frequency, however, remains unaltered. It is proposed to study the changes in respiration of the leaflets under different loads.

**Cytology.** Studies on the behaviour of isolated nuclei floating in the endosperm liquids of coconut and areca nut have been continued. The observations are made in a temperature controlled aseptic room. Mitosis in areca nuts nuclei do not continue when mounted on a slide. Coconut nuclei continue to divide under such condition, if the room temperature is kept between 26°–28°C. The stages in the division of nuclei from coconut is being studied. In arecanut nuclei the spindle undergoes changes in shape under the action of light; some similariy in behaviour under the action of light and of chemical agents like colchicine, coumarin are being followed.

**Radiation Genetics.** A new scheme on the mutagenic effect of radiation on groundnuts, sponsored by the Indian Central Oilseeds Committee, came into operation this year. The Weed Control scheme financed by the ICAR terminated at the end of the year. As reported in another place, a Cobalt 60 gamma radiation field has started operation from



the middle of February, 1959. Different economic plants like jute, sesamum, ground nuts are being raised in the gamma field. The effects produced will be available for study in course of 1959–60.

Observations on the mutagenic action of X-rays, and B-radiations from P-32 and S-35, on jute, sesamum, ground nut, paddy, and tomato are being continued. The results obatined are evaluated for their genetic significance and for their economic suitability. Each year selections are made from plants characterised either by noticeable morphological changes or for higher yielding characters ; the progenies produced are studied as morphological mutants or for their higher yielding behaviour.

Some of the mutants have dominant characters, that is, they are evident in the first generation of progenies raised from irradiated seeds, as in the case of the *C. olitorious* species, R-26, which following treatment with P-32 produced plants with pigment characteristics similar to that of the Tall Mutant obtained previously by X-ray irradiation of R-26. The new tall Mutants when selfed have segregated in to R-26, Tall Mutant, and Chinsurah Green types. The segregation of character of the selfed progenies is being followed up to the 3rd generation. The comparative yields of fibres have been reported in the last year's report.

With the oilseeds Sesamum and Mustard, a number of recessive morphological mutants have been obtained after irradiation with X-rays.

The Sesamum type 16 has given a white flowered mutant (isolated in $X_3$) and a small seeded type (isolated in $X_4$ generation). They have both bred true upto $X_9$. A "mosaic" multicoloured (with colours ranging from ash, to grey and white) seed mutants were isolated in 1954 from S 12.

In Mustard two morphological recessive mutants have been obtained during the present year (i) with 'Appressed' pods (isolated from Rai 5 in $X_3$ generation) and (ii) a 'Thickened' pod (isolated from Rai 5 in $X_2$ generation). Though their yield of oil is not higher than those from the controls, still on account of their nonshattering character they may withstand mechanical harvesting and thus prove economically useful.

Besides X-ray treatment, the different types of Sesamum and Mustard have been treated to B-radiation from P-32 and S-35. No observable morphological mutants have been obtained. In all instances progenies from morphological mutants, and from individual plants with higher yield characteristics but without morphological deviations, are examined for their economically important characters eg. number of seed pods, weight of seeds per plant, the oil content and its keeping qualities.

Similar studies are being made on the effect of different radiations on other economic plants (i) on *Aman* paddy *Rupsal* and Patna and (ii) in *Boro* and *Aus* paddy and (iii) in tomato.

In previous years it was noted that a large number of plants in the treated population of *C. olitorius* (Tall Mutant, R-26, Chinsurah Green) had 2–6 branches emanating from

near the soil level, when treated with different concentrations (0.5 m.c. to 2.5 m.c.) of P-32, as against unbranched plants in the control and in the S-35 treatment.

The progenies raised from selected seeds of such multibranched plants have mostly reverted to the normal single stemmed type; only a few double stemmed plants are continuing in succeeding generations.

During 1957–58 treatment with different concentrations of P-32 on the seeds *C. capsularis* var. D154 and D386, gave rise to few plants with thick stems in the $P_1$ generation. Anatomical studies with regard to the cells and average weight of fibres per selected plant gave the following relative results :

| | *Mean ratio for thick stems* | *control* |
|---|---|---|
| Number of fibre cells | 2.2 | 1.0 |
| Yield of dry fibre | 2.2 | 1.0 |

Selection made from seeds obtained from the thicker plants were grown without any further treatment, along with the controls, in the $P_2$ generation, with the following results :

| | *Progeny of thicker plants* | *Control* |
|---|---|---|
| Number of fibre cells (in lakhs) | 27.4 | 26.0 |
| Yield of dry fibre (in gms.) | 1.94 | 1.70 |

The two instances given above, in which jute plants of the species Olitorious and Capsularis treated with radioactive phosphorous, exhibited abnormalities in characters in the $P_1$ generation; the progenies in the $P_2$ generation have reverted to the control type. These are probably instances of somatic mutation which have been reported previously in other plants.

**Microbiology.** The investigations carried out in this department are being extended to the study of mutations induced in microorganisms by means of ultraviolet radiation and by chemicals. The mutants produced are studied for increase of yield of the particular substances secreted by them eg. in the case of *Streptomycin* $A_{21}$(460) the mutants produced by U.V. radiation are being studied for their yield of antibiotic stubstances. Both morphological and nutritional mutants have been isolated. In the majority of cases the yield of antibiotic substance increased; in some cases, however, there was diminution in the yield.

Ultraviolet induced mutants in *Aspergillus niger* has been studied both for the yield of citric acid, as well as from the genetic point of view. In this strain of *Aspergillus niger* reproduction is asexual, by means of spores. Nutritional mutants are formed during UV irradiation; these are distinguished by their requirements for one or more supplemental growth factors. By mixing the hyphae of two nutritional mutants requiring different growth factors, heterokaryons are produced by the fusion of two such nutritionally different mutants. When the heterokaryonic condition of the mycellium remain constant on a selected medium it is said to be balanced. The mechanism of segregation and recombination of heredity particles could be studied by this method without sexual reproduction.

Different strains of fungi belonging to *Ascomycetes* have been collected . These microorganisms pass through a sexual phase, whose occurrence depend upon nutritional factors.



Excepting for *Neurospore cressa* and *Aspergillus nidulens*, not much is known of their sexual behaviour. It is proposed to study such behaviour and its dependence on nutritional factors.

Using the *Neurospora* backmutation test as the model, attempts are being made to ascertain the mutagenic action of certain chemicals singly and in combination.

Other investigations being carried in this department are (i) isolation of bacteriophage from soil, and study of their action on some strains of root nodule bacteria, and (ii) ecology of soil fungi, specially of those found in the root regions of some crop plants.

**Zoology.** Investigations are being continued on certain substances which retard the metamorphosis in tadpoles of *Rana tigrina* and *Bufo melanostictus*. In previous reports the effect of penicillin as a retardant of metamorphosis, and of vitamin B-12 as an agent for overcoming such retardation effect has been reported. This year Thiourea has been found effective in retarding by several months the metamorphosis in tadpole of *Bufo melanostictus*. It is being investigated whether the vitamin $B_{12}$ can reverse this retarding effect also.

Another investigation on the increase in body weight of albino rats by treatment with penicillin and thyroxine has been commenced. As with some other animals on which positive results have been previously reported, it was found that daily doses of penicillin (200 units) and thyroxin (0.002 mg) have produced significant increase in body weight of young albino rats. The effect of penicillin is more significant of the two. Investigation using larger number of rats is being continued.

# A. PHYSICS

## A.1. New Apparatus

A.1.1. *Microphotometer*

To carry out direct measurements on the intensity of very feeble amount of light as well as to measure the amount of blackening produced on a photographic film due to exposure to light, a sensitive microphotometer with a null type indicator has been recently made in the laboratory.

The apparatus consists of the following units :

(1) constant intensity light source

(2) photomultiplier tubes types 931A and 6801A with (a) power supply, (b) amplifying unit, (c) indicating meters, (d) crossed slit system, for varying the transmitted light intensity.

Photomultipliers 931A and 6810A are used as light detecting device; they are housed in a lighttight metallic chamber. Light is allowed to fall on the sensitive part of the photomultiplier through a lens system. This lens is used as a diffuser of light in order to illuminate the entire photocathode surface.

(3) Alternately the transmitted light beam is allowed to fall on a photographic film and the intensity of blackening in different order of diffracted light is measured. For this purpose the light source is kept fixed, the blackened film is moved at an adjustable speed across the slit placed in front of the light source. The transmitted light is measured by a photomultiplier connected to a recording arrangement. There is an interlocking device by which the movements of the blackened film and of the recording drum can be adjusted to any convenient ratio.

The power supply units, amplifier and the indicating meters are housed in an iron rack. All the controlling devices are mounted on a panel fixed on the rack. The photomultiplier assembly, light source unit, slit system, film drive unit are mounted on a heavy platform. Provisions are incorporated in the platform for making correct optical alignments.

## A.2. Ultrasonics

A.2.1. *Measurements on absorption of ultrasonic radiation in different liquid media in relation to their structural characteristics*

By extending the spherical radiometer method (published in Trans. Bose Res. Inst., 19, 1953-55), ultrasonic energy densities were measured in two groups of liquids.

(1) Water, methyl alcohol, ethyl alcohol, normal propyl alcohol, normal butyl alcohol and (ii) Benzene, carbon disulphide, carbon tetrachloride.



Following results were obtained from such measurements of ultrasonic energy density. The strength of the primary source of ultrasonic energy was kept constant during all such measurements; the experiments were carried out under identical conditions.

| Liquid group (i) | Absorption of energy ergs/cm³ | Difference | Liquid group (ii) | Absorption of energy ergs/cm³ | Difference |
|---|---|---|---|---|---|
| Water | 6.209 | | | | |
| | | 5.791 | Benzene | 9.31 | |
| Methyl alcohol | 12.00 | | | | 7.04 |
| | | 2.07 | | | |
| Ethyl ,, | 14.07 | | Carbondisulphide | 16.35 | |
| | | 2.49 | | | 12.44 |
| *n*-propyl ,, | 16.56 | | ,, tetrachloride | 21.75 | |
| | | 2.06 | | | |
| *n*-butyl ,, | 18.62 | | | | |

These measured values of differences in energy absorption in the liquids in group (i) indicate the existence of some relation with the structure of the liquids. It is evident from these values that except in case of water there is a regularity in the increase in the values of energy absorption in the alcoholic series; some irregularity is observed when one $CH_2$ group is added to a water molecule to get a methyl alcohol molecule. Non-linearity of water molecule seems to be responsible for such a large deviation from the regularity. It is known from other measurements that water molecule is not linear and the present ultrasonic energy absorption measurements support this view. In the case of the alcoholic series starting with methyl alcohol, it is evident that the magnitude of energy absorption increases by approximately equal amounts, with the increase in length of the molecule due to addition of a $CH_2$ group to a molecule. In the case of three highly absorbing liquids in group (ii) this relation may also hold, if we can a assign a characteristic linear dimension with each of these molecules and correlate the magnitude of energy absorption with this characteristic linear dimension. It is expected that such measurements will show a maximum in the value of the absorption of energy, when the characteristic of the linear dimension equals half the wave length of incident ultrasonic radiation. To establish some such relation, the measurements with the alcoholic series are being continued.

A.2.3. *Accurate determination of the frequencies of ultrasonic radiation propogated through liquid media*

For continuing the investigations reported last year in A.1.3, on the separate determination of streaming and radiation pressure in a liquid medium, it was found necessary to make more accurate determination of the frequencies of ultrasonic radiation. For this purpose, an absorption type wavemeter using a IN34 germanium diode crystal, with tuning coil, a variable condenser with micrometer drive, and proper sheilding arrangement has been made in the laboratory. This is calibrated by comparison with a standard wavementer. Actual reading of the frequency is taken from the calibration curve plotted with angular rotation of the tuning condenser against frequency. With this wavementer, frequencies within the range 1 Mc/s to 15 Mc/s can be measured. Attempts are being made

to extend the range of this wavemeter by calibrating it in the higher frequency regions. Some modifications have been made to obtain greater stability and constancy of the source of ultrasonic energy. Measurements on the separation of streaming and radiation pressure with three highly absorbing liquids benzene, carbon tetrachloride and carbon disulphide, are being continued.

A.2.4. *Measurement of the distribution of intensity in the different order of diffraction of light propagated through a liquid traversed by ultrasonic waves*

The acoustical interferometer reported last year in A.1.4. has been equipped with the following additional features in order to extend the scope of application of this apparatus to study the various problems of ultrasonic propagation in liquid media.

(a) A rotating sector of variable aperture
(b) Leica camera fitted with a fine focussing attachment
(c) A green filter for isolating the mercury 5461A line

Following investigations on ultrasonic in liquid media by employing the apparatus described in the previous paragraphs are being pursued: (i) The study of the intensity distribution of light diffracted by ultrasonic waves, (ii) Determination of the velocity of ultrasonic waves in liquid media by diffraction method as well as by acoustic interferometer method.

To this end photographs of the diffraction pattern in benzene have been taken under different experimental conditions.

Intensity of the spectral lines of the diffraction pattern as obtained in the photographs in the above experiments will be measured by employing the microphotometer connected with a recorder described in A.1.1.

A.2.5. *Development of pulse technique for the production of pulsed ultrasonic radiant energy*

Investigations on the production of continuous beam of ultrasonic radiation as well as its detection by (i) thermal method, (ii) radiation pressure method and (iii)optical diffraction method have been carried out with success since 1948 in this Laboratory. It is now realized that continuous wave generation method is beset with many difficulties for application to problems of measurement over an extended region. The highly developed pulse technique method has already shown its applicability over a greater range of problems as well as its supremacy over other methods.

Some amount of progress was made in the development of electronic circuits required for such a technique during the year 1950-52. (vide Annual Report, Bose Institute, 1.1 of 1950-51 and A.1.2. of 1951-52).

The non-availability of some important standard type electronic instruments and accessories such as (i) signal generator, (ii) fast oscilloscope, (iii) calibrated attenuator, (iv) linear high gain amplifier, and (v) pulse transformer, stood in the way of continuing the work at that time.

Since the beginning of this year, attempts have been being made to import the above mentioned articles and design work for the entire assembly is in progress.



## A.3. Microwaves

Microwave investigations have been started in the newly developed microwave laboratory during the past year. The following main lines of investigations are being taken up.

A.3.1. *Development of millimeter wavelength technique*

(i) Exciting a cylindrical cavity of requisite dimensions by means of dipole type spark gap; the output being monochromatised by using waveguides of gradually diminishing dimension serving the purpose of filters.

By this technique it has been possible to generate microwave radiation at 1-cm wavelength range. Different crystals were employed to detect the energy of microwave generated and maximum indication was obtained with a crystal calibrated for 1-cm wavelength. Attempts have been made to measure the wavelength with an absorption type cavity wavemeter but the method requires certain modifications.

(ii) *Harmonics generation by utilising the nonlinear characteristic of crystal diodes*

The fundamental frequency generated by a Klystron oscillator fed its power to a waveguide system suitable for the frequency. This waveguide is coupled to another waveguide suitable for a higher harmonic frequency; a crystal for generation of harmonics being placed at the junction of the two waveguides. Detection of harmonic frequency is effected by means of the detector unit coupled to the waveguide propagating the harmonic frequency.

By employing this technique, attempts are being made to generate the harmonics of 3-cm wavelength microwave radiation. Sufficient progress could not, however, be made for want of suitable waveguides and detectors. Attempts have been made to make waveguides of required dimensions in the workshop. The effort in this direction has proved successful. As the technique developed in the workshop for making waveguides of shorter length shows sign of promise, it is hoped to achieve the goal by the next year.

By these means it is intended to cover a few octaves of microwave radiation wavelengths from 3 cm to 6 mm., so that a study of the dispersion phenomena in this region could be made

A.3.2. *Microwave Analog Spectrometer*

The setting up of a 3-cm microwave spectrometer has just been completed. The entire assembly consists of the following accessories :

(i) 723 *A/B Klystron used as 3-cm microwave generator.*

(ii) *Electronically regulated power supply*—having provision for + 300 V and -200 V controllable outputs.

(iii) *Pyramidal horn* used as antenna to radiate the microwave energy into space directed in a narrow beam.

(iv) *Tunable waveguide detector.*

(v) *Liquid absorption cell*—Cells for different thicknesses so as to have different lengths of path in the medium, have been made with ebonite side walls in order to minimise the loss by reflection.

(vi) *Calibrated wave meter*—This unit is incorporated in the main transmission channel. This is practically a wavemeter cavity and is used as an absorption instrument.

Technically the entire arrangement may be termed as a 'horn measuring set up'. This arrangement gives microwave transmission through space. It is being employed to conveniently determine the properties of horn antennas, the attenuation of space transmission and the dependence of signal strength upon distance between antennas.

Necessary accessories are being incorporated in the spectrometer, described above, to study microwave dispersion using artificial dielectrics. This has already been undertaken. This type of investigation, now-a-days known as 'microwave analog physics', owes its origin to the founder of the Bose Institute Acharya Jagadish Chandra Bose. The main idea is to revive and extend his pioneering work in this line by subjecting macroscopic models of molecules to microwave radiation and thus obtain information on analogous phenomena occurring in the submicroscopic region.

A.3.3. *Resonance Absorption Experiments*

As an application of spectorscopy at microwave frequency, a Scanners 3-cm complete test bench equipment incorporated with a modulator and power supply unit has been recently installed in the laboratory.

Work on the modifications of the apparatus and development of the measuring techniques are being carried out since last year. It is proposed to take up one or more investigations from the following groups.

(i) Paramagnetic resonance absorption due to ions in a crystalline lattice whose energy levels can be influenced by an external magnetic field. This will supplement the investigations of Dr. D. M. Bose on the effect of electric field of surrounding atoms and molecules on energy levels of paramagnetic ions in salts and solutions.

(ii) measurements, on free radicals produced in living tissues due to radiation and other physical treatments, on F-centres, and eletron resonance in metals and semiconductors.

In carrying out investigations of the type mentioned in sections (i) and (ii) fixed frequency, but variable external magnetic field principle will be employed. The magnetic field will be accurately determined by employing proton resonance technique. Progress is being made in building up the proton resonance field meter. Different types of resonant cavities to carry out such investigations are being made in the workshop.

## A.4. Cosmic Ray

A.4.1. *High altitude studies*

The pictures obtained at Darjeeling by triggering the multiplate cloud chamber with a penetrating shower trigger have been analysed mainly for the detailed study of the mass-spectrum of stopping secondaries by measuring their residual range, scattering and ionisation. The results of this analysis show clear maxima at proton, k-particle and pion mass-values; but the ionisation estimates so far have been only visual and thus subject to large



errors. We have therefore placed an order for a transparency density measuring photometer for an accurate determination of the ionisation along cloud tracks and these results will be published after a final check of the ionisation values with this apparatus. This study has been undertaken to find out if there is any evidence of particles of mass intermediate between that of a proton and a k-particle.

The cloud chamber at Darjeeling has now been set up to expand with a trigger initiated by a coincidence–anticoincidence set so as to photograph stopped particles in any of the eleven $\frac{1}{4}''$ (5.7 gm/cm$^2$) Cu plates. The purpose of this experiment is to study the anisotropy, if any, of the decay electrons emitted from stopped muons. As the plates are thin, this will facilitate the photographing of the decay electrons and thus give a clear visual estimate of the amount of anisotropy. The cosmic ray muons are expected to be partially polarised and these muons have already shown some asymmetry in emission of decay electrons observed at sea level. In the same experiment we shall get an independent estimate of slow muon intensity at Darjeeling and a mass spectrum of stopped particles.

A.4.2. *Sea level investigations*

Analysis of the previous pictures taken with our small cloud chamber at Calcutta has enabled us to find out the energy spectrum of electrons at this place from 10–300 MeV. The main conclusion in this analysis is that the electron spectrum falls down according to a power law with an exponent −1.2, whereas the muon intensity gradually increases to reach an almost constant value of $5.8\times10^{-6}$ sec$^{-1}$ gm$^{-1}$ steraed$^{-1}$ at 100 MeV. If the intensities per MeV of both electrons and muons are plotted against their energies the two curves intersect at 95 MeV, pointing thereby that 95 MeV muons and electrons are equally abundant at sealevel. The muon-spectrum at the extreme low-energy end agrees satisfactorily with the computed sealevel spectrum of Sands.

The data on the scattering of low energy muons obtained with the small chamber and published last year gave a clear evidence of an anomalous component at the low energy end much larger than that obtained at higher energies. Although this result is in agreement with that obtained by the Russian group, it is not consistent with those obtained by the British group. Because of this controversial nature of the results, we have decided to study the scattering of moderate energy muons in a big cloud chamber fitted with $\frac{1}{2}''$ iron plates. With this large chamber we hope to collect a good statistically significant data within a short time.

Considerable time has been spent to make the large surface of the big cloud chamber leak-tight. It has now been completely mechanised and continous operation of the apparatus will be started as soon as the room in which it is housed is air-conditioned.

Since the plates in this chamber are fairly thick (11.5 gm/cm$^2$ of Fe), we have decided to use helium gas inside the chamber so that large variations in ionisation densities upto 8 or 9 times minimum can be measured. The Atomic Energy department of the Govt. of India has kindly consented to provide us with two cylinders of He. This will enable us to construct a mass spectrum of stopped particles at sealevel where we have already obtained some evidence of a particle of mass $\sim$525 $m_e$ first discovered by the Russian group.

We have also decided to replace the anti-coincidence system by a velocity threshold selector Cerenkov counter placed just above the cloud chamber. We shall concentrate on building up this Cerenkov detector in the next few months.

A.4.2. *I. G. Y. Programme*

Intensity measurements by the two cubical meason telescopes and one neutron monitor pile installed at Mayapuri, Darjeeling, during the IGY programme are still being continued. On the expiry of the IGY period in December 1958, the programme of intensity measurements has been continued as part of the programme under the IGC (International Geophysical Co-operation) scheme.

The data obtained from the meson telescopes for the first eight months of the IGY period (July 1957 to February 1958) have been analysed. The tabulated data for the first five months (July-November, 1957) giving the bi-hourly intensity values have been sent to the World Data Centre at Minneapolis, U.S.A. through the Indian Co-ordination Committee.

A few interesting variations in cosmic-ray intensity which are typical effects of solar flares and magnetic storms have been recorded. Sudden decreases in cosmic-ray intensity were recorded on the following dates : August 29, and October 21, 1957; and February 11, 1958. These variations were of a world-wide characters. Similar variations have also been observed by other participating laboratories.

A.4.3. *Investigations on Cosmic Ray N-radiations*

The nuclear active property of cosmic ray N-radiations has been studied employing an ionisation chamber, (or, a cylindrical plastic phosphor) along with G. M. counters, as detectors of nuclear interactions. The selection criteria of nuclear events of interest, were modified from time to time as demanded by the energy spectrum under investigation, which extended from energies below $2\times10^{8}$ eV up to $2\times10^{11}$ eV. The data obtained so far have been analysed for events divided into two groups as follows :

(a) *The high energy band :* $(10^{10}\ \text{eV}\leqslant E\leqslant 2\times10^{11}\ \text{eV})$

The detector used for investigations in this high energy region was a combination of ion chamber and G. M. counters arranged to select penetrating showers. Energy estimates of the primary, initiating the nuclear events, were achieved from the calibrated pulse height measurements of the ion-chamber pulses, assuming that the pulse amplitude was entirely due to the cascade multiplication of electrons from the decay of $\pi^{o}$'s liberated in the energetic interactions.

The experiment was undertaken in order to ascertain some of the important characteristics of high energy N-radiaions, which are directly correlated with the development and propagation of nucleon cascades within the atmosphere. The analysis of data obtained so far was undertaken keeping in view an estimate of the following parameters : (i) the absorption mean free path, $\lambda_a$ (ii) the interaction mean free path, $\lambda_i$ (iii) the energy spectrum at the sea level observation, (iv) the charged to neutral ratio of the penetrating



shower primaries, (v) the elasticity parameters $\alpha$ in high energy nucleon-nucleon collisions, (vi) the zenithal distribution of N-radiation at great depths within the atmosphere.

Some of the results obtained are shown in Table I

TABLE I

| Energy range in Bev | $\lambda_a$ g/cm² | | $\lambda_i$ g/cm² | | Exponent |
|---|---|---|---|---|---|
| | Lead | Air | Lead | Aluminium | |
| 10–50 | 320±30 | 130±7 | 185±20 | 80±5 | 1.55±.14 |
| 50–200 | 290±27 | 125±10 | 160±15 | 81±6 | |

The values quoted above have not been corrected for the zenithal distribution of penetrating shower primaries.

The size-frequency distribution of nuclear events of the energy band $10^{10}$ eV to $2\times10^{11}$ eV has been found to obey a power law giving value of the exponent $\gamma=-1.55\pm.14$. This value of the exponent $\gamma$ for the energy spectrum of N-radiation at an atmospheric depth of 790 g/cm² (Darjeeling) shows that the primary spectrum, characterised by a similar power exponent $\gamma=1.5$ in the energy region $10^{10}$ eV to $10^{13}$ eV does not change appreciably as the primaries propagate down through the atmosphere. This essentially unchanged spectrum of high energy N-radiations at great depths within the atmosphere coupled with the fact that the essential mechanism of propagation is the cascades of nuclear interactions initiated by the primaries, and sustained by secondary N-radiations through successive generations, demand a constant absorption mean free path, $\lambda_a$, and a constant value of $\alpha$, the elasticity parameter throughout the entire energy spectrum. The constancy of $\lambda_a$ is supported by observations in table I.

Further analysis is now in progress for the determination of the charged to neutral ratio and the zenithal distribution of penetrating shower primaries with a view to evaluate the elasticity parameter $\alpha$, and establish the correlations between the various parameters referred above.

(b) *The low energy region*

Investigations on the nature of nuclear active particles in the low energy region were performed, employing an ionisation chamber both at Darjeeling (2200 m) and at Calcutta (sealevel), and, a flat cylindrical plastic phosphor only at Calcutta. In both experiments the selection of events by G. M. counter geometry was such that only low energy nuclear interactions occurring within the body of detectors (gas volume and the ionchamber wall, or, the phosphor body and its housing assembly) having no charged secondary ejected out of its body, were assured. In both cases the energy measurements were deduced from pulse

amplitudes calibrated against single relativistic $\mu$-meson pulses from the detectors. The results of our analysis are shown in table II.

TABLE II

| | Exponent $\gamma$ | $\lambda_a$ g/cm² Lead | air | % of charged primary |
|---|---|---|---|---|
| Ion chamber bursts | −3.2 | 300±30 | 153±7 | 25% at Darjeeling |
| Phosphor bursts | −2.6 | 296±22 | .. | 5% at Calcutta |

[A paper entitled 'On the absorption mean free path of *N*-radiations' has been reported in the Cosmic Ray Symposium held at Bombay on 9th–12th March 1959]

## A.5. Nuclear Physics

A.5.1. *Measurements on thermal neutrons*

Bound proton-slow neutron cross sections have been investigated using a neutron howitzer and an enriched $BF_3$ counter. Experimental values of the scattering cross-sections have been correlated to the structure of scattering molecules using mass tensor approximation theory of Sachs and Teller as interpreted by Kolos and Janik. Experimental values for the scattering cross section of $H_2SO_4$ at different dilutions show the existence of $OH_{3+}$ ions rather than free hydrogen ions at higher dilutions and also show the different stages of dissociation. The possibility of hydration of the acid at higher concentrations is not clearly indicated by the experiment. Further investigations are in progress.

Measured values of the cross sections for flurobenzene, benzene and monochlorobenzene showed that the scattering cross-sections per proton in these liquids are 50.8 ±0.5, 43.6±0.1 and 55.3±2.8 barns. From the cross-section for flurobenzene, the cross-section of OH in chlorophenol was calculated. The measured values of cross-section for p– and 0– chlorophenol showed general agreement with theory and indicated association effects of the molecules in the liquid form.

A.5.2. *Absolute measurement of Co60 photons in lead*

The spherical symmetry method, which has been developed in this laboratory, has been applied to determine the true absorption cross-section of Co60 photons in lead. We have taken into consideration the effects of multiple scattering, alteration of detector efficiency due to degeneration of photons, finite size of the source and other effects.

Using a G-M counter, the measured value of absorption cross- section of lead came out as 4.32±0.73 barns, which shows general agreement with theory. To reduce the error in the final result, we have developed a new type of scintillation photon detector which has practically constant efficiency for photons above 200 KeV. With this detector and a more precise set of apparatus, which are under construction, it is hoped to obtain a very accurate value of the total absorption and hence the photoelectric cross-section of MeV photons in heavy elements.



A.5.3. *Application of Scintillation Spectrometers to Cosmic Ray Investigations*

Continuing our work on cosmic rays using scintillation spectrometers, we have found that at sea level the size-frequency distribution of pulses produced by cosmic rays in a 2″ diameter, $1\frac{3}{4}$″ long NaI crystal follows a simple power law. A multi-channel pulse height analyser is under construction to study the fine structure in the spectrum.

A.5.4. *Radiocarbon dating*

The radiocarbon dating equipment has been removed to a more suitable room in the laboratory. Anti-coincidence guard ring as well as the proportional counter assembly has been constructed and tested. Possibility of synthesing p-cymene and other similar compounds for scintillation counter work is under investigation.

## A.6. Neutron Generator

It was stated in the last report that 14 MeV neutrons were generated in a powerful neutron generator, which had been constructed in addition to a prototype instrument. Neutrons were generated by $T^3$ (d, n) $He^4$ reaction.

The total number of neutrons released from the target in the forward direction *i.e.* in the direction of motion of projective deuterons, has been estimated. Increase in the yield of neutrons with increase of projectile energy has been studied.

A.6.1. Work of further increasing the efficiency of the high voltage machine has been undertaken. Increasing the number of voltage-multiplication stages in the Cockroft Walton high voltage generator is now being done for obtaining higher voltages. Application of this high voltage source for accelerating deuterons to produce 3.5 MeV neutrons by D(dn) $He^3$ reaction is being proposed.

A.6.2. The number of neutrons released from the Ti/Zr target (in which .61 cc. of tritium gas was absorbed) in the powerful neutron generator, has been estimated by foil activation method. A silver foil of normal isotopic composition of $Ag^{107}$ and $Ag^{109}$ in the ratio 51.4% and 48.6% has been attached to the atmospheric side of the target and this irradiated with fast neutron flux. $Ag^{107}$ and $Ag^{109}$ turn into radioactive $Ag^{106}$ and $Ag^{108}$ by (n 2n) reaction. The decay scheme are as follows:

$$Ag^{107} \ (n,2n)\ Ag^{106} \xrightarrow[24\ \text{min.}]{\beta^+} Pd^{106}$$

$$Ag^{109} \ (n,2n)\ Ag^{108} \xrightarrow[2.3\ \text{min.}]{\beta-} Cd^{108}$$

By absolute counting of the $\beta$ particles from the short lived isotope, no. of neutrons has been obtained from the following relation :

$$n = \frac{A}{\sigma.N},$$

A = equivalent activity
$\sigma$ = (n, 2n) reaction cross section
N = number of nucleii/cm² in the foil.

Relative increase in the yield of neutrons with increase of deutrons energy from 30 KeV to 100 KeV has also been obtained, and the curve fits with that obtained by Conner, Bonner and Smith (1952, Phys. Rev. 88, 468).

More efficient proportional counting of the fast neutrons has also been developed which employs scintillation counting of recoil protons, discriminating against $\gamma$-radiation.

Monitoring is being done by scintillation counters which has very low $\gamma$-sensitivity.

A.6.3. The following chart shows the performance of the neutron generators built up in the Institute.

| No. | High voltage obtained from | Total accelerating potential impressed during experiment | Max. ion-current drawn at any time | Yield | Detector |
|---|---|---|---|---|---|
| 1. | 150KV, 50 cycles rectifier-condenser voltage doubler unit | 52KeV | 165 $\mu$a | $1 \times 10^7$n. per micro coulomb of beam | Foil activation |
| 2. | Do | 62/KeV—100KeV | 1000$\mu$a | $2.4 \times 10^9$n, with 50 micro coulomb of beam | Foil activation also Scintillation counting. |
| 3. | 240KV Cockroft Walton type voltage multiplier unit | upto 125KV | 1000$\mu$a | | |

Work is in progress for increasing the efficiency of the present 14 MeV neutron generator by:—

(1) Increasing the efficiency of the target cooling system, so that more ion-current can be drawn without damaging the target,

(2) Increasing the stages of the voltage multiplier unit by another 8, thereby making it possible to obtain 320 K.V. for acceleration of the projectile particles. For this special corona shielding devices have been constructed.

For the housing of the neutron generator, providing for all shielding facilities, an underground room with air-conditioning and humidity control devices is nearing completion. The following investigations have been undertaken :

(1) Determination of (n, 2n) reaction cross section of various elements.

(2) Spectroscopy of emitted neutrons.

## B. CHEMISTRY

### B.1. Physical and Biochemistry

B.1.1. *Vapour Phase Chromatography*

A new vapour phase chromatography apparatus has been designed and constructed at the Institute. The entire glass-portion of the instrument is supported on a manifold. This glass-portion is divided into a number of easily detachable units connected by standard joints. A new type of thermal conductivity cell, designed and constructed at the Institute, constitutes the detector unit of the apparatus. The thermal conductivity cell has a pair of very sensitive thermistors as its sensing element. The instrument is so designed as to accommodate a number of chromatographic columns at a time, with the help of a carrier gas bypass system. The carrier gas may be selectively passed through one or more of the chromatographic columns containing different liquid phases. This special modification will enable one to pick out any desired fraction from a complex mixture of a large number of volatile compounds with different functional groups and at the same time to analyse in detail the chosen fractions. Only with this modification it is possible to analyse such complicated mixtures as the natural essential oils and mineral oils. It is proposed to analyse with the help of this apparatus the essential oils obtained from Indian tea and also the low-boiling fractions of Indian petroleum samples. Another new feature of this instrument is the sample collection unit. This consists of a number of small fluted tubes of narrow bore attached to the exit end of the instrument. The volatile components coming out of the chromatographic columns can be collected in these sample collectors in a very pure form by cooling the collectors in suitable low temperature baths. The chromatographic columns are placed in an air thermostat and can be kept at any temperature up to 200°C. The instrument has been calibrated with volatile liquids like *n*-pentane, *n*-hexane, ethyl and methyl alcohol, diethyl ether, etc. The columns containing decyl phthalate, silicone oil and tricresyl phosphate as liquid phase have been tested. The supporting medium in all these columns is C-22 fine brick powder (60 to 80 mesh).

B.1.2. *Studies on the Decomposition Kinetics of Ascorbic acid in Solution*

In this study it is attempted to evaluate the rate of decomposition of a very low concentration (0.002% to 0.004%) of ascorbic acid in ion-free water and to compare the value with those obtained in presence of catalytic amounts of $Cu^{++}$ ion ($10^{-6}$ gm./lit). The rates of decomposition were optically measured by means of a Beckman DU spectrophotometer. The decomposition rates were found to be of the first order and $Cu^{++}$ ion behaved as a positive catalyst for the decomposition.

B.1.3. *Metabolic Effects of Total Body Ionising Radiations in Animals*

(1) *Effect of Total Body X-ray Exposure:* Six male rats from the same litter were kept in individual metabolic cages and their normal urinary pattern was studied for one week. The various end products of nitrogen metabolism were quantitatively measured, such as

urea, uric acid, creatinine, total inorganic sulphates and total neutral sulphur and also the free amino-acids in urine. The importance of estimating sulphates lies in the fact that sulphur-containing amino-acids and peptides are known to be highly radiation sensitive and that they afford protection against radiation damage in animal. Urinary amino-acids, particularly taurine, have been reported to increase severalfold, following irradiation.

After a week, 4 of the 6 animals were irradiated with a dose of 600 r from a 200 KV therapeutic X-ray machine (target distance 10 c.m.,1 mm. Al, $\frac{1}{4}$th mm. Cu filters). The excretory pattern was studied daily with 24-hr. sample of urine for a period of two weeks. Two animals were kept as controls. None of the animals died during the experimental period.

Excretion of urea, uric acid, creatinine, inorganic sulphates and neutral sulphur increased. Increased excretion of the following amino-acids was noted : glycine, glutamic acid, alanine, tyrosine, valine, phenylalanine and taurine.

The experiment indicates that following radiation, apart from immediate tissue destruction, prolonged catabolic changes occur inside the body. The urine samples of irradiated animals also contained some amount of ammonia which could not be accounted for by the normally known constituents of waste products.

(2) *Effect of Total Body $\gamma$-Ray Exposure of Lethal, Semilethal and severe Exposures:* Similar experiment, as above, was conducted on 30 rats, (all females and of an inbred strain), in order to find the extent of departures from normal excretory urinary pattern as a result of varying degrees of damages from lethal, semilethal and severe dosages of $\gamma$-radiation.

These rats were divided into 5 groups of 6 rats each and kept in metabolic cages, on standard diet and distilled water. Normal patterns were studied for one week: group variations were found to be low.

After one week three of these groups were exposed to 1200 r, 600 r and 300 r respectively, and two groups were kept as controls. Urea, uric acid, creatinine, total inorganic sulphates, neutral sulphur and urinary amino-acids were determined. Urinary ammonia was also estimated.

Urea, uric acid and creatinine showed increase in all the irradiated groups. The rise was roughly proportional to dosage. The curves also showed two peaks, one immediately after radiation which came down on the 2nd/3rd day. The next rise began on 4th/5th day and continued till death or up to the end of the 2nd week. Total inorganic sulphates and neutral sulphur showed the same characteristics. Amongst the amino-acids glutamic acid, glycine, alanine, valine and phenylalanine showed significant increase following 1200r; however, no increase in taurine could be seen.

The interesting features were the different patterns following lethal radiation in comparison to those following semilethal and severe radiations. In lethal exposure, urea and creatinine, after an immediate rise, showed a secondary rise which was maintained at a high level till death. Uric acid excretion did not show the secondary rise. Total inorganic sulphate and neutral sulphur showed high secondary rise, which persisted till death.



The most interesting feature was the excretion of ammonia, which was very high. It will be worthwhile to study compounds, such as glutamine which has a labile $NH_2$ group, as the precursor of this ammonia.

(3) *$P^{32}$ Incorporation in the Body:* Six rats were given intramuscularly 1 millicurie of $P^{32}$ each. The urinary nitrogenous end products were estimated and the radioactivity of the urine was measured. The excretion of $P^{32}$ was exponential, showing that the whole amount of $P^{32}$ had gone into the metabolic pool.

B.1.4. *Isolation and characterisation of seed proteins.*

(1) *Studies on homogeneity and physicochemical properties of crystalline $\alpha$-globulin from sesame seeds* (Sesamum indicum)*:* In the annual report of 1956-57 a preliminary report on investigations on proteins from sesame seeds was published. During the period under review, work has been carried out mainly on the crystalline $\alpha$-globulin fraction which was prepared according to the method of Jones & Gersdorff (J. Biol. Chem. **75**, 213, (1927)) from the three varieties of sesame seeds, namely, brown, white and black. Attempts have been made to purify the material further by fractionation and recrystallisation in order to obtain a preparation which would be homogeneous by applying the usual tests of protein homogeneity, namely, electrophoresis, ultracentrifugation and solubility. The material crystallises in the form of tetragonal bipyramids. In buffers of ionic strength 0.1, it dissolves in the *p*H range of 2.5-4.25, in which it is very susceptible to denaturation with change of salt or protein concentration as indicated by precipitation of the protein and its loss of solubility. At higher *p*H values, the effect of salt or protein concentration is not so marked and solubility increases with salt concentration. In all experiments 3-6 times crystallised material was used.

*Electrophoresis:* Electrophoretic experiments were limited over the *p*H range 2.5-4.1, since at higher *p*H values the material is insoluble in buffers of low ionic strength. These studies were carried out in a Tiselius apparatus using a 11 ml. cell at 0°C. Symmetrical, single peaks were obtained at *p*H values 3.23 and 4.1; at *p*H 2.5, below which the material denatures, two partially resolved peaks were observed due probably to breakdown of the molecule. The mobilities at *p*H 2.45, 3.23 and 4.10 were determined respectively as $6.66\times10^{-5}$ (for the major component), $6.49\times10^{-5}$ and $6.04\times10^{-5}$ ($cm^2\ V^{-1}\ sec^{-1}$).

*Sedimentation studies:* Preliminary observations have been made with the Spinco model E analytical ultracentrifuge recently installed in the Institute. At *p*H 4.1 and ionic strength 0.1 the $\alpha$-globulin shows a single sedimenting boundary which diffuses very rapidly. On the other hand, at *p*H 6.7 in 0.062 M phosphate buffer containing 0.885 M sodium chloride two sedimenting components, the faster one of which is present in very small concentration, are observed. The value of the sedimentation coefficient $S_{20,\,w}$ for the major component in this solvent (protein concentration = 1.1%) was determined to be 12.69S (S = Svedberg units, $1S = 10^{-13}$ seconds), $S_{20,\,w}$ being the corrected sedimentation coefficient referred to water as solvent at 20°C. The value of the partial specific volume for calculations was taken as equal to 0.749 $cm^3/g$. from the tables (Svedberg and Pederson, "The Ultracentrifuge," Oxford, 1940).

*Solubility tests:* Variable solvent solubility test (Falconer & Taylor, Biochem J. **40**, 835, 1946) for determination of homogeneity was carried out at *p*H 6.7 in .02 M phosphate buffer containing 0.285 M sodium chloride with varying concentrations of ammonium sulphate from 0.45-0.85 M at 20°C. Forty hours were allowed for attainment of equilibrium. The protein concentration in the solution was determined by measurement of absorption at 280 m$\mu$. Several determinations indicated the presence of a second component precipitating almost simultaneously with the first one. Solubility curve obtained by constant solvent solubility test (single determination) did not show sharp breaks as was obtained in the variable solvent test. The nature of the curve resembled more or less those obtained in the cases of solid solutions.

It appears that the material, though crystalline, is not homogeneous according to the tests described above; its heterogeneity may be due to dissociation or to the presence of a second component detectable at higher *p*H values by sedimentation and solubility tests. Further purification and measurements on sedimentation and diffusion are in progress.

(2) Work on the isolation, fractionation and electrophoretic studies of the proteins of mustard seeds (*Brassica campestris*) was reported last year (Ann. Report 1957-58, p. 22). Two fractions (A) and (B) were obtained respectively with 20-22% and 30-80% saturation with ammonium sulphate of the 10% NaCl extract from the defatted meal. The fractions were further purified by reprecipitation, dialysis and precipitation by dilution. Sedimentation analysis, in phosphate buffer of *p*H 8, of the total precipitate obtained by 80% saturation of the extract with ammonium sulphate showed the presence of two components corresponding to the fractions (A) and (B). The fraction (A), however, after purification shows two sedimentation boundaries, the faster one of which represents a component present in very low concentration. This was not observed in the sedimentation pattern of the total precipitate nor was it due to incomplete separation of fraction (B) during preparation, as the sedimentation behaviour of (B) showed it to be a low molecular weight substance. The minor component of fraction (A) has also been observed on prolonged electrophoresis It is unlikely to be an artifact and the fact that it is not observed in electrophoretic or sedimentation patterns of the total precipitate may be due to its presence in very low concentration. The fraction (B), as has been mentioned, is a low molecular weight substance and gives single boundaries both by electrophoresis and sedimentation. It diffuses fairly rapidly. Possibility of its polydisperse nature is under investigation. The corrected sedimentation coefficient $S_{20,\,w}$ of the major component of the fraction (A) was found to be 11.37S with a protein concentration of 1% in phosphate buffer of *p*H 8.01 and ionic strength 0.1. The value of $S_{20,\,w}$ for the fraction (B) was determined to be 1.64S in the same solvent.

B.1.5. *Activities of bacteria-free preparations from* Vibrio cholerae

This work has been carried out as a joint programme of work with Professor S. N. De, Professor of Pathology, Medical College, Calcutta.

The cholera vibrio is at present believed to produce only an endotoxin which is liberated after autolysis in older cultures or after their disintegration in tissues of men or animals.



The measure of such toxicity has usually been its lethal effect after parenteral injection into animals. (De and Chatterjee, (J. Path. Bact. **66**, 559, 1953)), has devised a technique to demonstrate pathological effect of entire organisms like *V. cholerae* or *Bact. Coli* on ligated loops of rabbit's small intestine which swells up due to collection of fluid inside. This technique has now been utilised to show exterotoxic activity of bacteria-free products obtained from young cultures of *V. cholerae*. Bacteria-free filtrate from saline washings of *Vibrio cholerae* grown on nutrient agar medium dissolves mucin and haemolyses human red blood cells in presence of a little calcium salts and occasionally swells the ligated loop of rabbit's small intestine. That from culture in 5 p.c. peptone-water medium consistently shows the presence of mucinase and enterotoxic activities but has no haemolytic property. It can also cause fall of blood pressure in cats, but the fall is no greater than that with the control medium. The enterotoxic activity of this filtrate is retained in the ammonium sulphate precipitate and is not lost on dialysis, preservation at 2°–4°C for a week or on freezing and thawing but is destroyed on heating at 56°C for half an hour. The enterotoxic effect could not be checked by the use of several drugs and does not appear to be related to mucinase or haemolytic activity.

Sterile filtrate from washed suspension of *Vibrio cholerae* exposed to ultrasonic waves cuases fall of blood pressure but has no mucinase, haemolytic or enterotoxic activity. It shows three electrophoretic components, whereas the enterotoxic filtrate from centrifuged supernatant fluid from 5 p.c. peptone-water culture shows only two.

## B.2. Plant Chemistry

B.2.1. *Chemical investigation of* Helichrysum *flowers*

The flowers of *Helichrysum annum* furnished a flavonoid glycoside which unfortunately could not be obtained in crystalline state. The corresponding aglycone formed bright yellow rectangular plates, m.p. 312-316° (d), gave an acetate m.p. 226-228° and a methyl ether m.p. 167-168° and was identified as luteolin (5:7:3′:4′-tetrahydroxyflavone).

B.2.2. *Chemical investigation of the genus* Clerodendrum

A systematic investigation of different species of *Clerodendrum* has been undertaken and the following results have so far been obtained.

(1) *C. inerme* Linn.

Though the leaves of this plant are extremely bitter, no bitter principle could be isolated in crystalline state. $\beta$-Sitosterol has been isolated from its leaves.

(2) *C. siphonanthus*

No crystalline compound could be obtained.

(3) *C. phlomidis*

No bitter principle could be isolated from the plant. $\beta$-Sitosterol was isolated in low yield.

B.2.3. *Constitution of clerodin*

Work on the constitution of clerodin has been continued. A direct evidence for the presence of hemiketal structure has been adduced. Tetrahydroclerodin formed a crystalline 2:4-dinitrophenyl hydrazone and could be isomerised to iso-tetrahydroclerodin which gave positive test for $-CO.CH_3$ group. The iso-compound gave the same DNP as in the case of tetrahydroclerodin. A tentative structure of clerodin has been advanced.

Studies on molecular models of clerodin reveal that the two acetoxy groups are diequitorial trans. This has been experimentally supported by the observation that—

(a) Desacetylclerodin or the corresponding tetrahydro-derivative does not form acetonide derivative.

(b) Rate of oxidation of desacetylclerodin or tetrahydrodesacetylclerodin by lead tetra-acetate is very slow.

B.2.4. *On the constitution of mesuol.*

Of the five oxygen atoms of mesuol ($C_{23}H_{22}O_5$, m.p. 154°) two were reported to be phenolic and two lactonic (this Report, 1957-58). It has now been proved that mesuol does not contain any ketonic oxygen as neither dimethyl mesuol nor the methyl ester of its tri-O-methyl acid formed 2:4-dinitrophenyl hydrazone. The absence of an epoxide linkage or a keto-group in conjugation with an *iso*-propylidene double bond was proved by the inertness of dimethyl mesuol towards 5% oxalic acid. The I.R. spectrum of dimethyl mesuol (hydroxyl band at 3400 $cm^{-1}$) and active hydrogen estimation of mesuol (three hydroxyl groups) leave little doubt that the fifth oxygen is hydroxylic in nature. Attempts to acetylate dimethyl mesuol (reported earlier) and its tetrahydro derivative were unsuccessful showing the hindered nature of the hydroxyl group. Dimethyl mesuol was reduced under conditions specified by Rosenmund and Karg (Ber. 1942, **75**, 1850) to find out whether the third hydroxyl formed the part of a $C_6H_5$-CHOH- group. The I R spectrum of the hydrogenated product showed absence of the hydroxyl band and the U V spectrum of the compound showed band with $\lambda$ max at 280 m$\mu$ (log $\epsilon$ 4.07). On the basis of these observations it was inferred that mesuol contains a CHOH— group attached to the phenyl nucleus.

On quantitative hydrogenation mesuol consumed 2.1 moles of hydrogen. The I R and the UV data of tetrahydromesuol showed that on hydrogeneation the $\alpha$-pyrone double bond remained intact. Therefore, besides the $\alpha$-pyrone double bond, mesuol contanis two other double bonds. This is also supported by the absence of the *iso*-propylidene (832 $cm^{-1}$) and the end methylene (895 $cm^{-1}$) bands of mesuol in the I R spectrum of tetrahydromesuol.

Dimethylmesuol on hydrogenation gave a colourless tetrahydro-derivative ($C_{25}H_{26}O_5$, m.p. 122–23°). Tetrahydromesuol on methylation gave the identical product. The compound was optically inactive and gave the characteristic reaction for coumarins with alcoholic alkali. It gave pale yellow colour with tetranitromethane due probably to the $\alpha$-pyrone double bond. The UV absorption spectrum of the compound like that of dimethyl mesuol showed bands with a $\lambda$ max at 300 m$\mu$ (log $\epsilon$ 4.07). Thus the five oxygen functions and the unsaturation in mesuol are accounted for.



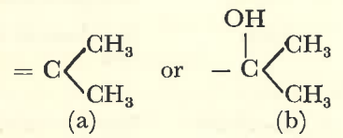
Previously it was reported that mesuol on hydrolysis with 40% aqueous caustic potash yielded acetone, acetophenone, and two other crystalline constituents of m.p. 268° ($C_{20}H_{18}O_4$) and 189° ($C_{16}H_{12}O_4$) respectively.

It was further observed that in addition to above four constituents, a steam-volatile acid was formed which was identified as *iso*valeric acid by paper chromatographic method.

The compound, $C_{16}H_{12}O_4$, m.p. 189°, was found homogeneous by paper chromatographic method. The I R spectrum of the compound showed it to be a phenolic coumarin with a C–$CH_3$ group in it. Dimethyl mesuol was found stable towards 40% aqueous caustic potash.

On fusion with caustic potash mesuol gave phloroglucinol (reported previously) and acetophenone. The formation of phloroglucinol and acetophenone may be taken as a satisfactory evidence for the presence of a 5 : 7-dihydroxy-4-phenyl coumarin skeleton in mesuol. This has also been supported by the results of alkaline hydrolysis of meusol. The behaviour of mesuol and its dimethyl derivative towards magnesium powder and conc. hydrochloric acid was found to be analogous to that of 5 : 7-dihydroxy-4-phenylcoumarin and its dimethyl derivative. (Ahluwalia, Mehta and Seshadri, Proc. Ind. Acad. Sc. 1957, **45,** 293).

It was shown that chromic acid oxidation of dimethyl mesuol yielded acetone which arose from either of the following groups:




(a) (b)

The oxidation of tetrahydromesuol with chromic acid yielded acetophenone but no acetone, thereby proving that mesuol contains the group (a), and not (b).

*iso*Caproic acid was isolated by oxidising tetrahydro dimethyl mesuol with alkaline hydrogen peroxide which is a proof for the presence of a $\gamma:\gamma$-dimethylallyl chain.

Thus the data so far obtained revealed the following.

(a) The basic skeleton of mesuol is 5 : 7-dihydroxy-4-phenylcoumarin.

(b) It contains a $\gamma:\gamma$-dimethylallyl side-chain.

(c) It has a hindered secondary hydroxyl attached to the benzene ring.

(d) Mesuol contains an end methylene group (=$CH_2$) in one of its side-chains.

The subtraction of 4-phenyl coumarin skeleton and the $\gamma:\gamma$-dimethylallyl chain leave behind a $C_3H_5O$-fragment which could be expanded to $CH_2$=CH-$CH_2$OH.

From the data presented and from the study of the probable molecular models of dimethyl mesuol, a tentative structure of mesuol has been suggested.

B.2.5. *Survey of Indian plants for coumarins*

Preliminary examination of the following plants was carried out for the detection of coumarins. The results are detailed below. The $R_f$ values reported in the table were

obtained by examining the plant extracts following the method of Chakraborty and Bose (J. Ind. Chem. Soc. 1956, **33,** 905) using water as solvent.

| Name of the plant | Part examined | $R_f$ values of fluorescent spots presumably due to coumarin | Other constituents detected if any |
|---|---|---|---|
| (a) *Clausena pentaphylla* (Roxb.)DC. | Leaves | 0.36 | — |
| (b) *Albizzia lebbeck* Benth. | Seeds | 0.66 | — |
| (c) *Glycosmis pentaphylla* Corr. | Leaves | 0.22 | — |
| | Root bark | 0.12, 0.31 and 0.80 | — |
| | Stem bark | 0.18, 0.60 | — |
| (d) *Murraya Koenigii* Spreng | Stem bark | One coumarin of m.p. 140° | A steroidal substance isolated |
| | Flowers | A phenolic coumarin with $R_f$ 0.56 | A steroidal substance isolated |
| (e) *Ferula* sp. | Roots | No coumarin | $\beta$-sitosterol identified as its acetate |
| (f) *Feronia elephantum* Corr. | Leaves | Bergapten isolated | — |
| (g) *Vicia faba* Linn. | Seeds | No coumarin detected | A steroidal substance m.p. 128-32° detected |
| (h) *Oryza sativa* Linn. | Seed husk | $R_f$ 0.50 and 0.70 | Steroidal substance |
| (i) *Triticum sativum* Linn. | Seed husk | $R_f$ 0.82, 0.61 | — |
| (j) *Phaseolus radiatus* Linn. | Seed husk | $R_f$ 0.50, 0.72 | — |
| (k) *Mesua ferrea* Linn. | Seed shell | No coumarin | A waxy substance m.p. 65° |

B.2.6. *Ultraviolet absorption spectra of the following coumarins were studied :*

Murrayin, angelicin, bergapten, psoralene, xanthotoxin, byakangelicin, marmesin, luvangetin, seselin, dalbergin, nordalbergin, wedelolactone, tri-O-methyl wedelolactone, mesuol, tetrahydromesuol, dimethyl mesuol, dimethyl tetrahydromesuol, 5 : 7-dihydroxy-4-phenylcoumarin.

(a) It was observed that furano-coumarins show characteristic absorption maxima at 205–10 m$\mu$ and 245 m$\mu$ regions. The absorption maxima are due to the presence of the benzofuran chromophore in the compounds.

(b) Chromeno-$\alpha$-pyrones show characteristic absorption band with a $\lambda$max. at 268 m$\mu$ region.

(c) 4-Phenylcoumarins show an absorption max. in the 205 m$\mu$ region which is due to the contribution of the phenyl group in position 4.



B.2.7. *Biochemical properties of coumarins*

(a) The effect of some coumarins on the growth of several micro-organisms was observed. The micro-organisms were *Micrococcus pyogenes* var. *aureus* (*Staphylococcus aureus*), *Bacillus friedlanderi*, *Escherichia coli*, *Eberthella typhosa*, *Vibrio cholerae*, *Mycobacterium phlei*, *Curvularia lunata*, *Aspergillus niger*, *Helminthosporium sativum*, *Alternaria solani* and *Fusarium vasinfectum*.

The coumarins tested were: Tetrahydro mesuol, dimethyl mesuol, dimethyl tetrahydro mesuol, dalbergin, nor-dalbergin, tri-O-methyl wedelolactone, 5 : 7-dihydroxy-4-phenyl coumarin, 5 : 6-dimethyl chroman-7-hydroxy-4-phenyl coumarin, seselin, umbelliferone, ayapin, herniarin, luvangetin, psoralene, *iso*psoralene, xanthotoxin, marmesin, byakangelicin.

It has been found that psoralene and seselin are fairly active against *C. lunata*. Xanthotoxin is active again *F. vasinfectum*. Seselin also inhibits growth of the bacteria, *S. aureus* and *M. phlei*.

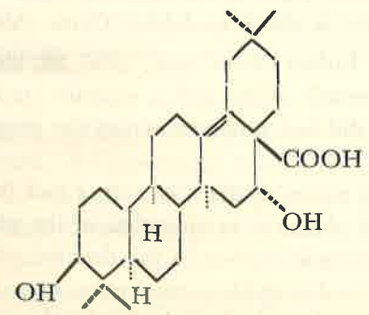
(b) The effect of psoralene on the tyrosinase activity was examined following the method of Lerner (Methods in Enzymologia, Vol. II, p. 872, 1955). Frog liver and skin slices were used as the experimental tissue. It has been found that psoralene accelerates the *in viro* liver tyrosinase activity. Further work is in progress.

B.2.8. *Survey of saponin-bearing plants of India*

Survey of the saponin-bearing plants has been extended and investigations on the structure of some saponins have been continued.

*Albizzia lebbeck* Benth.—In the previous report isolation of albigenic acid and a neutral sapogenin fraction was reported. Albigenic acid $C_{30}H_{48}O_4$ was shown to contain two secondary hydroxyl groups, one carboxyl group and a hindered double bond. It neither lactonised nor formed a bromolactone under conditions used for the formation of these derivatives of oleanolic acid. A clear evidence bearing on the position of the ethylenic double bond and the carboxyl group in albigenic acid came from the formation of a lactone diacetate, $C_{34}H_{52}O_6$, m.p. 281–282°; $[\alpha]_D$–24° with hydrobromic acid in glacial acetic acid. This compound was found to be identical with 18-*iso*echinocystic acid lactone diacetate.

Structure (I) was suggested for it and was confirmed by a partial synthesis of albigenic acid along the following lines.




I

Oxidation of diacetyl methyl echinocystate with selenium dioxide in glacial acetic acid furnished a crystalline compound, $C_{33}H_{50}O_5$, m.p. 236–237°; $[\alpha]_D$–134°, which showed triple UV absorption maxima at 244, 252 and 260.5 m$\mu$ (log $\epsilon$ 4.4, 4.44, 4.26) typical of the $\Delta^{11:12, 13:18}$ dienes of the $\beta$-amyrin series. Hydrogenation of this diene gave a compound $C_{33}H_{52}O_5$, m.p. 205–6°; $[\alpha]_D$–6.5° which on acetylation furnished albigenic ester diacetate.

Albigenic acid incidentally is the first example of a naturally-occurring triterpene acid with the double bond at the more thermodyanamically stable 13 : 18 position and is therefore of biogenetic interest.

The neutral sapogenin mixture was purified by chromatography of the acetate through Brockmann alumina when an acetate m.p. 211–212° was obtained in major yield and a higher melting fraction m.p. 242–248° was obtained in traces. The former appears to be a monoacetate and has the molecular formula $C_{31}H_{48}O_3$; $[\alpha]_D$–101° ($CHCl_3$). On hydrolysis with alcoholic caustic potash, it furnished an alcohol $C_{29}H_{46}O_2$; $[\alpha]_D$–114° ($CHCl_3$). It showed a UV absorption maxima characteristic of a carbonyl group which was confirmed by the preparation of 2 : 4-dinitrophenylhydrazine derivative, m.p. 266–268° (dec.). It responded to Liebermann-Burchard test. The compound appears to be a new triterpene and has therefore been named 'albigenin'.

Albigenin and its acetate consumed only one mole of perbenzoic acid within 24 hrs. and there was no further uptake on subsequent days. The resulting epoxide could be isolated in crystalline state, m.p. 241–242°, and did not show any colouration with tetranitromethane. On biogenetic grounds, albigenin is assumed to be a member of the $\beta$-amyrane group as it occurs in association with albigenic acid, echinocystic acid and oleanolic acid. The rapid consumption of perbenzoic acid and the strong laevo-rotation exhibited by albigenin suggest the possibility of the double bond being in the 13 : 18 position of the pentacyclic triterpene nucleus.

B.2.9. *Centella asiatica*

*Centella asiatica* is a common indigenous drug in India and is reported to be useful in diseases of skin, blood and leprosy. Asiaticoside (saponin) and hydrocotyline (alkaloid) have been reported to be present in the plant. Asiaticoside has been shown to be active in treatment of leprosy and certain types of tuberculosis. (Nature, 1945, 601; Amer. J. Pharm., 1949, 434). The plant is also insecticidal (Chem. Abstr. 1947, 2202).

Recently Bhattacharyya (J. Indian Chem. Soc., 1956, **33,** 893) reported the isolation of a new triterpene acid (indocentoic acid) and a saponin (indocentelloside) from the Indian variety of the plant. He did not, however, report the presence of asiaticoside and asiatic acid.

In view of the physiological action of the above drug and the recent observation by Bhattacharya (*loc. cit.*) a detailed chemical examination of the plant has been taken up in the department. From the alcoholic extract of the defatted plant, a good quantity of a triterpene acid was isolated as its dimorphic methyl ester, m.p. 170° and 220°. Besides this, a crude saponin was isolated and, on hydrolysis, furnished an acid sapogenin fraction.



The purified glycoside was found to have antibacterial action against organisms such as *Eberthella typhosa*, *Staphylococcus aureus*, *Vibrio cholerae* while no action was observed against *E. coli*. There was no antifungal action against *Penicillium notatum*, *Alterneria solani*, *Aspergillus niger*, *Fusarium* and *Curvularia* species.

B.2.10. *Alkaloidal survey*

The alkaloid potentialities of the local flora, especially the non-medicinal plants, are being surveyed. Of the twenty plants which were screened on a semi-quantative basis the following showed the presence of alkaloids. (a) *Crotalaria mucronata*, (b) *Acanthus ilicifolius* (bark), (c) *Gouania leptostachy*, (d) *Bassia longifolia*, (e) *Acacia concinna*.

B.2.11. *Aegiceradienol, a new triterpene alcohol from* Aegiceras majus *Gaertn*

The isolation of three triterpenoid sapogenins (of which the major constituent was genin A) and isorhamnetin, from the bark of *A. majus* (Syn. *A. corniculatum* Blanco) was reported earlier. Sapogenin I (*vide* previous report) has a m.p. 185–8°, $[\alpha]_D$+74° ($CHCl_3$) and analysed for $C_{30}H_{48}O$ ($\pm CH_2$). The presence of a hydroxyl group in it (I R bands in $CHCl_3$ solution at 3630, 1030 and 995 $cm^{-1}$) was confirmed by the formation of an acetate, m.p. 187–8°, $[\alpha]_D$+62° ($CHCl_3$) (I R bands in $CHCl_3$ solution at 1725, 1370 and 1250 $cm^{-1}$) and a benzoate, m.p. 231–3°, $[\alpha]_D$+81° ($CHCl_3$) (I R bands in KBr disc at 1710, 1275 and 710 $cm^{-1}$). The unsaturated nature of the sapogenin was shown by a brown colour with tetranitromethane and a rose-pink colour in the Liebermann-Burchard test. Since this triterpene alcohol has not been encountered in any other plant, we named it "aegiceradienol" and the elucidation of its structure was taken up.

Perbenzoic acid titration of aegiceradienyl acetate showed the presence of at least two double bonds and its ultraviolet absorption spectrum with maxima at 237 and 244 m$\mu$ (log $\epsilon$ 4.25 and 4.28 respectively) is indicative of a conjugated hetero-annular diene system in the compound.

Aegiceradienol, on oxidation under Sarett's conditions, formed the corresponding ketone, m.p. 127–9°, in which the carbonyl group is not in conjugation with a double bond as it exhibited an inflexion at 290 m$\mu$ besides the diene absorption as in the case of the parent alcohol. Further aegiceradienone gave a positive Zimmermann test characteristic of the 3-keto group in triterpenes. Hence the hydroxyl group in the sapogenin should occupy the ubiquitous 3-position and must be equitorial as suggested by its easy acetylation.

A formula of $C_{30}H_{48}O$ ($\pm CH_2$) with at least two double bonds suggests a tetracyclic structure for aegiceradienol. In agreement with this, it was found that aegiceredienyl acetate took up one molar equivalent of hydrogen over platinum and acetic acid forming the dihydro derivative m.p. 204-6°, $[\alpha]_D$+43° ($CHCl_3$), which on hydrolysis gave the alcohol, m.p. 221–4°. This shows the presence of a reactive double bond in the compound, which, however, is not exocyclic as aegiceradienyl acetate is resistant to attack by osmic acid.

The UV absorption data of this endocyclic system are similar to the characteristic 7:9-diene absorption of the lanosterol group which shows bands at 237, 244 and 251 m$\mu$ with the principal maximum, also appearing at 244 m$\mu$. But curiously enough, the hydrogenated product is transparent in the ultraviolet above 220 m$\mu$ though still giving a yellow colour with tetranitromethane. Obviously hydrogenation of one of the conjugated double bonds has taken place. This is contrary to the behaviour of the typical dienes of the tetracyclic group of triterpenes which are resistant to hydrogenation.

It may be presumed, on biogenetic grounds, that aegiceradienol which occurs along with genin-A might also belong to the $\beta$-amyrin group of pentacyclic triterpenes, a supposition which satisfactorily explains its reactions. The second double bond in conjugation with the 12 (13) double bond can occupy one of the positions 14 (15), 17 (18) or 18 (19). The physical constants of aegiceradienol and its derivatives agree closely with those of norolean —12:17—diene—3$\beta$-ol and this was confirmed by comparison of the mixed m.p. and I R curves of the benzoates.

Aegiceradienol, being the first $C_{29}$-triterpene encountered in nature, is of biogenetic interest.

B.2.12. *Minor constituents of* A. majus :

Petroleum ether extraction of the powdered bark of *A. majus* gave, besides a very large amount of waxy solid, traces of a sterol, m.p. 159-60° which formed an acetate m.p. 173-5°.

Attempts to locate any free flavonol in the bark, besides isorhamnetin present as a glycoside (*vide* previous report), resulted in the isolation of a small quantity of a phenolic acid, m.p. 207-8°. It gave no colour with ferric chloride or with magnesium and ethanolic hydrochloric acid. Its UV absorption spectrum exhibited a single maximum at 265 m$\mu$ which was shifted by ca. 5 m$\mu$ towards lower wave-length in the presence of anhydrous sodium acetate. This shows that the carboxyl group in the compound is either attached to or conjugated with an aromatic nucleus (L. Jurd, Arch. Biochem. Biophys., 1957, **66,** 284). In agreement with this its infrared absorption spectrum has a band at 1695 $cm^{-1}$ ($\alpha\beta$-unsaturated acid) besides bands at 3580 $cm^{-1}$ (hydroxyl) and 1615 $cm^{-1}$ and 1460 $cm^{-1}$ (aromatic bands). With diazomethane it formed a methyl ester, m.p. 82-3° which on hydrolysis gave a different acid, m.p. 170°. Its acetate melted at 189–90°.

The third triterpenoid sapogenin, $C_{30}H_{50}O_2$, m.p. 240–2°, $[\alpha]_D$-17° ($CHCl_3$) formed a diacetate, m.p. 196–8° and a dibenzoate, m.p. 233–5°. It was transparent in the ultraviolet above 220 m$\mu$ and the dibenzoate consumed 1 mole of perbenzoic acid in 24 hours showing the presence of one reactive double bond in it.

Further work on these componds is in progress.

## B.3. Radiochemistry

B.3.1. *Photosynthesis :*

(i) *Photosynthesis in* Hydrilla: The investigation on the utilization of malic acid in light by *Hydrilla verticillata*—an observation made by Sir J. C. Bose more than 35 years ago



—has been completed. Earlier investigations (this Report 1955-56 p. 23, 1957-'58, p. 32) had shown that the path of $CO_2$ fixation in *Hydrilla* is normal and there is an evolution of $CO_2$ when the plants are illuminated. Experiments on the utilization of malic acid-2, 3-$C^{14}$ in light have furnished interesting evidences. As expected, there was no radioactivity in sucrose since carbons 1 and 4 of malic acid were non-radioactive. But starch, sugar phosphates and phosphoenol pyruvate were tagged with $C^{14}$. The available evidences are in agreement with the following mechanism of utilization of malate as a substrate for photosynthesis: (*a*) Malic acid is decarboxylated, and a part of the $CO_2$ evolved is fixed back in to the plant along the conventional path. (*b*) Malic acid is incorporated into starch through phosphoenol pyruvate and reversal of glycolysis as has recently been demonstrated for some plant tissues. Other organic acids of the Krebs cycle are also decarboxylated.

(ii) *Thioctic acid and photosynthesis:* Thioctic acid, which has been found to play an important role in the Hill reaction of photosynthesis, has apparently no effect on the $CO_2$ fixation reaction (Nature, **181,** 1219). It might however be argued that thioctic acid plays an indirect role, namely, the coupling of the oxidation of the thiolform of thioctic acid to photosynthetic phosphorylation. This implies that (*a*) thioctic acid itself in light should promote photosynthetic phosphorylation and (*b*) in the green tissues there is a TPN-linked thioctic dehydrogenase, since TPNH is the reduced nucleotide involved in photosynthetic phosphorylation. To test the former possibility 5 day-old cells of *Scenedesmus obliquus* were incubated with 20$\mu$g./ml. of thioctic acid in light with or without DPN and TPN. After 1½ hr. $Na_2HP^{32}O_4$ was added. After further incubation for 1 hr. the cells were passed through a column of celite, washed with water and finally extracted in 80% ethanol followed by chromatography. No stimulation of photosynthetic phosphorylation was observed. To investigate the latter possibility a TPN-linked thioctic dehydrogenase was looked for in the green leaves of *Spinacea oleracea*. No indication of the TPN-linked enzyme was available, the DPN-linked enzyme, known to occur in animal tissues, was however present. It seems, doubtful, therefore, that thioctic acid plays any significant role in photosynthetic phosphorylation.

(iii) *Localisation of the products of photosynthesis:* An investigation has been undertaken to study the distribution and localisation of the products of photosynthesis in the leaves of Biloxi soyabean and *Antigonum leptopus* as a preliminary to an investigation of the Russian claim that the earliest product of photosynthesis is an iron-containing compound precipitable with 60% acetone.

Two week-old Biloxi soyabean seedlings and leaves of *A. leptopus* were allowed to take up $Na_2HP^{32}O_4$ from a nutrient solution of *p*H 6.0 with or without $C^{14}O_2$ in light. The leaves were homogenised in 0.5M sucrose solution. The chloroplasts were spun down by high speed centrifugation after removal of cell debris and the sucrose solution was treated with cold acetone to 60% saturation. The precipitate formed on standing for 24 hours at 0°C was collected following the usual procedure.

When $Na_2HP^{32}O_4$ was supplied through the roots of intact soyabean plants the specific activity of the acetone-precipitate was found to be 1½ times that of the chloroplast; 80% of

the chloroplast activity could be extracted with 80% ethanol. When allowed to photosynthesise in an atmosphere of $C^{14}O_2$ for 5 min. the two topmost leaves were found to possess a photosynthetic rate higher than the lower ones. When the compounds were separated on paper chromatograms, compounds in the nucleotide area were found to be formed at a rate much higher than the phosphate esters in these leaves.

When $Na_2HP^{32}O_4$ was fed through the petioles of *A. leptopus* for 90 min. followed by photosynthesis in $C^{14}O_2$ for 5 min. the distribution of radioactivity in chloroplasts was found to possess a relatively small fraction of total radioactivity. 20% of the water-soluble products could be precipitated with acetone. Kinetic studies on $C^{14}O_2$ fixation with leaf discs indicated a linear relationship between the incorporation of $C^{14}$ in the acetone precipitable substances and time. In other water-soluble components, however, the rate was relatively slow for the first 3 mins. followed by a sharp rise. Activity in the chloroplasts also increased with time. Analysis of the acetone precipitable substances indicated a preponderance of phosphate esters.

The acetone precipitate also contains some proteins. This has been revealed in experiments in which the leaves were fed $Na_2S^{35}O_4$ for several hours in light, and the acetone precipitate chromatographed two-dimensionally. At least three different proteins or peptides were observed. The one near the origin apparently contains Fe, since similar experiments with $Fe^{55}$ also revealed the presence of such a compound on the autoradiograms. The role of this iron containing compound in photosynthesis is now under study.

B.3.2. *Tracer studies on auxin-induced growth :*

The following aspects of auxin-induced growth have been studied: (i) $CO_2$ fixation; (ii) Acetate metabolism; (iii) Phosphorus metabolism; (iv) Sulphur metabolism; and (v) Nucleic acid synthesis.

(i) *$CO_2$ fixation:* In continuation of previous experiments it has been observed that DPN, TPN, coenzyme A, thioctic acid, sucrose, aspartic acid and ATP also stimulate $C^{14}O_2$ fixation, but the pattern of distribution of $C^{14}$ into the cellular components is not identical with that observed with IAA. The promotive effect of IAA agrees in large measure to that of DPNH. Attempts to demonstrate the formation of DPNH with tissue homogenates have so far been unsuccessful. It has also been observed that the IAA stimulation of $CO_2$ fixation cannot be demonstrated with homogenates; apparently some of the factors involved are highly labile.

In experiments involving 20 min., IAA stimulates $C^{14}$-incorporation into the pectates but $C^{14}$-activity in protopectin and polysaccharides decreases. With longer periods of incubation the pattern changes. In the alcohol-soluble fraction for relatively short periods of experimental duration the compounds, which accumulate considerable $C^{14}$-activity, are not similar to those observed with relatively long periods when malic acid is the most heavily tagged product. There is thus a shift in the pathway of $CO_2$ fixation as time passes. The most prominent compound detected in short-term experiments is one which occupies a position near about alanine on the paper chromatograms. It disappears with the addition of a little uridine triphosphate. It is not identical with uridine diphosphoglucose, glucuronic acid or galacturonic acid. Addition of xylose markedly inhibits $CO_2$ fixation in short-term experiments, even in presence of IAA, ATP or UTP.



(ii) *Acetate metabolism:* The accumulation of malate as an effect of low concentrations of IAA led to an investigation on the possibilities of its accumulation by a stimulation of the glyoxylate cycle. Avena coleoptiles were incubated with acetate-2-$C^{14}$ with different concentrations of IAA, glyoxylate and *iso*citrate. No stimulation of malate synthesis was observed. High concentrations of glyoxylate and *iso*citrate resulted in a decrease in the $C^{14}$-activity in malate. The products of acetate metabolism in presence of IAA is very much similar to those of $CO_2$ fixation.

(iii) *Phosphorus metabolism in potato tubers:* Phosphorus metabolism in potato tubers was studied at 5° and 30°C, with or without auxin and mannitol. At 5°C water uptake is very slow and mannitol is an inert metabolite which can control water uptake. The auxin used was naphthalene acetic acid (NAA). Water uptake is known to be promoted by auxin and there is a lag phase for the first 2 days. The reactions going on during this lag-phase are not well-understood and in order that the relationship between growth and metabolism *vis-a-vis* water uptake is correctly interpreted it is important to study both the lag phase and the phase of active water uptake following it.

Potato discs 5 mm. in diameter were incubated with $Na_2HP^{32}O_4$ with the following concentrations of NAA : $5\times10^{-3}$M, $5\times10^{-5}$M and $5\times10^{-7}$M, at 30°C and 5°C for $2\frac{1}{2}$ hrs. At 30°C, the curve for phosphate absorption was found to be similar to the two-phase concentration curve usually encountered in auxin-induced growth. When the discs were extracted in hot 80% ethanol it was found that low concentrations of the auxin stimulated the incorporation of $P^{32}$ into the alcohol-insoluble fraction but the activity in the alcohol soluble fraction decreased. Similar results were also obtained with indole acetic acid (IAA). In certain studies on $CO_2$ fixation conducted in this laboratory indications have been obtained that in the *Avena* coleoptile one of the effects of IAA is to generate the reduced form of diphosphopyridine nucleotide (DPNH). To study whether similar reactions also take place in the lag phase of water uptake in potato tuber, experiments were carried out replacing the auxin with DPNH or DPN. It was found that DPNH or DPN stimulates $P^{32}$ incorporation into the alcohol-soluble fraction. Apparently the reactions during the lag phase of water uptake are not identical with those obtaining in the *Avena* coleoptile during the period of active growth. Paper chromatographic analysis revealed that $P^{32}$ was present in at least 6 different compounds, but high concentrations of the order of $5\times10^{-3}$M markedly decreased the number of compounds.

At 5°C also the same picture was revealed. The most remarkable change was that with $5\times10^{-5}$M NAA, 98% of the radioactivity was located in a compound with $R_f$ values of 0.36 and 0.33 in phenol-water and *n*-butanol-acetic acid-water respectively. Apparently, the metabolisation of this compound is affected by high concentrations of NAA at low temperatures.

The occurrence of several $P^{32}$-labelled compounds on the chromatograms for the 5°C experiment indicates that metabolic changes go on in the potato tuber even at this

low temperature. Earlier observations that the decreased water uptake at low temperature is due to a cessation of metabolic reactions thus appear to be incorrect. It has also been found that the absorption of phosphate during the lag phase is similar to the two-phase concentration curve characteristic of growth. The metabolic changes are however not identical even though there are certain similarities in some of the reactions involved.

(iv) *Phosphorus metabolism in* Avena *coleoptiles:* To study phosphorus metabolism in *Avena* coleoptile 5 mm. subapical sections of 4-day old seedlings grown in darkness with occasional red light at 25°C were used. The sections were incubated with 0, $10^{-7}$M, $10^{-5}$M, $10^{-3}$M IAA and 100$\mu$C of $Na_2HP^{32}O_4$ at *p*H 6 for 2 hrs. After the experiment the tissues were washed thoroughly with water and extracted with hot 80% ethanol followed by chromatography and autoradiography.

In the alcohol-insoluble fraction no effect of IAA was observed for the first hour, during the second hour, however, $P^{32}$ was incorporated at a much higher rate. When 2,4-dinitrophenol (DNP), the uncoupling agent of phosphorylation, was incorporated in the experimental solution with or without IAA the incorporation of phosphorus was markedly inhibited in the absence of IAA. When low concentrations of IAA which promotes phosphorylation were added in addition to DNP, the inhibitory effect was countered, the net effect depending on the concentration of the IAA used.

Similar results were also obtained with the alcohol-soluble fractions. Paper chromatograms revealed the presence of phosphorus in 5 different compounds which included the nucleotides as also the sugar phosphates. If the sum total of the esterified phosphorus and the phosphorus present in the nucleotides are taken as a measure of the total amount of ATP synthesised, it is observed that IAA stimulates phosphorylation in a manner similar to that observed in growth. This tracer study thus furnishes conclusive evidence regarding the validity of the hypothesis that auxins lead to metabolic changes resulting in the synthesis of high-energy phosphate bonds needed for the growth process which include water uptake and the energy consuming metabolic reactions.

(v) *Nucleic acid synthesis:* The auxin stimulation of $P^{32}$-incorporation into the nucleic acids of coleoptile tissues has now been confirmed with *Avena* coleoptiles (*cf.* this Report 1957-58, p. 40). When however the coleoptiles were incubated with formate -$C^{14}$, bicarbonate -$C^{14}$, acetate-1-$C^{14}$, acetate-2-$C^{14}$ and glycine-1-$C^{14}$ which are known to contribute to the carbon skeletons of the purine and pyrimidine bases no enhancement of the $C^{14}$-incorporation into the nucleic acids was observed. This is due to the fact that auxins stimulate the phosphorylation step in the synthesis of nucleotides (see above) which are used as building blocks for nucleic acid synthesis but not the purine and pyrimidine bases or the sugar moieties.

(vi) The effects of auxins on sulphur metabolism in *Avena* coleoptiles and potato tuber were studied with $Na_2S^{35}O_4$. The coleoptile sections and potato discs were incubated with sulphate-$S^{35}$ in phosphate buffer of *p*H 6 with or without auxins followed by extraction in alcohol and chromatography. Incorporation into the amino-acids of proteins was studied by hydrolysing the alcohol-insoluble fraction in sealed tubes at 110°C



for 36 hrs. Sulphate was quickly reduced by these tissues and in two hours a variety of compounds were formed including a number of sulphur amino-acids. A concn. of $10^{-3}$ M IAA had a drastic inhibitory effect on the reduction and metabolisation of sulphate. Since sulphur-containing compounds are known to play important roles in growth, it is suggested that one of the ways, by which the inhibitory effects of relatively high concentrations of auxins are exercised, is through a severe retardation of sulphur metabolism in these tissues.

B.3.3. *Tracer studies on photoperiodism :*

In continuation of previous experiments on the metabolic changes induced by photoperiodic treatments, $CO_2$ fixation and acetate metabolism in the short-day plant *Rupsail* rice has been studied. In *Rupsail* rice, when sown in summer months, 100% flowering of the main shoot is observed with 4 weeks short-day treatment and the effect of the photoperiod on the shoot apex is quantitative.

Three week-old seedlings were subjected to 8 hr. photoperiods (in 24 hr. cycles) for 1, 2, 3 and 4 weeks. Groups of seedlings from each set were exposed to $C^{14}O_2$ in light for 15 min. The plants were then divided into four batches: one set was extracted immediately with hot 80% ethanol; a second set was exposed to darkness for 6 hrs.; in the third set the 6-hr. dark treatment was followed by $\frac{1}{2}$-hr. red light and in the fourth the dark treatment was terminated after 16 hrs. by extraction with hot 80% ethanol. Examination of the alcohol-soluble fraction indicated a marked decrease of $C^{14}$ activity during the 6-hr. dark treatment in non-induced or partially induced plants. With 3 or 4 weeks, induction treatment an increase was observed. This effect of dark treatment could be reserved with $\frac{1}{2}$-hr. of red light. Continuation of the dark period of 16 hrs. resulted in a sharp increase of $C^{14}$ activity in the alcohol-soluble fraction in induced plants.

Earlier observations with Wintex barley and *Xanthium* that photoperiodic treatment does not lead to the synthesis of new compounds in the buds as indicated by $C^{14}$-tracer studies, have now been extended to *Rupsail* rice. Intact rice plants were allowed to metabolise $C^{14}O_2$ in light on the 9th day of the photoperiodic treatment and the treatment continued till the end of the 3rd week when the floral primordia begin to be initiated. It was observed that the root system of induced plants had six times, the leaves twice and the shoot apices four times the $C^{14}$-activity of non-induced ones. Though there were important quantitative changes there was no qualitative difference.

Studies on the translocation pattern of $C^{14}$-labelled compounds have been extended to Biloxi soyabean. Detached soyabean leaves were allowed to photosynthesise in an atmosphere of $C^{14}O_2$ for 30 min. and subjected to dark treatment. The petioles of the leaves were immersed in a beaker containing distilled water and samples of the diffusate were withdrawn periodically. It was observed that the rate of translocation was initially very slow, followed by an increase, but decreased again in the second half of the long inductive dark period. Subsequent exposure to high intensity light resulted in a sharp increase. At the end of this treatment, there were few compounds in the diffusate, but a large number was present inside the leaves.

Since simpler compounds translocated to the buds might lead to the synthesis of more complex molecules, *e.g.*, proteins and nucleic acids, greater attention is now being paid to metabolic reactions in the buds themselves. Acetate-1-$C^{14}$ metabolism studies with isolated *Rupsail* rice apices have revealed marked qualitative changes in certain unidentified compounds as an effect of induction treatment. The $C^{14}$-labelled proteins of the apices of intact plants fed $C^{14}O_2$, on hydrolysis. yield similar $C_{14}$ amino-acids but in different amounts. Since the sequence of occurrence of such amino-acids in the proteins of the shoot apex still remains to be elucidated, conclusions must be deferred for the present.

## B.4. Enzyme Chemistry

B.4.1. *Preparation of analytical enzymes :*

Analysis of biochemical events is dependent on the availability of analytical tools. Since isolation procedures and chemical determinations often lack accuracy and specificity, enzymologists turn to enzymes as an aid in assays of intermediary metabolites. The projects undertaken involve work at the enzymatic level; so the routine preparation of analytical enzymes was considered essential. The following enzymes have been prepared either by the usual or by modified methods: (1) enzymes prepared from rabbit muscle—aldolase, hexokinase, α-glycerophosphate dehydrogenase, lactic dehydrogenase, triose phosphate dehydrogenase, phosphoenolpyruvate kinase, phosphomannose isomerase, and myokinase; (2) enzymes prepared from dried baker's yeast (kindly supplied by the Central Food Technological Research Institute, Mysore)—zwischenferment, inorganic pyrophosphatase and alcohol dehydrogenase; (3) enzymes prepared from plant sources—phosphatase from potato and phosphoriboisomerase, phosphoribulokinase, C-1 diphosphatase and dihydrolipoic dehydrogenase from *Spinacia oleracea*. The last two enzymes are new.

B.4.2. *Oxidative and reductive pentose phosphate pathways in plants :*

Two steps in the photosynthetic cycle (reductive pentose phosphate pathway) involve removal of the C-1 phosphate from a diphosphate. An enzyme which removes the C-1 phosphate of both ribulose and fructose diphosphates (RuDP and FDP) has been purified about 17 fold from *Spinacia oleracea*. The rates with the two substrates are found to be the same and competitive inhibition experiments indicate that one enzyme is involved in the two reactions. The properties of the enzyme have been investigated in detail. It is a $Mg^{++}$-independent phosphatase, and displays maximum activity at *p*H 5.5. Reaction products have been isolated and characterised. In case of FDP, fractose-6-phosphate (F6P) is the product while in case of RuDP a mixture of ribose-5-phosphate (R5P) and ribulose-5-phosphate (Ru5P) is obtained due to the presence of phosphoriboisomerase. The function of the enzyme in photosynthesis is evident. In one case it controls the pool of RuDP, the key intermediate in the photosynthetic cycle, in the other it makes the cycle operative by converting FDP to F6P.

The occurrence of the direct oxidative pathway in the germinating mung bean seedling has been demonstrated by showing the presence of all the enzymes of the pentose phosphate pathway in the extracts of the seedlings. The presence of both glucose-6-phosphate (G6P) and 6-phosphogluconate (6PG) dehydrogenases has been shown by the



reduction of triphosphopyridine nucleotide (TPN). The first enzyme has been twelve-fold purified and its properties are found to be similar to those of the yeast enzyme. During the initial period of germination the amounts and specific activities of the two dehydrogenases increase with time up to 72 hr. and then decrease. The presence of phosphoriboisomerase, the next enzyme in the cycle, was shown by the cysteine-carbazole colour reaction of the keto-pentose formed. The presence of phosphoketopentoepimerase (PKPE) was demonstrated by measuring the disappearance of xylulose-5-phosphate (Xu5P), which was prepared from R5P using the enzymes, phosphoriboisomerase and phosphoribulokinase. The transketolase activity was confirmed by measuring the formation of glyceraldehyde-3-phosphate (Ga3P) from R5P and Xu5P. Ga3P was estimated by phosphotriose isomerase and α-glycerophosphate dehydrogenase from muscle. The addition of sedoheptulose-7-phosphate (S7P) and Ga3P (generated in the system) to the seedling extract resulted in the formation of F6P (measured by phosphohexoseisomerase and zwischenferment) indicating the presence of aldolase. The last enzyme, phosphohexose isomerase, which completes the cycle, is also present in the extract, as shown by the formation of F6P from G6P, measured by both chemical and enzymatic methods.

B.4.3. *A stereospecific dihydrolipoic dehydrogenase and the mechanism of α-keto-acid oxidation :*

Recently considerable light has been thrown on the role of lipoic acid in the oxidation of α-keto-acids. It takes places in a number of integrated steps of which the last two are mediated by dihydrolipoic transacetylase and a DPN-linked dihydrolipoic dehydrogenase. In contrast to dihydrolipoic transacetylase, dihydrolipoic dehydrogenase does not exhibit optical specificity. A new stereospecific dihydrolipoic dehydrogenase has been isolated in this laboratory from the acetone powder of *Spinacia oleracea*. The enzyme which is DPN-linked and completely inactive with TPN has been 30–40 fold purified. It displays maximum activity at *p*H 8.0. The rate of reaction with dihydrolipoamide is faster than that with dihydrolipoic acid. The enzyme is inactive with (+)dihydrolipoic acid and fully active with (−)dihydrolipoic acid. It has not been possible to demonstrate the reversibility of the reaction using dihydrolipoic acid as the substrate even by coupling with alcohol dehydrogenase. The reaction is, however, fully reversible with lipoamide. Since even by coupling with lactic dehydrogenase or alcohol dehydrogenase not more than 50% of the (±) dihydrolipoic acid was oxidised, the stereospecificity of the enzyme was suspected. (−)Dihydrolipoic acid was oxidised to an extent of about 90%. Interesting results have been obtained using arsenite as an inhibitor. Arsenite at comparatively higher concentrations inhibits the assay system to certain extent, but pre-incubation of the enzyme with much lower concentrations of arsenite completely inhibits the enzyme. The incubation with the enzyme without arsenite does not result in any loss of activity.

With the isolation of this stereospecific dihydrolipoic dehydrogenase a new way has been opened for the investigations into the mechanisms of α-keto-acid oxidation where a 'bound' form of lipoic acid is involved. New assay systems are being developed to measure the oxidation of pyruvic and α-ketoglutaric acids by plant mitochondria and the soluble extracts. Attempt will be made to purify the enzyme complexes involved in these oxidations and then to study the mechanism of action with the individual enzymes.

B.4.4. *Microbial synthesis of protein in relation to the biogenesis of nucleic acid :*

The steps postulated in the synthesis of protein are: (1) the incorporation of the free amino-acids into the pools within the cell; (2) the activation of these amino-acids with adenosine triphosphate (ATP); (3) the incorporation of the activated amino-acids into the ribonucleic acid; and (4) the formation of the protein on the nucleic acid template and its subsequent release as the fully formed protein. Evidences have already accumulated in favour of the first three steps but the last step is very little understood as yet.

Burma and Burris (J. Biol. Chem., 1957 **225,** 723), demonstrated the incorporation of $C^{14}$-amino-acids into protein in the cell-free preparations of *Azotobacter vinelandii*. It was expected that the different steps involved in the protein synthesis would be operative in this case as well. A project has been undertaken to study in detail each and individual step and to throw more light on the last two steps where the ribonucleic acid is supposed to play the direct role.

(1) *Incorporation of the free amino-acids into the pools.*—*A. vinelandii* is a Gram-negative bacteria and as such its pool of amino-acids is rather small. An extract of *A. vinelandii* prepared by shaking in the Nossal disintegrator showed the presence of glutamic acid, alanine, valine, methionine, leucine and phenylalanine of which the amount of glutamic acid was maximum. *A. vinelandii* grows on molecular and combined nitrogen and is known not to use amino-acids under ordinary conditions. Preliminary experiments, however, have shown that radioactive glycine is transported within the cell.

(2) *Activation of amino-acids.*—The amino acid-dependent exchange of $P^{32}$–$P^{32}$ with the terminal phosphates of ATP was used as a criterion to show the formation of amino-acid adenylates. Since the bacterial extracts had considerable amount of free amino-acids some endogenous exchange always took place. Seventeen amino-acids were used for these exchange experiments. Activating systems for aspartic acid, leucine, valine, lysine and cysteine are found to be present in considerable amounts. The other amino-acids were more or less activated. In case of aspartate, the formation of adenyl aspartate could be demonstrated by the less sensitive hydroxamate method. Attempt is being made to purify this amino-acid activating enzyme using the hydroxamate assay.

(3) *Incorporation of amino-acids into ribonucleic acid.*—Since RNA and DNA from other sources failed to stimulate the amino-acid incorporation into proteins, it has been planned to isolate the RNA and DNA in the native form from *A. vinelandii* and use them in the incorporation studies. Exploratory experiments are nearing completion.

(4) *Direct role of nucleic acid in protein synthesis.*—Before using cell-free preparations preliminary experiments have been carried out with whole cells using $P^{32}$ as tracer. Growing and resting cells of *A. vinelandii* readily incorporate $P^{32}$ into the nucleotide pool and the nucleic acids. In case of growing cells the nucleotide pool shows no tendency of saturation while in case of the resting cells it becomes rapidly saturated. 90% of $P^{32}$ incorporated into the TCA-insoluble fraction has been shown to be present in the nucleic acids. It is interesting to record that chloromycetin at growth inhibitory concentrations does not at all inhibit this incorporation. Study of the incorporation of $S^{35}$ into proteins under similar conditions will throw considerable light on the assumed parallelism between nucleic acid and protein synthesis.

## C. BOTANY

### C.1. Plant Biochemistry and Physiology

C.1.1. *Studies on Crown Gall Problem*

C.1.1.1. *Causal organism : Avirulent strain.* In an attempt to search for avirulent bacterial strains, 432 single cell pure cultures were isolated from mature galls produced by *Agrobacterium tumefaciens*, strain nos. B23, and BI 16. The virulence of these isolates, as indicated by initiation and development of gall tumour on Balsam, Bryophyllum, Marigold and Hollyhock plants as hosts, was tested by using both multiple and single needle puncture methods. Each isolate was inoculated on each of the four hosts taking three plants at a time. Only in cases of unfavourable reactions, the inoculation was repeated thrice for confirmation. The infected hosts were examined at regular intervals of 7 days. From the observations it was found that the isolates, though derived from the same strains, differed widely in their virulence, 3.5% of them had mild to strong reaction depending on the host whereas 16% of them, though reacting strongly in some hosts, failed to produce any gall tumour in others. No isolate, however, was found to be avirulent as far as all the four hosts tried were concerned.

C.1.1.2. *Host range for Agrobacterium tumefaciens.* In continuation of the survey for new hosts for strain nos. B23, and BI 16, 35 new plant species belonging to different families were inoculated. Of these, 4 plants were found to be highly susceptible, while 3 plants displayed mild reactions.

The cytology of oncogenesis in plants offers a vast scope of study and by the middle of the period under review detailed study of this aspect was contemplated. As most of the plants, observed earlier as susceptible to the strains in use, possessed a large number of chromosomes, they were not considered very suitable for the purpose. So the reaction of about 22 different plant species possessing a comparatively smaller number of chromosomes of large size was studied. Of these *Vicia faba* ($2n = 12$), *Pisum sativum* ($2n = 14$), *Centaurea cyamus* ($2n = 24$) and *Coreopsis sp.* produced good reactions. It may be noted that none of the monocotyledonous plants tested in this experiment showed any sign of tumour initiation. At the time of writing this report, bacteria-free tissues have been obtained from these hosts by antibiotic treatment and transferred to antibiotic-free medium in agar slants. The rate of growth is still slow but it is expected that a few passages through the "Tobacco" medium will enable them to recover sufficiently for *in vitro* culture in liquid medium.

C.1.1.3. *Secondary gall tumour.* Various workers have reported the occurrence of secondary tumours, which generally grow spontaneously above the point of inoculation on stem, petiole, and leaves. Their occurrence is irregular and generally unpredictable and the cause or the mechanism of their induction is not known definitely. In the present series of experiments secondary tumours were not observed in earlier experiments perhaps

because comparatively older plants were inoculated. During the current period, an attempt was made to induce secondary galls in *Impatiens Balsamina*. For this purpose about 300 seedlings were linesown and grown to the second internode stage, when inoculations were made by single puncture method at a point between the first and the second leaves. After a month from the date of inoculation gall tumours were growing normally at the points of inoculation but in about 60 cases secondary tumours were observed on the second or third internode above the point of inoculation. In a few cases secondary tumours were also observed on the petioles. On examination, 95% of them were found to be bacteria-free.

Young Bryophyllum plants were also treated in the same way but they failed to produce any secondary gall. However, it was interesting to observe that in some of the Bryophyllum plants, mature galls, about a year old, gradually withered off in summer due to excessive heat, but in a few cases secondary tumours started growing spontaneously with the onset of rains at a point above the position of the necrosed primary tumour.

C.1.1.4. *Medium for continued in vitro culture of bacteria-free crown gall tissue.* In last year's report, the superiority of "Tobacco" medium over "Sun-flower" medium for the *in vitro* growth of bacteria-free Hollyhock crown gall tissue was described. During the year under review the *in vitro* growth of this tissue in "Tobacco" medium under various conditions was studied. The tissues were grown in all cases at 25°C. Considering the volume of the medium it was noted that 10 ml. of medium per flask produced the best growth compared to 5, 15, 20 or 30 ml. in cases when the flasks were shaken gently at 20 strokes per minute, whereas 5 ml. of medium per flask resulted in the best growth when the flasks were stationary. In all cases, however, the gentle shaking of the flasks resulted in a better growth for the same volume of medium; the better aeration on shaking was perhaps responsible for this better growth. Changing the rate of shaking it was observed that, for a volume of 10 ml. of medium per flask, a shaking rate of 30 strokes per minute yielded better results than with 5, 10, 20 or 40 strokes per minute. Higher rates of shaking generally produced fragmentation of the inoculum tissue and perhaps too much of surface injury whereas the lowest rate, namely, 5 strokes per minute probably limited the quantity of available oxygen. Hence, for further studies, 10 ml. of medium per flask shaken at 30 strokes per minute was adopted as the standard condition.

An attempt was made to improve the medium for the best growth of Hollyhock tissue. For this purpose several combinations of salts with varied concentrations selected on the basis of "triangular selection method" were tried. For each experiment the concentrations of three salts were varied at four different levels providing ten different combinations on the triangle, while the concentrations of the other salts remained the same as in normal "Tobacco" medium. Each combination had 25 replicates. In each experiment a parallel control with normal "Tobacco" medium having the same number of replicates was also run. The tissues were allowed to grow for 14 days and the ratio of the final weight to the initial weight provided the "Growth value" for comparison of growth. In later experiments the "triangular selection method" was abandoned in favour of a "quadrangular



selection method" designed in this laboratory, which permitted a simultaneous variation of a maximum of four components providing a maximum of 25 different combinations for each set.

The Hollyhock tissue displayed a higher requirement for potassium chloride and boric acid, and lower requirement for sodium sulphate, magnesium sulphate, manganese sulphate, thiamine and glycine. It was practically indifferent to the concentrations of potassium nitrate and zinc sulphate within the physiological limits. Total absence of calcium nitrate, magnesium sulphate and sodium biphosphate resulted in complete failure of growth. Thiamine at a concentration above 2 mg. per litre and pyridoxine even in lower concentrations were found to be toxic. A tentative "Hollyhock medium" based on the results of these experiments is now being tried out in comparison with "White's salt", "Tobacco" and "Sunflower" media.

In all the above experiments sucrose was used as the source of carbohydrate. In a recent experiment other sugars, and the polysaccharides isolated from the bacteria, and normal and crown gall tissues of Hollyhock are being tried as carbohydrate source for the hollyhock tissue in culture.

The effect of *p*H of the nutrient medium on the growth of Hollyhock crown gall tissue was also studied over a range of initial *p*H of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0. Initial *p*H of 4.5 resulted in the best growth. It is however of interest to note that within the range of initial *p*H of 4.0 to 6.0, the reaction of the media changes to about 6.7 by the third day of tissue growth and remains more or less constant thereafter, whereas with initial *p*H of 6.5 and 7.0 the shift is to 7.0 and 7.3, on the third day and a constant value thereafter. It is intriguing to explain the optimum growth at the initial *p*H of 4.5 only on the basis of hydrogen ion concentration when in all cases ranging between 4.0 to 6.0 the *p*H of the medium attains practically the same value on the 3rd day and remains constant thereafter. It may be presumed that the early growth of the tissue produces some substance or substances which raises the *p*H and brings about a buffering of the medium and this substance may also stimulate growth. That being so, a lower *p*H, unless too unphysiological, is likely to produce this substance or substances in larger quantity and would thereby produce a better growth. The identification of such a substance would necessitate a thorough and precise analysis of the culture medium at different stages of growth of the tissue and may be undertaken soon.

C.1.1.5. *Growth characteristics of Hollyhock Crown Gall Tissue.* The *in vitro* growth characteristics of Hollyhock gall tissues cultured in synthetic nutrient medium have been established. The rate of growth over a period of 42 days has been determined by weighing the tissues at every three days' interval. Effects of change of medium at different intervals, of tissue disturbance without change of medium, and of prolonged culture in the same medium on tissue growth have also been studied both in liquid medium and solid media of different concentrations of Agar. From the data of these experiments it is now possible to predict the growth of a tissue sample in a given time provided the physiological condition of the tissue at the beginning of the experiment is known. For any definite period

Hollyhock tissue growth has been found to be proportional to its mass with a constant 'growth value' when the culture is started from a freshly cut inoculum. Attempt has also been made to correlate the total increase in weight with 'growth value' (Ratio) and the volume of the tissue in liquid and semi-solid media. Further work to confirm the findings is in progress. For all the experiments reported here more than 8,000 individual tissue pieces were weighed under aseptic conditions.

C.1.2. *Gibberellic acid and plant growth*

C.1.2.1. *Effect of Gibberellic acid on Plants under subtropical conditions.* In continuation of last year's observations on the rapid growth in length induced by Gibberellic acid (GA) in *Tagetes patula* and *Impatiens balsamina* under tropical conditions, the same species were treated with GA in the Mayapuri research station of this Institute in Darjeeling at an altitude of about 7,000 feet above sea-level, thus subjecting them to a colder environment. During the period of experiments, extending from April to July, 1958, the temp. of the glass-house remained round about 22°C. (29.5–18.5°C) by day and about 17°C (19–15°C). at night. Young plants were treated with 2–3 drops of a $10^{-4}$ molar solution of GA at intervals of 3 to 4 days, so that each plant received a total amount of nearly 25 $\mu$g. GA. Under the action of GA, *Tagetes patula* exhibited a 100% increase in length and earlier setting of flower-buds. There was no significant increase in fresh wt. Like *Tagetes*, two other species, Salvia and Cosmos, also responded by rapid elongation. Under identical conditions, *Impatiens* however showed no promotion of growth and no earliness in flowering with applied GA. Height, flowering date, leaf-number, fresh wt. and branching were not significantly different from those of the controls. Only the floral stalks were about 50% longer than those in the controls. The difference in the behaviour of *Tagetes* and *Impatiens* to GA demonstrates once more clearly that plants vary widely in their response to GA. Whether the failure of *Impatiens* to react to GA of fungal origin (as used in these experiments) can be traced back to the level of heteroauxin or to the presence or absence of other Gibberellin-like substances, particularly in the growing region, remains to be investigated.

C.1.2.2. *Gibberellic acid and breaking of seed dormancy.* Gibberellic acid has been found to be able to act as substitute for red light in breaking dormancy of *Mazus rugosus* seeds. Seeds of this common weed of Bengal plains germinated on moist filter paper in petri dishes within 2–3 days in the presence of artificial light from fluorescent lamps, whereas in complete darkness they remained dormant for three weeks. The seeds remained viable without germination even for longer periods unless they were killed by fungi or bacteria.

GA at concentrations of $10^{-4}$ M and $10^{-5}$ M could bring about nearly a 100% germination in darkness while concentrations lower than $10^{-5}$ M were hardly effective. The action of GA seems to be rather specific; citric acid, malic acid and $\beta$-indole acetic acid could not substitute for light in breaking dormancy.

This acceleration of germination as induced by GA is all the more interesting since GA is supposed to contain a lactone ring. Other lactones are all known to be germination inhibitors. Hexenolactone was shown to inhibit germination of *M. rugosus* seeds, both in dark and in light, and its inhibitory action is much stronger than the stimulating action of GA.



While studying the effect of GA, it was also observed that sometimes 1 or 2% of the control seeds could germinate even in the dark. Cause of this is unknown. A probable genetic mechanism can be conjectured, but it can be established only by further experiments.

## C.2. Plant Physiology

In the previous report some preliminary observations on the electrical changes following stimulation in Mimosa and Desmodium have been mentioned under separate headings. Extension of the investigation during the period under review is given below:

C.2.1. *Electrical pulsation of Desmodium*

Simultaneous study of the mechanical and electrical pulsations of Desmodium showed that corresponding to each mechanical pulsation there is a concomitant electrical version. It was again found that when the movement of the leaf is mechanically stopped the normal electrical component persists. But under the action of an anaesthetic like chloroform both the mechanical and electrical pulsations disappear almost simultaneously. Next it was proposed to find out whether the mechanical and the electrical pulsations are initiated simultaneously or one precedes the other, at the time when the leaf regains its activity after a temporary cessation.

Attempt was made to bring about a temporary cessation of pulsation by the application of a small dose of chloroform. But this was not successful, as a small dose could not totally abolish the pulsatory activity, whereas, a stronger dose produced a fatal effect on the leaf. As no intermediary dose could be ascertained which could stop pulsation without killing the leaf, a different course was followed to study the phenomenon.

It was found that when water is withdrawn the pulsation of the leaf gradually stops after a time. By irrigation at such condition the pulsatory activity is revived after some time. Continued recording of the mechanical and electrical behaviours of the leaf shows that in the revival of the pulsatory activity, the electrical phenomenon of pulsation reappears a considerable time ahead of the initiation of the mechanical movement. So far, only a few observations have been made. To understand the definite correlation between the electrical and the mechanical activities of the leaf more experiments have been proposed to be performed in the next monsoon when the plants are in vigorous condition.

C.2.1.1. *The Electrical pattern of Desmodium pulsation*

It has been mentioned previously that the electrical pattern of the Desmodium pulsation more or less corresponds to that of the animal heart beat in the expression of the P.Q.R.S.T. phenomenon. It is known how the P.Q.R.S.T. phases of the electrical impulse of the animal heart beat are related with the initiation of the electrical potential and its

spreading from node to node through different tissues of the heart. Similarly, if it were possible to record simultaneously the change of potential at different points of the pulvinule, necessary clues for correct interpretation of the characteristic impulse in Desmodium would be available.

It is a difficult job to make more than one electrode connection to the tiny pulvinule. Attempts were made to make connections by inserting very fine platinum pins at the upper and lower sides of the pulvinule for studying independently the variations of potential of the two sides, but it was not successful. Pulsatory activity of the leaf does not revive after the pin prick. Fresh attempt will be made by modification of surface connection arrangement so that the electrical changes of the different portions of the pulvinule can be studied simultaneously.

C.2.2. *Electrical changes in Mimosa*

Studies on some of the aspects of electrical transmission of excitation in Mimosa were described in the previous report. It was mentioned there that no definite result could be obtained in an attempt to pursue the reflex arc process by following the electrical transmission of excitation. In those experiments two surface connections were made on the petiole, one near the pulvinus and the other at a distance from it towards the periphery, through R.C. coupling. It was expected that after the fall of the pulvinus outgoing waves might be detected preceding the closure of other three subpetioles. But as no such wave could be detected, some changes in the experimental method were subsequently made. Petiole connections were made with platinum pins and they were connected with the amplifiers through D.C. coupling. By this method some detectable electrical waves of smaller amplitude have been recorded after the fall of the pulvinus. The impulses are more pronounced at the basal than at the apical connection. The apical impulse is not always conspicuous but in some cases, where the two impulses can be distinguished to be of the same origin, the velocity of transmission seems to be very high. The time difference of the initiation of impulses at the two different points lying apart 1.5 to 2 cm. is in some cases quite indistinguishable. In some cases, however, the slight difference that could be detected, indicates that the impulses are propagated from the pulvinus towards the periphery. It is expected that in the healthy condition of the plant during the monsoon more definite results can be obtained.

C.2.3. *Cellular electric pulsation*

J. C. Bose showed that the active cells in the interior of the plant have a rhythmic behaviour which, when connected with a galvanometer, is identified in the alternate deflections of positivity and negativity. According to him this electrical change of the active cell is correlated with the periodic increase and decrease of turgor due to alternate expansion and contraction. Further extension of study in this line was proposed to be undertaken with the pen recorder. Attempts were made with herbaceous plants and the records indicated the presence of electrical pulsations. But they cannot be prominently displayed under the amplification available with this apparatus. In the present recorder



1 mm. movement of the pen indicates 5 mv. whereas, the generated E.M.F. hardly exceeds 2 mv. In order to undertake detailed study in this line the amplification of the apparatus requires to be much increased.

C.2.4. *Responsive movement of Mimosa under condition of drought*

This experiment was conducted with a petiole-pulvinus preparation of Mimosa, with a piece of stem attached to it. It was arranged that the piece first absorbed water; after full recovery, the supply of water was withdrawn and its responses to a measured stimulus and recovery thereafter, at intervals of twenty minutes, were continuously recorded. With gradual dehydration due to evaporation the responses on and on became smaller but the pulvinus retained its power to respond and partial recovery for about three hours.

Similar specimens kept on a balance indicated the amount of loss of water that was gradually taking place under the condition. The plant material seemed to lose about 40% of its water within a three-hour period. The total water content of similar specimens was determined by dry weight method.

The experiment reveals how the pulvinar tissue can recover partially by absorbing water from the adjoining tissues which are undergoing a process of gradual deficit of water under protracted drought condition.

It was proposed to study how the Electrical response of the pulvinus is modified under such drought condition. But in performing the experiment application of measured electrical stimulus entails great difficulty. A suitable means of application of electrical stimulus without risk on the apparatus has not yet been found out.

C.2.5. *Effect of load on the pulsatory movement of Desmodium leaflet*

The investigation was undertaken to observe how the leaf could work against a load, when it is in the form of either a pull from above or a pull from below, and how much pull it can withstand in keeping its normal activity unimpaired. In our normal recording method the lever is not completely balanced; an over-weight of about 5 m.g. is maintained on the other side of the fulcrum where the leaf is attached, to make it slightly unbalanced. The leaf in its downward movement has to work against the upward pull. Similarly, the leaf has to work against its own weight or a pull from below in its upward moevement. To observe the effect of increasing upward pull, weight was suspended on the other side of the lever, at a place equidistant from the attachment point of the leaf. The weight was gradually increased. To observe the effect of downward pull similar weights were attached to a silk cocoon previusly suspended from underside of the leaf.

It has been found that by increased upward pull, from 10 m.g. onward, the amplitude of pulsation is progressively decreased but the frequency remains the same. The pulsatory activity is not totally abolished even at a pull of 100 mgs. After the withdrawal of weight the normal amplitude of pulsation is regained almost immediately.

When such weights were added to the underside of the leaf, exerting downward pull on it, the leaf exhibited a different behaviour in its pulsatory activity. By the introduc-

tion of weight upto 10 mg., the amplitude of pulsation is increased by 60 to 80%; with 20 mg. weight the amplitude comes down to that of normal pulsation. With further increase of weight the amplitude is progressively reduced but it is not totally abolished even with 100 mgs. With addition of weight, though the amplitude changes, the frequency remains unaffected. With the removal of the downward pull the pulsation comes back to normal amplitude.

When 10 mg. pull in the upward direction sufficiently reduces the amplitude, 10 mg. downward pull added in that condition enlarges the amplitude up to nearly the normal size.

Similar results were obtained with different specimens of Desmodium leaflet. Further experiments will be undertaken in this line, in which, the modification of the respiratory rate of the leaflet as well as the electrical pattern of its pulsation during the movement of the leaflet with load, have been proposed to be studied.

Workers in the laboratory together with the instruments used by them had to take part in the preparation of a documentary film as well as in the exhibition held during the Birth Centenary Celebration of Acharya Jagadish Chandra Bose.

## C.3. Cytology

A temperature controlled sterilized chamber meant primarily to house de Fonbrune's micromanipulator was completed in the period under review.

C.3.1. *Mitotic Figures in Free floating Nuclei*

The micromanipulator has been put to some preliminary use; among the results obtained being perforation and disruption of the nuclear membrane in coconut endosperm as well as isolation of single nuclei from the meristematic syncitium.

The behaviour and nature of the spindle during mitosis was noted in Coconut and Areca (betel nut). In the latter, giant polyploid nuclei occur which have from time to time been observed at polar view and therefrom the feature of the living metaphase plate and arrangement of chromosomes on it was noted. This type of plate was photographed at all magnifications, inluding oil immersion. Unlike coconut, areca nuclei do not continue with mitosis when mounted. Many features of the sequence of division was noted in coconut among them being the spindle orientation and its local structural differences, the appearance of chromosomes during the anaphase movement and the gradual development of the cleavage plate. In addition the spindle undergoes typical changes in shape as division progresses. Mitochondria are found to wander all through the endospermal milk and generally to bear no relation to the resting nuclei, but to show a characteristic and localized attraction when these same nuclei were undergoing division. They always occur outside of the nuclear membrane.

The existence of a persistent nuclear membrane, which is not disturbed during division, was observed.



Spindles are found to undergo certain changes in shape under the action of external agents, both physical and chemical. It appears that whenever any agent interferes with the even course of division, it is the spindle orientation that is first destroyed. But though the linear orientation may be lost, bipolarity is not immediately destroyed and may persist for some time. Limitations to the disorientation of the spindle are found to exist in two categories, firstly, that all stages of mitosis are not equally sensitive and secondly, that there may be portion which remain comparatively resistant to returning to plasmagel.

The airconditioned chamber when first built was kept at 19°C and division could not continue at this temperature; finally with the installation of a thermostatic switch the temperature was kept at 26—28 C, and this helped not only to keep the mounts viable and active for a prolonged period of time, but also helped the storage of fruit in a meristematic condition for a number of days.

Photomicrographs were taken with slow speed fine grain Adox film on a Leitz ortholux microscope with a Leica Camera and attachment.

C.3.2. *Mimosa pulvini*

Work on sensitive pulvini was continued on a particular aspect, namely, the absence of the typical chloroplast in the green active cells, and observation of the bodies that substitutes for it, these bodies change shape and under certain conditions may develop into true chloroplasts; the latter again may revert to the above mentioned particulate bodies. Thus the chloroplast may not be a stable structure as it is generally supposed to be. The changes it undergoes are found to be related to the presence and absence of light and on sugar content.

The contractile vacuole, so characteristic of *M.pudica* and determined with Brilliant Cresyl Blue staining, was demonstrated to be present in all categories of motor pulvini.

C.3.3.

Chromosomex members of some sensitive Mimosa species were determined. It was found that the *M.pudica* variety with smaller sized vegetative parts, collected from Bangalore on a previous trip, turned out to be an octoploid, as against the tetraploid of the common Bengal variety which has larger vegetative parts.

## RADIATION AND CHEMICAL MUTAGENESIS, CYTOGENETICS, PLANT BREEDING, ANATOMY AND WEED CONTROL

### C.4. Radiation mutations

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

This is a research project which is being financed by the Indian Central Oil-seeds Committee since 1950, for inducing mutants with desirable economic characters in Til and *Mustard*, by using X-rays and beta radiations. Both the dominant and recessive mutants with increased number of fruits, seeds and oil content and/or early flowering, isolated from the irradiated progenies every year, are being grown in their succeeding generations, to study their behaviour.

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

The season was favourable for the growth of the *Sesamum* plants.

The following varieties and types of *Sesamum orientale*, viz: *types* 10, 12, 16 and 20 from West Bengal and T. M. V. I from Madras, were studied during the year.

C.4.1.a.1. *Breeding behaviour of the recessive mutants.* During the year under report, the behaviour of the *white flowered mutant* isolated in the $X_3$ generation from the 20,000 r X-ray treatment in 1952 and the *small seeded mutant* isolated in the $X_4$ generation from the 14,400 r X-ray treatment in 1953 from *S. orientale type* 16, which were in the $X_9$ generation, were studied. The data on their oil content, peroxide value and flowering behaviour are included in Table 1.

TABLE 1

| Control and Mutants | $X_9$—1958 | | | |
|---|---|---|---|---|
| | Mean percent of oil over control ± S.E. | Peroxide value | | Difference in time of flowering ± S.E. |
| | | Treatment | Oil kept for a month | |
| Control | 100.00±0.86 | 6.4 | 12.2 | — |
| White flowered Mutant | 102.90±0.86 | 1.2 | 4.4 | +*4.6±0.22. |
| Small seeded Mutant | 114.10±0.86 | 1.5 | 5.0 | −*2.5±0.24 |

*Significant at 1% level.

It will be seen from the above table that in the $X_9$ generation, there is increase of about 3.0% and 14.0% in the oil content in the *white flowered* and *small seeded* mutants over the control, as against the $X_8$ generation where the increase was 4.0% and 17.0% respectively.



The keeping quality of the oil of these mutants continued to be better than the control, as indicated by their relative peroxide values of 6.4, 1.2 and 1.5 in the freshly extracted oils of the control, *white flowered* and *small seeded mutants* respectively. Oils kept for a month gave peroxide values of 12.2, 4.4 and 5.0 respectively.

While the *white flowered mutant* flowered about 5 days later, the *small seeded mutant* flowered 2.5 days earlier than their respective controls.

C.4.1.a.2. *Breeding behaviour of higher yielding selections.* The breeding behaviour of two of the selections made for higher yield in the previous years from *Sesamum types* 12 and 16 are included in Table 2.

TABLE 2

| Type and Locality | Control and Treatment | Plant population | Highest yielding plant | | Age of the plant at the time of flowering, in days |
|---|---|---|---|---|---|
| | | | No. of fruits | Wt. of seeds in gms | |
| Type 12 (W. Bengal) | Control | 288 | 212 | 31.90 | 39 |
| | 14,500 r | 298 | 348 | 31.95 | 39 |
| | 20,000 r | 290 | 281 | 40.40 | 39 |
| Type 16 (W. Bengal) | Control | 311 | 208 | 23.00 | 39 |
| | 14,500 r | 299 | 237 | 23.22 | 44 |
| | 20,000 r | 316 | 232 | 27.60 | 42 |

It will be seen from the table 2 that the best plants in $X_9$ generation in 14,500 r and 20,000 r treatments in *type* 12, gave 31.95 and 40.40 gms of seeds as against 31.90 gms of control. Similarly in *type* 16, the two treatments gave 23.22 and 27.60 gms of seeds as against 23.00 gms of control. All these selections are the progenies of the best selections of the previous years.

The breeding behaviour of the selections which are in the $X_8$, $X_6$, $X_5$, $X_3$, $X_2$ and $X_1$ generations during the year under report, is being summarised as follows:—

In the $X_8$ generation, selections in both *types* 12 and 16 gave lower fruit and seed yields.

In the $X_6$ generation of *Sesamum types* 10, 12, 16 and 20 of the 40,000 and 56,000 r treatments, some of the selections in *types* 12 and 16 *only*, gave higher seed yield.

In the $X_5$ generation of *Sesamum type* 10 which had received 10,000, 12,500, 56,000 and 80,000 r, doses of X-rays initially, the best selection in 80,000 r only, gave higher seed yield of 37.60 gms, as against 33.40 gms in the control.

In the $X_3$ generation of *Sesamum type* 10 where 40,000 and 1,20,000 r treatments were initially given , the best plant in 1,20,000 r only gave higher seed yield of 34.20 gms. as against its control with 31.34 gms.

In the $X_2$ generation of *Sesamum types* 10, 12, 16, 20 and *TMV I* of the 1,00,000 r treatments, all the types except *type* 10, gave plants with higher fruit and seed yields.

C.4.1.a.3. *Flowering behaviour of the selected plants.* The selections of *types* 12 and 16 which were in the $X_9$ generation during the season, did not show any significant difference in the date of flowering (Table 2).

The selections of *types* 12 and 16 in the $X_8$ generation, *types* 10,12, 16 and 20 in the $X_6$ generation, *type* 10 in the $X_5$ and $X_3$ generations and *types* 10, 12, 16, 20 and *TMV I* in the $X_2$ generation also, did not give significant variations in dates of flowering.

In the $X_4$ generation, selections in 8,000, 16,000, 24,000 and 40,000 r treatments flowered 2 to 4 days earlier than their controls.

C.4.1.a.4. *Multicoloured seed mutant.* The estimation of the yield of the oil in various 'mosaic' selections from *Sesamum type* 12 has been carried out. The results obtained show confirmity with the previous year's data in increased oil percentage in the differently coloured seeds such as brown, black, brownish black, ash, grey and dull white.

C.4.1.b. *Effects of radiosulphur ($S^{35}$) in Sesamum*

C.4.1.b.1. *Yield behaviour of previous selections.* In the $S_3$ generation of *Sesamum type* 12, where two activities, 89 and 123 $\mu$cs were utilised for treating seeds for 48, 72 and 96 hours, the selections in 123 $\mu$c/48 hours, 89 and 123 $\mu$cs/72 hours and 89 $\mu$c/96 hours, gave higher yield of seeds than the control.

Seeds of *Sesamum type* 16 and its mutants, *white flowered* and *small seeded*, were treated with 630 and 1960 $\mu$cs of radiosulphur for 24, 48 and 72 hours. In the $S_1$ generation of *Sesamum type* 16, only one selection each in 630 $\mu$c/24 hours and 1960 $\mu$c/48 hours gave higher yield of seeds. In the *white flowered mutant*, the treatment with 1960 $\mu$c/24 hours and 1960 and 630 $\mu$cs/48 hours, gave selections with higher seed yield.

C.4.1.b.2. *Flowering behaviour of selections.* The selections of *Sesamum type* 12 which were in the $S_3$ generation, flowered more or less on the same day as that of the control.

Slight differences were observed in the date of flowering in the treatments and controls of *type* 16 and *white flowered* and *small seeded* mutants in the $S_1$ generation. However, two to three early flowering selections have been made in all the three types.

C.4.1.b.3. *Percentage of sterility and diameter of pollen grains.* In *Sesamum type* 16 in $S_1$ generation treatments, 630 and 1960 $\mu$cs/24 hours, 630 $\mu$c/48 hours and 1960 $\mu$c/72 hours, gave 2 to 4 percent higher sterility than the control. However, all the treatments except 630 $\mu$c/24 hours gave greater pollen diameter than the control.

In *white flowered* mutant in $S_1$ generation, all the treatments except 1960 $\mu$cs/48 and 72 hours gave greater pollen sterility. All the treatments gave greater pollen diameter than the control.

In *small seeded* mutant in $S_1$ generation, all the treatments gave lower sterility than the control. Only the treatments with 1960 $\mu$cs/48 and 72 hours gave greater pollen diameter than the control.



C.4.1.b.4. *Characteristics of the oils*

It will be seen from the table 3 that there is no difference in the percentage of oil between control and treatments. But the treatments show slight decrease in the red pigment. All the other characteristics of oil, such as, refractive index, saponification value, iodine value, non-sap and components of mixed fatty acids (linoleic and oleic), remain practically unchanged. It is interesting to record that in most of the treatments, the peroxide values in the freshly extracted oil are higher than the controls. But when kept for a month, although there is corresponding increase in the value both in the controls and treatments, it is lower in the latter. It indicates therefore that the oils of the treatments can be kept for a longer period, specially so, of the 96 hours duration.

C.4.1.c. *Effects of radio-phosphorus ($P^{32}$) in Sesamum*

C.4.1.c.1. *The yield of previous selections.* In the $P_3$ generation, although most of the selections both in *types* 12 and 16 gave higher number of fruits than the control, in seed weight, only a few of these selection in 750 $\mu$c/48 hours and 300 and 750 $\mu$cs/72 hours in *type* 12 were better than their controls.

In the $P_2$ generation, some of the selections in 572 $\mu$c/48 hours and 127 $\mu$c/96 hours in *type* 12 and 182 and 116 $\mu$cs/96 hours in *type* 16, continued to give higher yield of seed than the control.

In the current treatment ($P_1$ generation), treatments 1830 $\mu$cs/48 and 72 hours and 720 $\mu$cs/24 and 48 hours in *type* 16, gave selections with higher seed weight. In the *white flowered mutant*, better selections were not obtained. In the *small seeded* mutant, the treatment 1830 $\mu$c/48 hours only, gave selections with higher seed yield than the control.

C.4.1.c.2. *Flowering behaviour of selections.* In the $P_3$ generations, selections of *Sesamum type* 12 in 750 $\mu$cs/48 and 72 hours and 1000 $\mu$c/72 hours, flowered 3 to 4 days earlier than the control. In *type* 16, the selection in 750 and 380 $\mu$cs/48 hours, flowered 2 to 3 days earlier than the control.

In the $P_2$ generation, selections of *Sesamum type 12*, in 800$\mu$c/24 hours, flowered 4 days earlier and in 352$\mu$c/48 hours and 216$\mu$c/96 hours, flowered 2 days earlier than the control. In *Sesamum type 16*, the selections did not flower earlier.

In $P_1$ generation, like $S_1$ generation, there was no deviation in flowering behaviour of the treatments from that of control.

C.4.1.c.3. *Pollen grains sterility and diameter.* The pollen grain sterility and diameter in the control *type 16* has been found to be about 12 per cent and 61$\mu$ respectively. The treatments 720$\mu$cs/24 and 48 hours and 1830$\mu$c/72 hours gave higher sterility, ranging from 13.7 to 18.4 per cent and greater diameter of pollen grains, ranging from 63 to 66$\mu$. Similarly, in *white flowered* control, the respective figures are 13 and 60. All the treatments in all the durations gave higher sterility, ranging from 11 to 41 per cent and greater diameter ranging from 62 to 65$\mu$.

TABLE 3

| Type and Locality | Duration of treatment in hours | Control and activity in $\mu c$ | Percentage of oil | Colour by Lovibond scale | Refractive index $N_D$ 20°C | Saponification Value | Iodine Value | Percent Non-sap | Linoleic acid | Oleic acid | Peroxide value: Fresh | Peroxide value: Oil kept for one month |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type 12 (W.Bengal) | (Dry) | Control | 38.52 | Y = 1.0 R = 0.2 | 1.4676 | 191.7 | 108.6 | 1.8 | 36.0 | 50.9 | 3.6 | 30.2 |
| | 48 | " | 38.59 | Y = 1.0 R = 0.3 | 1.4672 | 191.9 | 108.4 | 1.7 | 37.8 | 49.2 | 3.4 | 30.0 |
| | " | 89 | 38.48 | Y = 1.0 R = 0.1 | 1.4598 | 192.6 | 104.9 | 1.5 | 36.4 | 50.2 | 5.0 | 25.4 |
| | " | 123 | 38.64 | Y = 1.0 R = 0.1 | 1.4617 | 191.4 | 1.5.6 | 1.3 | 33.3 | 48.6 | 3.5 | 20.4 |
| | 72 | Control | 30.49 | Y = 1.0 R = 0.1 | 1.4667 | 191.6 | 108.0 | 1.7 | 35.8 | 51.7 | 3.7 | 30.6 |
| | " | 89 | 38.93 | Y = 1.0 R = 0.2 | 1.4615 | 192.8 | 105.4 | 1.1 | 35.8 | 50.4 | 8.8 | 21.9 |
| | " | 123 | 38.58 | Y = 1.0 R = 0.2 | 1.4611 | 189.6 | 105.2 | 1.4 | 34.2 | 48.4 | 6.5 | 27.6 |
| | 96 | Control | 38.49 | Y = 1.0 R = 0.3 | 1.4670 | 191.2 | 107.9 | 1.7 | 35.6 | 50.9 | 4.0 | 31.2 |
| | " | 89 | 36.58 | Y = 1.0 R = 0.1 | 1.4589 | 912.6 | 104.7 | 1.1 | 36.8 | 47.9 | 7.5 | 10.0 |
| | " | 123 | 37.48 | Y = 1.0 R = 0.1 | 1.4584 | 192.5 | 104.5 | 1.1 | 34.6 | 48.1 | 7.3 | 13.0 |



C.4.1.c.4. *Meiotic aberrations.* In the current treatments with both $S^{35}$ and $P^{32}$ in *Sesamum types 16*, "*white flower*" and "*small seeds*" the common aberrations rccorded were clumping of the chromosomes, lagging bivalents and univalents and rare occurrence of bridges.

C.4.2. *Effects of X-rays and beta-rays in mustard (Brassica)*

C.4.2.a. *Effects of X-rays in Mustard.* The season was favourable for the growth of the mustard plants.

C.4.2.a.1. *The yield of the previous selections.* It will be seen from the Table 4 that the $X_7$ selections of *Rai 5* in all the treatments except 40,000 r, gave greater number of fertile seeds per fruit than the control. The seeds in all the treatments are also heavier than the control. The treatments seem to have lesser seed sterility than the control.

TABLE 4

| Type and Locality | Control and Treatment | Behaviour of selection: $X_6-1957$ No. of fruits | $X_6-1957$ Total weight of seeds in gms | $X_7-1958$ No. of fruits | $X_7-1958$ Size of sample = 10 fruits: No. of seeds Fertile | No. of seeds Sterile | Wt. of fertile seeds in gms |
|---|---|---|---|---|---|---|---|
| Rai 5 (W. Bengal) | Control | 615 | 8.10 | 886 | 123 | 28 | 0.240 |
| | 20,000 r | 947 | 22.00 | 500 | 145 | 18 | 0.270 |
| | 26,000 r | 339 | 8.72 | 692 | 165 | 4 | 0.330 |
| | 40,000 r | 415 | 4.60 | 533 | 118 | 17 | 0.245 |
| | 56,000 r | 718 | 10.20 | 558 | 128 | 14 | 0.270 |

Although the $X_6$ selections of *Tori 7* gave lesser number of fruits than the control, most of these had higher number of seeds than the control, which in a selection each in 26,000 and 80,000 r treatments also weighed more than the control. Only one $X_6$ selection of the 70,000 r treatment in *Rai* 5, gave higher number of fruits, but in seed weight, practically all the selections were better than the control. In *Brown Sarson*, however, a selection in 26,000 r treatment gave higher number of fruits and another gave higher weight of seeds.

Some of the $X_5$, $X_4$, $X_3$ and $X_2$ selections in the mustard types under investigation, gave higher number of fruits and seeds. The latter in most of the selections weighed more than the control.

C.4.2.a.2. *Flowering behaviour of selections.* Most of the early flowering selections in the different generations referred to in the previous report under this head, continued to flower early. Besides, some more early flowering selections have been isolated in $X_7$, $X_6$, $X_5$ generations of *Rai 5* and *R. L. 9* during the year under report.

C.4.2.a.3. *Percentage and peroxide value of oils.* There was no appreciable increase in percentage of oil in $X_4$ selections of *T.* 185 and $X_3$ selections of *Rai 5*, *R. L.* 9 and *T.* 185. However, as before, these oils gave lesser peroxide value than the controls and hence have better keeping quality.

C.4.2.a.4. *Behaviour of early selections.* Progenies of early selections in *Rai* 5 and *R. L.* 9 continued to be as early as 2 to 9 days, with increased yield of fruits and seeds.

C.4.2.a.5. Two recessive mutants, "Appressed pod" (isolated from *Rai* 5, in $X_3$ generation of 1,00,000 r treatment) and "Thickened pod" (isolated also from *Rai* 5, in $X_2$ generation of 40,000 r treatment), did not give higher fruits or oil yield than the control *Rai* 5. Seeds of "Thickened pod mutant" are heavier than the control. Both are non-shattering types and might withstand mechanical harvesting.

C.4.2.b. *Effects of radiosulphur ($S^{35}$) in Mustard*

C.4.2.b.1. *Yield behaviour of selections.* The $S_1$ and $S_2$ selections of *Rai* 5 and *Brown Sarson*, in a few of the treatments, gave higher number of fruits and seeds. Some of them appear to have heavier seeds than their controls.

C.4.2.b.2. *Chemical characteristics of oils.* A number of $S_2$ selections of *Rai* 5 in 453, 679 and 905$\mu$cs activities were analysed for the chemical characteristics of their oils. No appreciable change in the percentage of oil was observed, except in two selections in 905$\mu$c/72 hours treatment, where it was higher than the control. Some of the selections gave lower Lovibond value for red pigment of the oil, which makes the oil appear slightly clearer than the control. As regards the peroxide value of oil, these selections initially do not show significant deviation from the control, but after about a month, the difference is marked, with lower peroxide values in the treatments. Hence these oils, like those of X-radiated ones, have better keeping quality than their controls.

C.4.2.b.3. *Pollen grain sterility and pollen diameter.* In the current treatments with 1970, 1480, 990 and 490$\mu$cs in *Rai* 5, no appreciable changes in the pollen diameter and sterility have been recorded. In *Brown Sarson*, a slight increase in the size of pollen grains has been observed.

C.4.2.c. *Effects of radiophosphorus ($P^{32}$) in Mustard*

C.4.2.c.1. *Yield behaviour of selections.* Most of the selections of *Rai* 5 and *Brown Sarson* referred to in the last year's report, continue to give increased yield of fruits and seeds. Higher yielding selections have also been isolated from the current treatments.

C.4.2.c.2. *Flowering behaviour of selections.* Some of the early flowering selections of the last year were early this year also. Some new early flowering selections have been isolated from the current treatments.

C.4.2.c.3. *Chemical characteristics of oils.* The result of analysis of $P_2$ selections of *Rai* 5 in 127, 379, 514, 880, 1321 and 1761$\mu$cs activities show that percentage of oil decreases with the increase in activity and duration of soaking. Erratic data have been obtained with regard to the decrease in the red pigments. But consistent results have been obtained regarding peroxide values. The oils of durations 24 and 48 hours in all the activities are better than the treatments in 72 hours and much better than that of control.



C.4.2.c.4. *Pollen grain sterility and pollen diameter.* The current treatments with 1830, 1370, 910 and 460$\mu$cs in both *Rai* 5 and *Brown Sarson*, show overall increase in the diameter of pollen grains, with no significant difference in their sterility.

C.4.2.c.5. *Cytological observations.* In the current treatments, meiotic irregularities, such as, univalents and trivalents, plain and beaded bridge, laggards, clumping and extrusions have been observed.

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

This is a scheme of research financed by the Indian Central Jute Committee, for inducing favourable mutations with ionizing radiations and is in the third year of investigations, during the year under report.

C.4.3.1. *Sowing season.* As in the previous year, the monsoon was late and the fields had to be artificially irrigated soon after sowing.

C.4.3.2. *Treatments.* Dry seeds of *Tall Mutant*, *R*–26 and *Chinsura Green* belonging to *C. olitorius* and *D*–154 and *D*–386 belonging to *C. capsularis* were irradiated with 1.5, 3.0 and 4.5 mc initial activities of $P^{32}$ and $S^{35}$. These freshly irradiated seeds, their respective controls, and the seeds collected from previous year's promising plants, were sown in replicated plots at the Falta Experimental Station.

C.4.3.3. *Flowering behaviour.* As in the previous years, the time taken for first flowering (in days) was studied for individual plants in the control and treated population. In the $P_1$ generation, earliness of 1.5 to 4.8 days and of 1.2 to 7.1 days in mean time of flowering over the control, was recorded in the varieties *Tall Mutant* and *Chinsura Green* respectively. In the variety *D.* 154 lateness ranging from 1.5 to 1.9 days also in mean time of flowering over the control was observed. The differences between the control and the treatments in the variety *R*–26 were negligible. The range of flowering in most of the treatments in the varieties *R*–26, *Chinsura Green* and *D* 154, was greater compared to their respective controls.

In the $S_1$ generation, earliness of 1.4 to 3.8 days and 1.4 to 4.4 days in mean time of flowering over the control was observed in the varieties *R*–26 and *Chinsura Green* respectively. The differences in the treatments in mean time of flowering with respect to the control in the varieties *Tall Mutant* and *D* 154 were small, being less than one day.

In the $S_2$ generation, the mean time of flowering in the selections from all the five treatments in all the varieties was late, ranging from 0.5 to 6.5 days. However, a maximum earliness of 6.4 days was recorded in the selections made from 2.5 mc $S^{35}$ treatment, in the variety *Chinsura Green.*

C.4.3.4. *Induced variability.* During the course of the investigations on the flowering behaviour of jute, it has been observed that exposure to ionizing radiations like X-rays, $P^{32}$ and $S^{35}$ brings about increased variability in the population. The increase in varia-

bility in the treated population may or may not be associated with change in mean time of flowering. From a study of a large number of treatments and generations after treatments with the above mutagens, a general hypothesis has been formulated, which indicates that if the time taken for first flowering is earlier than the control, the variability is large, while if it is later, the variability is small.

The variability once induced in the first generation after treatment followed by repeated selections from extreme ends was found in some of the selections to be diminishing in subsequent generations, while in others and particularly in early flowering selections the increased variability continued for as many as eight generations.

C.4.3.5. *Behaviour of the early flowering types.* (a) *X-rays.*—Out of the six promising early flowering selections, which were isolated during the previous years from the X-irradiated population of the varieties *Tall Mutant* and *R*–26, five have been found to be significantly early.

TABLE 5

SHOWING THE DIFFERENCE IN (i) MEAN TIME OF FLOWERING (IN DAYS) AND (ii)STANDARD DEVIATION IN THE EARLY FLOWERING SELECTIONS (FROM X-IRRADIATED POPULATION) WITH RESPECT TO THOSE OF THE CONTROL

| Variety | Selection Number | Difference in: mean time of flowering ± S. E. (days) | Difference in: standard deviation ± S. E. |
|---|---|---|---|
| Tall Mutant | $T_1X$ | −48.2±1.52** | −18.94±1.080** |
| | $T_2X$ | −57.6±1.39** | −16.23±0.980** |
| | $T_3X$ | −63.2±1.44** | −17.40±1.023** |
| | $T_4X$ | −53.6±1.49** | −18.23±1.054** |
| R–26 | $R_1X$ | − 3.5±1.88** | −4.30±1.330** |
| | $R_2X$ | −13.2±2.03** | −7.79±1.435** |

— in mean time of flowering indicates earliness.
— in standard deviation indicates treatments having larger standard deviation.
** = P<0.01

In the former variety, all the four selections had significantly earlier mean time of flowering, ranging from 48.2 to 63.2 days over the control. All these selections have a long flowering period and the differences in the variability were highly significant. These selections have been grown for as many as eight generations and selections from extreme ends failed to reduce the variability. Similarly, in the variety *R*–26, significant earliness was observed in the selection No. $R_2X$. But both the selections showed significantly increased variability.



(b) $P^{32}$.—Six selections were also made in the previous years from the $P^{32}$ treated population of the varieties *Tall Mutant* and *R*–26 and were in the fourth generation after treatment, during the year under report. All these selections had earlier mean time of flowering than their respective control (Table 6).

TABLE 6

SHOWING THE DIFFERENCE IN (i) MEAN TIME OF FLOWERING (IN DAYS) AND (ii) STANDARD DEVIATION IN THE EARLY FLOWERING SELECTIONS (FROM THE $P^{32}$ TREATED POPULATION) WITH RESPECT TO THE CONTROL

| Variety | Selection Number | Difference in: mean time of flowering ± S. E. (Days) | Difference in: standard deviation ± S. E. |
|---|---|---|---|
| Tall Mutant | $T_1P$ | −33.0±1.47** | −17.86±1.042** |
| | $T_2P$ | −40.2±1.62** | −20.62±1.142** |
| | $T_3P$ | −30.5±1.37** | −15.90±0.968** |
| R—26 | $R_1P$ | −28.0±1.80** | −2.24±1.271 |
| | $R_2P$ | −29.5±1.90** | −4.51±1.336** |
| | $R_3P$ | −20.3±2.20** | −11.59±1.559** |

— in mean time of flowering indicates earliness
— in standard deviation indicates treatments having larger standard deviation.
** = P<0.01

In the variety *Tall Mutant*, the earliness in mean time of flowering in the three selections over the control varied from 30.5 to 40.2 days, while in the variety *R*–26, it ranged from 20.3 to 29.5 days. All these selections, excepting $R_1P$ showed increased variability.

(c) *Colour aberrants.*—It has been reported earlier that changes in pigmentation of plants occurred following treatment with $P^{32}$, particularly in the variety *R*–26. The treated population produced a few plants resembling the pigmentations of *Tall Mutant* and *Chinsura Green*, while the rest of them appeared as *R*–26. These colour aberrants in subsequent generations segregated with respect to the pigmentation. Single plant cultures were made with a view to obtaining populations of uniform colour.

The most interesting of them was those which resembled *Tall Mutant* and were named as *Tall Mutant II*. During the year under report, the flowering behaviour of these lines were studied along with other selections, when it was observed that *Tall Mutant II* is an

early flowering mutant compared to *Tall Mutant* (obtained after X-irriadiation on *R*–26 in 1947) having the mean time of flowering and the flowering period as 119.8±1.46 and 117 days in the former and 145.80.±0.43 and 36 days in the latter respectively. These differences on statistical analysis were found to be highly significant.

C.4.3.6. *Morphological abnormalities.* As observed during the previous years, a large number of morphological abnormalities like forking of leaves, two or three leaf blades arising from a single petiole and the blades being attached with each other at the base, fasciation of stem, epiascidia arising directly from the stem or from the abaxial surface of a leaf and maintaining the law of inversion, double leaves, etc., were obsderved in $P_1$, $P_2$, $S_1$ and $S_2$ generations of all the five varieties of jute. During the year under report, a large number of $S_2$ populations of varieties *D* 154 and *D* 386 showed morphological abnormalities. Not only their $S_1$ populations did not have these abnormalities during the previous year, but abnormalities were earlier found to be rather few in the treated population of the varieties of *C. capsularis*.

One plant in the $P_1$ population of the variety *D*-154 showed fusion of two petioles, which arose from two branches resulting from a bifurcation of the stem. The petioles fused at the top and bore a common bi-lobate lamina. Anatomical studies of the petioles however did not show any marked change from the normal. Anatomical studies of other abnormalities like epiascidia, forking of leaves, double leaves etc., were again continued and revealed changes in the organisation and course of vascular bundles from the petioles to the leaf blades.

C.4.3.7. *Pollen grain sterility.* Pollen grains were collected from plants in the $P_1$ generation and their controls and studied with respect to the induced sterility. Observations made on about 5,000 pollen grains from each treatment revealed no significant difference between the control and the treatment. But in the variety *D.* 154, there was an increase in sterility with increase in the activities of $P^{32}$, the maximum sterility of 6.17% being recorded in the 4.5 mc treatment. These results show that the treatments had no effect in inducing greater sterility in pollen grains in the first generation excepting in *D.* 154, indicating thereby that there were no chromosomal abnormalities during meiosis.

C.4.3.8. *Cytology.* Cytological investigations with reference to meiosis in some of the early flowering types have been made. Meiosis was normal in most of the cases studied. However, non-disjunction and movements of one or two bivalents to the same or either poles during anaphase I, was quite frequent. Nuclei with different chromosome numbers at second division were not observed and most probably the nuclei with unequal number of chromosomes or with normal chromosome number, but differing in unbalanced genetic constitution, being deficient in some chromosomes and having double number of other chromosomes, degenerated at interphase.

C.4.3.9. *Anatomy of thicker-stemmed plants.* During the year 1957-58, treatments with various activities of $P^{32}$ on the seeds of *Corchorus capsularis* var. *D.* 154 and *D.* 386 gave rise to a few plants with unusually thick stems in the $P_1$ generation. Anatomical studies with regard to the number of ultimate fibre cells revealed increase of fibre cells over those of the



control plants in the ratio of 2.2 : 1.0. The yield of dry fibre from the same plants showed an increase in the thicker plants in the ratio of 2.0 : 1.0.

Selections were made from the thicker plants and grown without any further treatment along with the control, as $P_2$ generation during the year 1958-59.

Anatomical studies of the $P_2$ generation revealed no significant differences between the control and the selections from the treated plants as regards the yield of dry fibre and in the number of ultimate fibre cells, when analysed statistically. The mean yield of fibre (in gms.) and the mean number of ultimate fibre cells (in lakhs) in the treated plant being 27.4 and 1.94 as against 26.0 and 1.70 in the control., respectively.

C.4.4. *Radiation mutation in Groundnut (Arachis hypogea)*

This scheme of research in groundnut, financed by the Indian Central Oilseeds Committee, for inducing useful mutations by X-rays and radioisotopes was started in April, 1958.

C.4.4.1. *Varieties and treatments.* Nine varieties collected from different sources were selected and treated with X-rays, and beta-rays from $P^{32}$ and $S^{35}$, according to the following schedule.

TABLE 7

| Variety | Source | Treatment | | |
|---|---|---|---|---|
| | | Radiation | Dose | Duration |
| T.M.V. 1 | Tindivanam, Madras | X-rays | 20,000 r | — |
| AK. 10 | Akola, Bombay | | 40,000 r | |
| Spanish | Kopargaon | | 60,000 r | |
| T.M.V. 2 | Tindivanam, Madras | Beta-rays (from $P^{32}$) | 1.2 mc | 48 hrs. |
| | | | | 96 ,, |
| Kopargaon | Kopargaon | | 2.2 mc | 48 ,, |
| | | | | 96 ,, |
| Small Japan | Coimbatore | | 3.2 mc. | 48 ,, |
| | | | | 96 ,, |
| Coimbatore | Coimbatore (Local) | Beta-rays (from $S^{35}$) | 1.0 mc | 48 hrs. |
| | | | | 96 ,, |
| T.M.V. 3 | Tindivanam, Madras | | 2.0 mc | 48 ,, |
| | | | | 96 ,, |
| AK. 12–24 | Nagpur | | 3.0 mc | 48 ,, |
| | | | | 96 ,, |

C.4.4.2. *Experimental results.* Observations have been made on the germination, survival at maturity, flowering behaviour, growth habit, yield of pod and kernels of the control varieties and their treated progenies.

C.4.4.3. *Flowering behaviour of the $X_1$, $P_1$ and $S_1$ generations.* From the study of the flowering behaviour of the treated progenies and their control, it was apparent that X-ray

treatments had very little effect on the mean time of flowering in T.M.V. 1 and AK. 10. But a different response was observed in case of $P^{32}$ and $S^{35}$ treatments, as beta rays, in general, was found to cause late flowering. With longer duration of the treatment, flowering is further delayed. Soaking the seeds in water for longer duration also had delayed effect on the flowering time.

C.4.4.4. *Yield of pods.* The yield of pods per plant seems to be affected by the application of X-rays. Irradiated progenies of all the treatments gave lower yield in comparison with the controls and the depression in yield in the case of 60,000 r is not as low as in other two treatments, viz., 20,000 r and 40,000 r. (Table 8). Among the 3 varieties, Spanish appared to be affected most.

TABLE 8

| Mean yield of pods (in gms.) per plant in X-ray treatment | | | | | |
|---|---|---|---|---|---|
| Variety | Control | 20,000 r | 40,000 r | 60,000r | Average |
| T.M.V. 1 | 44.8 | 39.7 | 40.9 | 42.5 | 42.0 |
| Spansih | 45.2 | 36.0 | 29.3 | 36.0 | 36.6 |
| AK. 10 | 54.8 | 40.0 | 48.0 | 53.8 | 49.2 |

In the treatments with radioisotopes, there was no systematic trend in the mean yield of pods per plant. (Table 9 and 10). But some of the treatments in the varieties showed better performances than the control in $P^{32}$ and $S^{35}$ treatments. Longer duration (96 hrs.) seemed to have an inhibitory effect, on the yield, as compared to the shorter duration (48 hrs.), except in the $S^{35}$ treatment in T.M.V. 3, where longer duration showed better results than the shorter one. (Table 10).

TABLE 9

| Mean yield of Pod (in gms.) per plant in $P^{32}$ treatments | | | | | | |
|---|---|---|---|---|---|---|
| Variety | Duration | Treatment | | | | |
| | | Control | 1.2 mc | 2.2 mc | 3.2 mc | Average |
| Kopargaon | 48 hrs. | 11.2 | 10.2 | 14.7 | 12.8 | 12.2 |
| | 96 hrs. | 10.7 | 9.4 | 8.9 | 9.4 | 9.6 |
| T.M.V. 2 | 48 hrs. | 9.5 | 11.5 | 6.4 | 8.5 | 8.8 |
| | 96 hrs. | 8.1 | 10.2 | 8.6 | 4.3 | 7.4 |
| Small Japan | 48 hrs. | 10.4 | 6.9 | 8.8 | 9.8 | 8.8 |
| | 96 hrs. | 12.8 | 4.0 | 9.9 | 6.4 | 8.3 |



TABLE 10

| Mean yield of Pod (in gms.) per plant in $S^{35}$ treatments | | | | | | |
|---|---|---|---|---|---|---|
| Variety | Duration | Treatment | | | | |
| | | Control | 1.0 mc | 2.0 mc | 3.0 mc | Average |
| Coimbatore | 48 hrs. | 9.0 | 11.7 | 8.2 | 3.6 | 8.9 |
| | 96 hrs. | 3.3 | 7.7 | 6.4 | 4.0 | 5.6 |
| T.M.V. 3 | 48 hrs. | 15.8 | 25.8 | 17.1 | 12.6 | 17.8 |
| | 96 hrs. | 16.6 | 25.5 | 31.8 | 10.5 | 21.2 |
| AK. 12-24 | 48 hrs. | 5.9 | 7.6 | 6.6 | 11.6 | 7.9 |
| | 96 hrs. | 5.8 | 8.0 | 6.4 | 5.4 | 6.2 |

C.4.4.5. Studies on the effect of different doses of X-rays and beta-rays on the pollen sterility and yield of groundnut are in progress.

C.4.4.6. *Isolation of some variant plants from the irradiated progenies of different varieties* A few morphological variants that have been isolated from the population of first generation of the different varieties are as follows:—

(1) *Creeping habit*—One creeping plant has been isolated from the variety AK. 10 from 40,000 r treatment.

(2) *Curled leaf and dissected margin of leaf-lets.*—This plant has been isolated from $P^{32}$ treatment of 3.2 mc for 96 hrs. in the variety T.M.V. 2.

(3) *Small leaf and small flower of lighter yellow colour*—This plant was isolated from the $S_1$ progeny of the variety T.M.V. 3 from 3.0 mc for 48 hrs.

In addition to these, several higher yielding plants have been isolated from the progenies of X-ray and $S^{35}$ treatments.

The behaviour of these selected plants will be studied in subsequent generations to find out their breeding behaviour.

C.4.4.7. *Cytogenetics.* Cytogenetical studies of the $X_1$, $P_1$ and $S_1$ plants have been undertaken to correlate the observed variations with pollen sterility and yield. Some interesting observations made, are summarised below:—

(1) About 60% of the pollen mother cells of one plant in the $P_1$ generation of the 2.2 mc 96 hours treatment, frequently showed a ring of four chromosomes at metaphase I and diakinesis or a chain of four. The rest of the chromosomes paired normally. This is assumed to be due to reciprocal translocation involving two non-homologous pairs of chromosomes. The identification of the pairs of chromosomes involved in the translocation by comparative karyotype study, is in progress.

(2) One plant has been isolated from the $P_1$ progenies of 3.2 mc 96 hours treatment, in which almost all the pollen mother cells showed cytomixis even at diakinesis, metaphase I and II. It was noted that the extra chromosomes moving to another cell did not mingle with the normal chromosomal set of the cell, but formed an isolated group.

(3) *Delayed separation of a bivalent* was noted from the P.M.C. of one plant from $P_1$ progeny of 2.2 mc 96 hours treatment. In this, one bivalent was found to be late in dis. joining at anaphase I in about 20% of the pollen mother cells. But this bivalent ultimately disjoined and was not left in the inter-pollar region like laggards.

The progenies of all these aberrant plants are being followed to study their genetical behaviour.

C.4.5. *Investigations on induced mutagenesis in paddy (Oryza sativa) using X-rays and beta rays from radioisotopes*

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

C.4.5.1. *Irradiation experiments on aman paddy.* During this year, fresh irradiations with radiophosphorus were given to the seeds of two winter varieties of paddy, namely, *Rupsail* and *Patnai*. Besides, the seeds collected from previous year's irradiated population were grown as plant progenies in $P_2$ and as panicle progenies in $S_2$ and $X_2$ generations. The behaviour of two short grain types isolated in the $P_2$ generation in the variety *Patnai* was also studied in the third generation.

C.4.5.1.a. *Treatments.* For fresh treatments with $P^{32}$, 450, 900 and 1350 $\mu$cs were the initial activities used, in which the paddy seeds were kept immersed for 24 hours. Seeds kept in distilled water for the same period, served as control.

C.4.5.1.b. *Sowing.* All these freshly treated seeds and those collected from previous year's irradiated population were sown in seed-beds in June, and transplanted to the field in July, 1958.

C.4.5.1.c. *Studies on the $P_1$ generation.* The effects of different dosages of $P^{32}$ on the percentage of survival at the time of transplantation, mean time of flowering, heights of plants, number of panicles, length of panicles and yields of grain and straw, were noted.

C.4.5.1.d. *Survival.* Following treatments with radiophosphorus in the varieties *Rupsail* and *Patnai*, there was a fall in the percentage of survival of plants. However, there was no correlation between this and the dosages.

C.4.5.1.e. *Mean time of flowering (in days).* Treatments with $P^{32}$ in the first generation did not produce any significant difference in the mean time of flowering. There was however an increase in the variability of the treated population in both the varieties, as can be seen from the increase in the magnitude of S.E.s in the treatments in the Table I. This implies that the range of flowering was longer than that of the control.



TABLE 11

$P^{32}$ TREATMENT ($P_1$ GENERATION)

| Treatment \ Variety | Rupsail | Patnai |
|---|---|---|
| Control | 159.4±0.16 | 160.6±0.15 |
| 450 $\mu$c | 159.2±0.17 | 160.4±0.19 |
| 900 $\mu$c | 159.0±0.20 | 160.0±0.20 |
| 1350 $\mu$c | 159.1±0.18 | 160.0±0.19 |

C.4.5.1.f. *Heights of plants, number of panicles, length of panicles and yields of grains and straw.* Variations were observed in the treated population of both *Rupsail* and *Patnai* as regards heights of plants, number of panicles, length of panicles and yields of grains and straw, as can be seen from Tables 12 and 13.

TABLE 12

$P^{32}$ TREATMENT ($P_1$ GENERATION)
VARIETY RUPSAIL
MEANS AND STANDARD ERRORS (S.E.)

| Treatments \ Characters | Height of plants (cms.) | Number of panicles | Length of panicles (cms.) | Yield of straw (gms.) | Yield of grains (gms.) |
|---|---|---|---|---|---|
| Control | 153 | 7.9 | 27.7 | 416 | 191.5 |
| 450$\mu$c | 146 | 7.2 | 26.8 | 357 | 145.5 |
| 900$\mu$c | 150 | 7.7 | 28.2 | 403 | 159.0 |
| 1350$\mu$c | 150 | 6.9 | 27.5 | 383 | 126.1 |
| S. E. | 1.38 | 0.78 | 0.42 | 44.2 | 17.86 |

TABLE 13

$P^{32}$ TREATMENT ($P_1$ GENERATION)
VARIETY PATNAI
MEANS AND STANDARD ERRORS (S. E.)

| Treatments \ Characters | Height of plants (cms.) | Number of panicles | Length of panicles (cms.) | Yield of straw (gms.) | Yield of grains (gms.) |
|---|---|---|---|---|---|
| Control | 152 | 6.8 | 23.7 | 419 | 196.3 |
| 450$\mu$c | 148 | 5.3 | 22.6 | 316 | 140.5 |
| 900$\mu$c | 147 | 5.6 | 23.1 | 341 | 148.2 |
| 1350$\mu$c | 149 | 5.4 | 23.5 | 362 | 141.6 |
| S. E. | 1.34 | 0.38 | 0.29 | 25.2 | 13.18 |

It will be noted from the above two tables that the $P_1$ population showed decrease in all the characters; however, the differences in heights of plants in the variety *Rupsail* and yield of grains in the variety *Patnai* only proved significant.

C.4.5.1.g. *Cytological investigations.* Pollen mother cells in the treated population in *Patnai* variety revealed chromosome abnormalities like bridges, lagging, etc., at anaphase I. The percentage of abnormalities was 0, 4.0, 17.5 and 6.0 in the control, 450, 900 and 1350$\mu$c treatments respectively. In the same variety, one plant grown after treatment with 450$\mu$c $P^{32}$ showed ring of four chromosomes at diakinesis, suggestive of translocation. In the variety *Rupsail*, one of the treated plants showed the presence of one extra chromosome during first division of meiosis. This chromosome remained as univalent and ultimately lagged behind during anaphase I.

C.4.5.2. *Studies in the second generations*

C.4.5.2.a. *Chlorophyll deficiencies.* Chlorophyll deficiencies like albino, xantha, virescent, etc., were observed in the seedlings in the second generations of X-ray, $P^{32}$ and $S^{35}$ treatments. Counts of the chlorophyll deficient seedlings in the control and the different treatments were taken 8–10 days after sowing. The albino and xantha, which were entirely white and yellow respectively, are lethal and did not survive beyond the two or three leaved stage. The virescents which were yellow-green in colour, became fully green later and survived as normal plants.

In the variety *Rupsail*, the percentage of occurrence of chlorophyll mutants was found to be as high as 9.84%, 7.61% and 3.18% in the 15,000 r, 3.2 mc $S^{35}$ and 3.2 mc $P^{32}$ respectively.

*Semi-sterile plant*—Root-tip squash study of the semi-sterile plant isolated in *Patnai* after 4.0 mc $P^{32}$ treatment in 1957, showed no abnormality and the chromosome number was found to be $2n = 24$.

C.4.5.2.b. *Heights of plants, number panicles, length of panicles, yield of grains and straw.* The heights of plants, number of panicles, length of panicles, yield of grains and straw, were recorded in the second generations of X-ray, $P^{32}$ and $S^{35}$ treatments.

There was an increase in all the above characters in the second generation of $P^{32}$ treatments in the variety *Rupsail*. The increase in the yield of straw only was statistically significant. In the variety *Patnai*, on the contrary, decrease in all the characters in almost all the $P^{32}$ treatments were noted. However, the variations noted amongst the control and the treatments proved significant only in case of the length of panicles. The treatments in the second generation of X-ray in the variety *Patnai* yielded significantly lesser panicles than that of the control.

C.4.5.2.c. Four aberrant plants were isolated in the $S_2$ generation of variety *Patnai* after 2.4 and 3.2 mc $S^{35}$ treatments. These plants showed change in the colour of the husk from light-yellow to red. In three of these, the colour of the husk was red throughout while, in the fourth, only the tip was red. Moreover, the plant where only the tip of the husk and in another where the entire husk was red, also had a change in the colour of the stigma, which was black as against yellow in the control, while the other two plants where the entire husk was red, had the normal yellow colour of the stigma. At the same time, there was reduction in the size of the grains in all these four plants. In the latter, one plant had four out of eight panicles, showing change in the morphology of the spikelets. In the first panicle, all the thirteen spikelets were sterile and at the same time one of the outer empty glumes in each spikelet was unusually longer than the other; in the second, all the twenty spikelts were sterile and twelve of them showed enlargement in the outer empty glume, while in the third and fourth panicles, only one spikelet out of 112 and 59 respectively, showed the change in the length of the outer empty glume as above, but apparently these two flowers appeared normal and seeds have been formed. Detailed studies of these two seeds will be undertaken, if they are found viable.



C.4.5.3. *Studies in the $P_3$ generation*

The two short grain types which were isolated in the second generation in 1957-58, after 100$\mu$c $P^{32}$ treatment in the variety *Patnai* were compared against the control in a compact family block design in the $P_3$ generation, with special reference to the yield of grains, height of plants and number of panicles. There was no significant difference in yield between them, but the number of panicles was greater in the control.

C.4.5.4. and 5. *Irradiation studies in Aus and Boro paddy.*

During the year under report, irradiations with X-rays and radiophosphorus were given to the seeds of three varieties of Aus paddy, namely, *Marichbati*, *Jhanjee* and *Dular*. Besides, X-rays were applied to one variety of Boro Paddy, namely, *Chinsura Boro II.*

C.4.5.4. *Investigations in Aus paddy*

C.4.5.4.a. *Treatments.* Dry seeds of three varieties of Aus paddy, namely, *Marichbati*, *Jhanjee* and *Dular* were irradiated with 25,000, 50,000 and 75,000 r X-rays and 1 mc, 2 mc and 3 mc initial activities of radiophosphorus for 24 hours.

C.4.5.4.b. *Studies on $X_1$ generation.* The effects of the different doses of X-rays and radiophosphorus on the growth of radicles, percentage of survival of plants, mean time of flowering, height of plants, number of panicles, length of panicles and weight of straw in the three varieties were noted.

C.4.5.4.b.1. *Growth of radicles.* The length of the radicles grown from the control as well as the X-irradiated seeds were measured. With increase in the doses of X-rays there was decrease in the length of the radicles.

C.4.5.4.b.2. *Survival.* The plants which survived in the control and the treatments at the time of transplantation were counted. There was a fall in the percentage of surviving plants in the treatments as against the control.

C.4.5.4.b.3. *Mean time of flowering (in days).* The mean time of flowering (in days) did not show any significant variation in the treated population. However, differences were noted among the varieties, *Dular* being early flowering, *Marichbari* mid-flowering and *Jhanjee* late flowering.

C.4.5.4.b.4. *Heights of plants, number of tillers, number of panicles, length of panicles and weight of straw.* No appreciable differences were noted between the control and the X-ray and $P^{32}$ treatments as regards height of plants, number of tillers, number of panicles, length of panicles and weight of straw. Variations were, however, observed among the varieties with respect to the above characters.

C.4.5.4.b.5. *Morphological aberrants.* (1) In the 50,000 r treated population of the variety *Jhanjee*, one off-type plant was isolated where the grains were long with awns, as against short awnless grains in the control. The mean length of grains of the changed type is 8.01 mm. as compared to 7.90 mm. in the control.

(2) With 3 mc $P^{32}$ treatment, an aberrant plant was noticed in the variety *Marichbati*, where the colour of the husk was changed from normal golden yellow to black. The length/breadth ratio of the grains was also changed, being 2.67 as against 2.28 in the control.

(3) With 3 mc $P^{32}$ treatment in the variety *Dular*, one chlorophyll deficient plant was observed, in which the lateral sides of the leaves along the mid-veins being white.

C.4.5.4.b.6. *Cytological observations.* Root tip squashes of the $P^{32}$ and X-ray treatments revealed abnormalities like ealry separation, bridges and fragments. There was increase in the percentage of abnormalities with increase in the dose of X-rays. No such correlation was however found with the $P^{32}$ treatments. The maximum anaphase bridges (22.5% as against 1.2% in the control) were found in the variety *Jhanjee* after 75,000 r treatment. 12.5% anaphase bridges were observed in the same variety after 3 mc $P^{32}$ treatment.

C.4.5.5. *Investigations in Boro paddy*

The seeds of the variety *Chinsura Boro II* were irradiated with 5,000, 10,000 and 15,000 r X-ray doses after being soaked in distilled water for 24 hours.

C.4.5.5.a. *Studies in $X_1$ generation.*

C.4.5.5.a.1. *Survival.* There was a linear fall in the percentage of surviving plants with the increase of X-ray doses.

C.4.5.5.a.2. *Growth of radicles, plumules and Ist and 2nd leaves.* With lower doses of X-rays such as 250, 500, 750 and 1,000 r, there was a stimulation in the growth of the radicles in the treated population when compared to the control. But with the higher doses like 5,000, and 10,000 r, there was an inhibition in the growth of the radicles in the treated materials. Similarly, in the growth of plumule and 1st and 2nd leaves, there was a stimulation with lower doses of X-rays like 250, 500, 750, 1,000 r and an inhibition in the growth of leaves in the 5,000, and 10,000 r X-ray treatments.

C.4.5.5.a.3. *Morphological abberrants.* (1) *Emergence of the panicles.* Large number of X-irradiated plants were observed in which the panicles did not emerge out of the leaf sheaths. In the 15,000 r treatment, the panicles did not emerge in 21.4% of the treated population, as against 3.4% in the control.



(2) *Dwarf plant.* In the 15,000 r treatment in the variety *Chinsura Boro II*, one dwarf plant was isolated which was found to be completely sterile and the leaves of which were narrow and incurved.

(3) *Finer grains.* Two plants with finer grains and with change in the colour of the husks were isolated, from the 15,000 r X-ray treatment. The length/breadth ratio of the grains in the two selections was higher than that in the control.

C.4.6. *Irradiation studies in tomato (Lycopersicon esculentum)*

Irradiation studies with Tezier's Prim, a French variety of cultivated tomatoes, which were started three years ago, were continued during the year under report. During the previous years, dry seeds, two-day water soaked seeds and germinated seeds were irradiated with ultraviolet, X-rays and beta-rays from $P^{32}$ and $S^{35}$. Several mutants, including a high yielding selection from the $X_1$ generation had been isolated from the treated population. In continuation of these studies, the following investigations were taken up during the year under report: (1) Fresh irradiation with X-rays on dry seeds, (2) Behaviour of the dwarf mutant, (3) Comparative effects of $P^{32}$ and $S^{35}$ in the production of variants and (4) The behaviour of the high yielding selection in the $X_3$ generation.

C.4.6.1. *Fresh irradiations. Lethal range.* Dry seeds stored for 18 months were irradiated with 2,000 r, 4,000 r, 8,000 r, 16,000 r, 24,000 r, 32,000 r, 40,000 r, 48,000 r, 56,000 r, 64,000 r, 72,000 r and 80,000 r of X-rays. The lethal range was found to be between 24,000 r and the 32,000 r. A similar experiment in the previous year showed that the lethal dose was above 48,000 r, indicating that storing of seeds brings about low germinability and increased sensitivity to X-rays.

C.4.6.2. *Morphological abnormalities.* For the first time, several treated plants were noted with a single dichotomous branching of the main axis. The branching occurred at about the forth node and the flowering of such plants was delayed. Fasciated stems were also noted as in the previous years, but in greater frequency.

C.4.6.3. *Stimulative effects.* Critical evidence was obtained to show that certain relatively low dosages stimulate the vegetative development of the plant and the time of flowering. Precocious flowering was noted in two seedlings only, following the exposure of the germinated seeds to 8,000 r. These seedlings flowered at the fifth and the sixth internodes respectively, as against the eighth internode in the control. An increase in the rate of formation of the leaves on the main axis without any significant changes in the first flowering node, was however noted in several treatments. The differences in the flowering dates between these treatments and the controls were analysed statistically and found to be significant and a maximum difference of 2.4 days earliness was obtained following the exposure of the two-day water soaked seeds to 4,000 r X-rays.

Relatively low dosages also stimulated the elongation of the internodes. A maximum increase of 30 per cent was obtained, following the exposure of the two-day water soaked seeds to 4,000 r X-rays.

C.4.6.4. *Behaviour of the dwarf mutant.* This mutant in the $X_4$ generation during the year under report, isolated from the $X_3$ generation of the previous year from the 8,000 r

X-ray treatment on dry seeds, was found to breed true. A comparative developmental study was made of the control and the mutant. The mutant seedlings were distinguishable from the first true leaf onwards. The internode, the petiole, the petiotule and the rachis of the leaf frond showed inhibition in their elongation. No changes were observed in (1) characters of the seed, (2) percentage and rate of germination of the seed, (3) elongation of the hypocotyl, (4) size of the cotyledons, (5) rate of formation of the leaves on the main axis and (6) the first flowering node.

The anatomy of the mutant plants showed interesting changes. These were: (1) fewer xylem elements, (2) irregular development of the secondary xylem, (3) poor lignification of the xylem and (4) reduction in the size of the cells.

Flowers were fewer per cluster and many of the first borne flowers aborted before or after anthesis, resulting in delayed and decreased fruit set. The pollen grains were normal, as could be studied from the shape, size and contents. Several plants were unfruitful and the rest had small fruits, from one to three in number.

Thus the evidence seems to show that the dwarfing gene is pleiotropic in nature.

Attempts were made to find out if this dwarfism could be overcome by application of growth substances like Indoleacetic acid and Gibberallic acid. Four-week old seedlings were treated with 5 mg/l, 10 mg/l and 20 mg/l and 0.5 $\mu$ gm/l, 1.0 $\mu$ gm/l and 2.5 $\mu$ gm/l, aqueous solutions of I.A.A. and Ga respectively. Thin films of the growth substances were drawn on young, expanding leaves and the growing apical meristems. There were no changes in the growth of the treated plants. However, treatment with Ga produced change in the shape of the margins of the leaves from dentate to entire.

C.4.6.5. *Comparative effects of $P^{32}$ and $S^{35}$.* Investigations with $P^{32}$ and $S^{35}$ revealed that treatments with $S^{35}$ produced a greater frequency of recessive lethal seedlings than $P^{32}$, while treatment with $P^{32}$ produced variants of horticultural and of economic importance. The variants produced following treatments with $P^{32}$ were having (1) absence of green shoulder in the fruit, (2) changes of colour in the unripe fruit, (3) fruits with a thick cuticle, (4) small fruits and (5) changes in the fruit shape. The variants which were observed following $P^{32}$ treatment did not occur so far, in any of the X-ray treated populations. Apart from the increase in frequency of the variants, no major changes were observed in the $S^{35}$ treated population, as compared with the control.

C.4.6.6. *Selection of a higher yielding line.* The high yielding selection was initially made in the $X_1$ generation during the season 1955-56, following the exposure of dry seeds to 4,000 r of X-rays. The behaviour of this selection was reported partially, during the previous years. A progeny of 32 individuals from this selection in the $X_2$ generation gave an increased yield of 40% over the control. Twelve selections were made from this $X_2$ family and grown in a replicated plot with four selections of the control population. In the $X_3$ generation, ten selections gave higher yield than the control. The best selection from the treatments gave an increase of 34% yield over the control. The four selections made from the control population were uniform with regard to the yield of fruits. Studies on the above selections in the $X_4$ generation are in progress.



### C.5. Chemical mutagenesis

During the previous year, the effects of certain concentrations of amine and butyl ester of 2,4-D (2,4-Dichlorophenoxy acid), M.C.P.A. (2, Methyl chlorophenoxy acetic acid), 2,4,5-T (2,4,5-Trichlorophenoxy acetic acid) and 3-A T (Aminotriazole), in producing aberrations in the chromosomes in *Crotolaria* and *Pisum* were studied and briefly reported.

During the year under report, systematic studies have been undertaken with different concentrations of $(CH_3)\ 1SO_4$ (di-methyl sulphate), ranging from 0.00625.M/L to 0.05000 M/L, with special reference to the temperature during the treatment, to find out critical concentration and temperature in effecting 50% germination in the seeds of jute—*Corchorus capsularis* and *C. olitorius*. Preliminary experiments in this connection were conducted in petri-dishes and in wooden boxes containing soil. On the basis of these findings, treatments were made on the seeds of the above species of jute and sown in proper lay-outs at the Experimental Farm of the Bose Institute, at Shyamnagar and regular field data regarding the germination and survival percentages, the flowering behaviour and the morphological pecularities, are being collected.

Cytological studies of the roots of the treated materials showed chromosome abnormalities like bridges, laggards, etc.

On the basis of these findings, detailed experiments will be planned and conducted with the above and other chemical mutagens, during the next season.

### C.6. Cytological and cytotaxonomical studies

C.6.1. *Cytotaxonomical studies in some species and varieties of Oryza*

Cytological and cytotaxonomical studies in seven species and four varieties of *Oryza* namely, *O. sativa* var. *Rupsail*, var. *Boro*, var. *Zuiho* and var. *Fukoku*, *O. glaberrima*, *O. perennis*, *O. australiensis*, *O. officinalis*, *O. minuta* and *O. eichingeri* have been made. All the species studied except *O. minuta* and *O. eichingeri* are diploids ($2n = 24$) and the latter two species are polyploids ($2n = 48$). These species are classified on their morphological characters and habitat.

The somatic chromosomes of the seven species and 4 varieties have been analysed thoroughly on the basis of the morphology, number of satellited chromosomes and total length of the haploid complement. Very rarely, variation in the diploid number and chromosome bridges were observed.

The meiosis of 5 species, namely, *O. sativa*, *O. glaberrima*, *O. australiensis*, *O. minute* and *O. eichingeri* have been investigated.

The nucleoli and nucleolar chromosomes have been studies in detail in all the species.

From the above studies, a tentative scheme of evolution and the speciation in the genus *Oryza* has been made. It has been found that there is a phylogenetic reduction in the length of the chromosome complement, accompanied by the shifting of the centromere

from the median to submedian position. It has also been observed that the cultivated species, namely, *O. sativa* and *O. glaberrima* have shorter chromosomes than the wild species of *Oryza*.

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

During the year under report, twelve varieties of *Capsicum annum* L., namely, 'Koppal' (125) $WB/C_{116}$; 'Soli' (3) $WB/C_{25}$; Var: 'Yellow' (12) $WB/C_{31}$; 'Round' (13) $WB/C_{32}$; 'Contai Local' (135) $WB/C_{132}$; 'Boah Morich' (6) $WB/C_{34}$; 'Boah Sitki' (17) $WB/C_{54}$; 'Boah Barasat' (14) $WB/C_{51}$; 'Black Chilli' (90) $WB/C_{64}$; 'Local Malda' (1) $WB/C_{20}$; 'Sutton's Chilli' $BI/C_1$ obtained from M/S Suttons, Calcutta, were grown at the Experimental Station of the Bose Institute at Shyamnagar, for studying their morphological differences in the following characters such as, height of the plants, size of the leaves, size of the petiole, colour of both the surfaces of the leaves, colour of the nodes and internodes of the stem, shape of the stem (in T.S.), colour of the flowers, size of the calyx and corolla; number of the stamens, colour of the anthers, colour, size and shape of the fruits and the orientation of the fruits.

C.6.2.a. *Cytological investigations*. For studying the mitotic chromosomes, the root tips were stained in aceto-orcein after pretreating in asculin. The chromosome number has been found to be $2n = 24$ in all the varieties. Chromosome morphology has been studied, which differed in the different varieties. In one variety, one heteromorphic pair of chromosomes has been noted.

Detailed studies from diakinesis to tetrad formation in P. M. Cs., were made. In 'Boah Marich' (6) $WB/C_{34}$, 'Boah Sitki' (17) $WB/C_{54}$ and 'Soli (3) $WB/C_{25}$, certain abnormalities, such as, asynaptic condition, lagging, dicentric bridges and interstitial chiasma, etc., were observed.

C.6.2.b. *Hybridization experiments*. Varieties having marked morphological dissimilarities were selected for hybridization work for studying their inheritance. Crossing among the varieties, viz., 'Local Malda' (1) $WB/C_{20}$, 'Black Chilli' (90) $WB/C_{64}$, 'Round' (13) $WB/C_{32}$, Var: 'Yellow' (12) $WB/C_{31}$ etc., are in progress.

C.6.3. *Cytotaxonomic studies in some genera of Malvaceae*

During the year under report, investigations on the somatic chromosomes of the following species and varieties of *Gossypium*, *Sida* and *Abutilon* belonging to the family *Malvaceae*, have been carried out.

A. Genus—*Gossypium*
   (1) *Gossypium hirsutum* L. var *Andrews*

B. Genus—*Sida*
   (1) *Sida rhombifolia* L.
   (2) *Sida rhomboidae* L.
   (3) *Sida acuta* L.
   (4) *Sida veronicaefolia* Lam.

C. Genus *Abutilon*
   (1) *Abutilon indicum* L.



A. (1) *Gossypium hirsutum* var *Andrews*. This species has a 2n complement of 52 chromosomes having 3 pairs of satellited chromosomes. The nucleolar study of the variety with Feulgen—Fast green technique reveals that there are six small nucleoli at the early prophase, indicating that all the satellited chromosomes are nucleolar in nature.

Abnormalities like laggards and bridges at the anaphase stage were frequent in this variety.

B. (1) *Sida rhombifolia*. The 2n chromosome complement of this species is 14 of which one pair is satellited. The study of nucleolus at the prophase stage shows that there are two small nucleoli, suggesting that the 2 satellited chromosomes are nucleolar in nature.

Cytological abnormalities like laggards, bridges, extrusions and polyploid cells with somatic pairing, were observed in this species.

(2) *Sida rhomboidae*. 2n chromosome number of this species is 14. The somatic complement consists of one pair of satellited chromosomes. Two nucleoli have been found, using Feulgen-Fast green technique, showing that the two satellited chromosomes are nucleolar.

No cytological abnormalities have so far been recorded in this species.

(3) *Sida acuta*. This species has 2n complement of 28 chromosomes, of which 2 pairs are satellited. The number of nucleoli found is four in the prophase stage, indicating that the satellited chromosomes are nucleolar in nature.

No cytological abnormalities have been recorded in this species.

(4) *Sida veronicaefolia*. The 2n chromosome number of the species is 34 and the somatic complement consists of 2 pairs of satellited chromosomes. The nucleolar study in the early prophase stage with Feulgen-Fast green technique reveals that there are four small nucleoli, suggesting that all the satellited chromosomes are nucleolar in nature.

Chromosome abnormalities like bridges and laggards have been observed in this species.

C. (1) *Abutilon indicum*. In this species, the 2n chromosome number is 42, of which 3 pairs are satellited. The nucleolar study using Feulgen-Fast green technique shows that there are six nucleoli at the early prophase, indicating that all the three pairs are nucleolar in nature.

Excepting for the rare occurrence of bridges in the anaphase stage no other abnormalities have been recorded in this species.

## C.7. Plant anatomy

C.7.1. *Abnormal ascidium and foliar teratology in Ageratum conyzoides* L.

*A. conyzoides* bears abnormally stalked ascidium (cup) composed of two opposite leaves of the terminal node, united laterally along the margins of both the petioles and blades. Variations occur in the degree of fusion. The ascidial stalk is nearly equal in length to the petiole of the normal leaf at the same height of the plant. The basal region of the cupstalk is generally conical and engulfs the terminal bud, which rarely grows up into a leafy shoot, if a slit is made or damaged otherwise at the base of the ascidial stalk.

Sometimes, two leaves of the same node are united along their two adjacent margins to form a "double leaf". Variations in the degree of cohesion also occur here. An ascidium is produced if a double leaf cohere by both margins. Double leaf has six leaftrace vascular bundles, against three in the normal leaf. Detailed anatomical studies have been completed. It is concluded from morphological and anatomical evidences that the ascidium is a product of fusion of the terminal pair of leaves, by both the margins.

## C.8. Weed Control

C.8.1. *Mechanisms of herbicidal action and selectivity of auxin herbicides*

With a view to explain the phenomena of herbicidal action and selectivity of auxin herbicides from the anatomical point of view, a detailed study was started last year on the structural derangements brought about in *Croton bonplandianum* Baill, *Ludwigia parviflora* Roxb, *Imperata arundinacea* Cyrill and *Cyperus rotundus* L, by various selective herbicides like Cornox—D, Cornox—M and Weedone 2, 4, 5-T. A major contributing factor for the death of plants was shown to be the destruction of phloem and the difference in the susceptibility between monocot and dicot plants was partly explained on the basis of the distribution of the phloem tissue. In addition, the following observations were made during the year under report.

Blocking the lumina and distinct suppression of development of the phloem have also contributed to the death of the plant. The degree of protection of the phloem tissue also seems to have played a prominent role, for the difference in the susceptibility of dicot and monocot plants.

Formation of a gummy substance, plugging the lumina of the conducting xylem elements of plants treated with herbicides in lethal concentrations and its role in the death of plants having perennial under-ground stems have been shown. Plugging of xylem elements has been suggested as indicative of mass movement of herbicides into water conducting channels.

Root tip modifications, such as, its enlargement, resulting from herbicide treatment, have been histologically analysed. Irregularity and frequency of cell division together with the direction of cell elongation, are suggested as histological factors for the inhibition of the growth of roots and root primordia. Cessation of growth of roots followed by their decay have been shown to contribute to the death of the plant.

From the study of anatomical responses of plants to the herbicides applied, the differences in the susceptibility of plants have also been attributed to the relative abundance of sensitive tissues, the degree of absorption and the extent of transport.

Certain morphological factors, such as, the nature of protection of buds, wettability and orientation of leaves, have also been found to be responsible for the wide range of susceptibility of the plants investigated.

It has been found that all the chemicals used induce adventitious root formation from the stem, leaf, etc. and the rooting response to herbicide concentration has been shown to be a quantitative one. The necessity of a minimum concentration of each herbicide for adventitious root formation has been observed.



An increase in the number of vascular strands in the auxin induced roots of *Croton* has been observed. The increase in the number of strands has been interpreted in terms of the dimensions of the procambial tissues, where the formation of vascular pattern takes place.

A careful study of the persistence of herbicide toxicity was made on *Croton* basing on the number of malformed leaves developed after spray treatment with equal quantities of the various chemicals. It was found that Weedone 2, 4, 5-T persisted in the plant for a considerably longer period, Cornox—D and Cornox—M showing almost equal degree of persistence. The degree of persistence of a herbicide in a plant seems to be indicative of its herbicidal property.

Among the diversity of structural changes due to herbicide treatments, a similarity of modification among dicot and monocot plants has been observed. In both classes of plants, the stage of ontogeny of a tissue at the time of treatment determines the character of modifications. Development of root primordia from proliferating tissues and suppression of phloem development are some of the other common responses.

## Departmental Seminars

27 Departmental Seminars were held during the year, in which the research workers of this department discussed the results of their investigations.

## Research publications

| | |
|---|---|
| (1) Papers published .. .. .. | 10 |
| (2) Papers accepted for publication .. .. | 1 |
| (3) Papers communicated to the 46th Session of the Indian Science Congress .. .. .. | 7 |

# MICROBIOLOGY

## D.1. Production of antibiotic substances by microorganisms

D. 1.1. *Production of Antibiotic from Streptomyces sp.* $A_{14}$ (475)

A comprehensive screening programme of freshly isolated cultures of *Actinomycetes* from soils of various parts of India was undertaken. The actinomycetes cultures isolated were tested by the agar-streak method to study their antibiotic producing capacity against bacteria and fungi.

Of the isolated active antibiotic-producing strains of *Streptomyces*, $A_{14}$ (475) was found to exhibit a remarkable antibiotic-producing capacity against gram positive and gram negative bacteria and fungi. This strain showed best growth at temperature 28–30°C and at pH 7.4. Altogether 15 different fermentation media were used to select suitable ones for production of the active material.

Antibiotic spectra of the strain $A_{14}$ (475) was noted from the 3rd day to the 11th day in different fermentation media (*p*H 7.4) and the highest antibiotic yeild was noted on the 7th and 8th day of the incubation at 28°C. The media tried here include those that were previously worked out in this laboratory together with some new ones suggested by different workers in this line. The accompanying table shows a comparison of some of the suitable media against test organisms.

TABLE 1

ANTIBIOTIC SPECTRA OF $A_{14}$ (475) ON THE 8TH DAY OF INCUBATION IN VARIOUS FERMENTATION MEDIA, AGAINST DIFFERENT TEST ORGANISMS

| Test organisms | Media* | | | | | | |
|---|---|---|---|---|---|---|---|
| | A13 | A7 | A16 | A5a | A20a | M12 | Ac. St. |
| | Zone of inhibition in mm. | | | | | | |
| 1. S. aureus | 20 | 20 | 19 | 19 | 17 | 16 | 16 |
| 2. B. subtilis | 21 | 20 | 20 | 19 | 18 | 17 | 18 |
| 3. B. anthracis | 15 | 16 | 14 | 15 | 12 | 16 | 15 |
| 4. E. coli | 20 | 20 | 19 | 19 | 17 | 17 | 18 |
| 5. Eb. typhosa | 14 | 12 | 10 | 12 | + | 12 | 10 |
| 6. Myc. tuberculosis | 22 | 20 | 20 | 18 | 12 | 22 | 16 |
| 7. Curvularia sp. | 37 | 37 | 32 | 37 | 35 | 32 | 28 |
| 8. Alternaria solani | 33 | 32 | 29 | 30 | 30 | 27 | 21 |
| 9. Fusarium vasinfectum | 17 | 18 | 18 | 18 | 16 | 17 | 16 |
| 10. A. niger | 18 | 15 | 16 | 18 | 13 | 17 | 15 |
| 11. H. sativum | 22 | 17 | 20 | 20 | 14 | 18 | 18 |

*Warren, H. B. et al ; Anti & Chemo., **5**, 6, 1955.

In view of the fact that many species of *Streptomyces* are known to grow well in synthetic media with a hexose as carbon source and single amino acids as the nitrogen source, two basal media containing different carbon and nitrogen sources in each case were utilised. The amount of carbon and nitrogen added are equal in proportion to those present in the basal media. It has been observed that the organism can utilise a wide range of hexose as carbon source, but utilisation of nitrogen sources is relatively restricted. Of the 24 amino acids added to the media as nitrogen source, antibiotic production is limited to the utilisation of only four amino acids, aspragine, glycine, histidine and arginine. However, the growth of the organism takes place in the presence of all the amino acids used.

It was found that the growth and antibiotic production of the *Streptomyces* sp. was better with amino acids in a number of fermentation media. Of these the medium $M_{12}$ was selected on account of its simple composition which ensures consequently easier purification of the antibiotic.

Direct solvent extraction of the antibiotic has been attempted, but on account of its extreme solubility in water, it could not be extracted by any water immiscible low boiling organic solvent. However, the active substance was found to be adsorbed completely with 'Norit' charcoal at neutral *p*H and was later on eluted with acidic methanol at *p*H



2.0 subsequently to bring it to the neutral range, the extract was passed through an ion-exchange resin 'Amberlite' IR-4B. Large amount of impurities that is being eluted with the active principle, however, presents a difficult problem of its being isolated in a pure form. It was found that some of the impurities could be removed from the elute by passing it through a basic alumina column. The eluate after vacuum evaporation was found to retain its antibiotic activity.

The method of extraction was further improved by using Amberlite IRC-50 in the H-form. Filtered broth was passed through the resin column and the active substance was found to be completely adsorbed. It was then eluted with N/50 HCl and collected in 20 cc. Vol. The active substance was found to be present in the 6th to 10th tubes admixed with deep brown coloured impurities. Aqueous solution on evaporation yields dirty yellow-colour substance. It is partially purified with acid-washed alumina.

A white coloured active substance probably in the form of sulphate can be obtained. Eluted portions obtained from resin IRC-50 is acidified with (N) $H_2SO_4$ to *p*H—2.0 and shaken with equal volume of ethyl alcohol, a white ppt. is thus obtained. This is purified by redissolving the mass in water and precipitated again with ethanol. A white amorphous substance in the form of sulphate is finally obtained which is antibiotically active.

D.1.2. *Studies on the production of a broad-spectrum antibiotic from a species of Pseudomonas*

An isolate of *Pseudomonas* sp. was obtained in this laboratory from soil plates. The bacteria showed marked antifungal and antibacterial activities when tested against *Curvularia* spp., *F. vasinfectum*, *A. niger*, *S. aureus*, *Eb. typhosa*, *E. coli* and *V. cholerae*. In nutrient agar slants, it produced blue-green pigment. The optimum growth of the cells and antibiotic production occurred at 25°C and at *p*H 7.0-7.5 in stationary flasks using nutrient broth as the medium. Harvesting period was 3-5 days. In shake flasks, growth of the cells occurred, but no production of pigment was noted even at 25°C. An interesting phenomenon was noted that at 37°C, there was practically very little production of the antibiotic substance, although cells grew abundantly. In Czapek-Dox medium, the bacteria multiplied easily, without any antibiotic production.

In Czapek-Dox medium nitrate was replaced by peptone, and glucose concentration was kept below 0.1%. Under this condition the bacteria could synthesize the antibiotic substance.

It was also observed that the bacteria possessed reducing enzyme system by which they reduced the pigment in the broth culture during antibiotic production. Cell suspension of the culture even reduced methylene blue. It could be mentioned here that antibiotic production at different temperatures had some relation with the reducing activity of the cells. The results are given below :

| Temperature | Zone of inhibition (A) | Reductase activity (B) |
|---|---|---|
| 25°C | 20 mm | 100% reduction |
| 28 ,, | 15 ,, | 75 ,, ,, |
| 30 ,, | 13 ,, | 60 ,, ,, |
| 37 ,, | 11 ,, | 10 ,, ,, |

(A) Zone of inhibition of the crude broth was noted against *S. aureus* by the usual cup-assay method.

(B) Reducing activity of the cells was noted in Thunberg tubes using methylene blue (50 γ/ml) as the substrate and keeping at 25°C for 24 hours.

The bacterial isolate is a gram negative, straight rod.

An attempt is now being made to characterize the active principle and its applications in bacterial and fungal infections.

## D.2. Ultraviolet-induced mutation in micro-organisms

### D.2.1. *Heterokaryosis in Aspergillus niger*

Heterokaryons are formed by the fusion of two different kinds of hyphae and contain two or more different kinds of nuclei. When the heterokaryotic condition of the mycelium remains constant on a selected medium, it is called a balanced heterokaryon. Essential

TABLE 1

*UV* MUTANTS OF *A. niger* USED IN THE COURSE OF STUDY AND THE SOURCE OF THE STRAINS

| Parent | Single mutants + | Double mutants ++ | | Triple mutants | | |
|---|---|---|---|---|---|---|
| $V_{35}$ | 21 a | 21 a | | *2 a | me | |
| | | *2 a | me | 1 a | me | ad |
| | | 3 a | arg | 8 a | me | pr |
| | | 4 a | lys | 13 a | me | thi |
| | | 10 a | ad | 22 a | me | arg |
| | | | | 31 a | me | arg |
| | | | | 34 a | me | leu |
| | | | | 45 a | me | bio |
| | | | | 46 a | me | cyt |
| | | | | 48 a | me | paba |
| | | | | 49 a | me | lys |
| | | 51 g | | | | |
| | | 6 g | arg | | | |
| | | 10 g | thi | | | |
| | | 11 g | leu | | | |
| | | 12 g | iso | | | |
| | | 15 g | paba | | | |
| | | 22 g | me | | | |
| | | 28 g | ad | | | |
| | | **29 g | py | | | |
| | | 34 g | hist | | | |
| | | 35 g | ino | | | |
| | | | | **29 g | py | |
| | | | | 8 g | py | arg |
| | | | | 42 g | py | inos |
| | | | | 44 g | py | ad |
| | | | | 53 g | py | paba |
| | | | | 54 g | py | lys |

+ Detection of colour mutants was by macrospical examination and nutritional mutants by total isolation.

++ The notation shows the isolation number on the left and symbols denote the following on the right.

a = avallaneous colour
ad = adenine
arg = arginine
bi = biotin
cyt = cytosine
g = serpentine green colour
hist = histidine
ino = inositol
iso = isoleucine
leu = leucine
lys = lysine
me = methionine
paba = para-amino-benzoic acid
pr = proline
py = pyridoxine
thi = thiamine



feature of a heterokaryon is that mechanism of segregation and recombination of hereditary particles could be studied without recourse to sexual reproduction. The technique is adapted in the study of genetics of *A. niger* which are without any sexual reproduction.

A dark-coloured strain, of *A. niger* ($V_{35}$) was selected from which successive sub-strains were obtained by Ultraviolet radiation (Table 1).

The heterokaryons were formed by mixing the conidia of two biochemical mutant strains requiring different growth factors, and inoculating heavily on agar containing limiting concentrations of the required growth factors, so that there was just a little growth which helped in coalescing the hyphae. The plates were incubated at 28°C for about 8 days and heterokaryotic mycelia formed were found to emerge out at several points. Table shows the balanced heterokaryons between different strains.

TABLE 2

| Number | Balanced heterokaryons | | | | | |
|---|---|---|---|---|---|---|
| 1 | 3 a | arg | + | 10 g. | thi | |
| 2 | 2 a | me | + | 6 g. | arg | |
| 3 | 3 a | arg | + | 11 g. | leu | |
| 4 | 4 a | lys | + | 12 g. | iso | |
| 5 | 10 a | ad | + | 15 g. | paba | |
| 6 | 3 a | arg | + | 22 g. | me | |
| 7 | 2 a | me | + | 28 g. | ad | |
| 8 | 3 a | arg | + | 29 g. | py | |
| 9 | 4 a | lys | + | 34 g. | hist | |
| 10 | 10 a | ad | + | 35 g. | ino | |
| 11 | 4 a | lys | + | 28 g. | ad | |
| 12 | 2 a | me | + | 29 g. | py | |
| 13 | 48 a | me | paba | + | 8 g. | py arg. |
| 14 | 46 a | me | cyt | + | 8 g. | py arg |
| 15 | 45 a | me | bio | + | 44 g. | py ad |
| 16 | 22 a | me | arg | + | 42 g. | py ino |
| 17 | 13 a | me | thi | + | 54 g. | py lys |
| 18 | 49 a | me | lys | + | 44 g. | py ad |
| 19 | 34 a | me | leu | + | 54 g. | py lys |
| 20 | 31 a | me | arg | + | 53 g. | py paba |
| 21 | 1 a | me | ad | + | 8 g. | py arg |
| 22 | 8 a | me | pr | + | 44 g. | py ad |

The colour of the sporing surface of the heterokaryon was a combination of serpentine green, avallaneous and black. The heterokaryons could grow on minimal medium and were maintained by hyphal tip transfers or growing mycelial fragments, but when transfers were made by conidia there was segregation into the two parent types.

Several million conidia of the heterokaryon *4a lys+12g iso* were plated on minimal medium. Only a few colonies arose, quite a good number of them were new heterokaryons presumably developing from hyphal fragments plated together with the conidia, others were with dark sporing surface like the original parent (wild type). These were purified and inoculated individually in the centre of a Petri dish containing (CM) complete medium. Some of them (table 3) gave rise to sectors of the two parental colours as well as growth deficiency. This showed that they were heterozygous diploids.

TABLE 3

THE HETEROZYGOUS DIPLOID CONIDIA ARISING FROM THE PLATING OF THE HETEROKARYON (4a LYS + 12g iso)

| Number of plated conidia | Growth of total colonies on M M | Heterokaryons (isolated) | Dark colonies (Probable Heterozygous diploid) |
|---|---|---|---|
| 5600,000 | 79 | 36 | 43 |

Out of the 43 probable heterozygous diploid colonies 39 showed colour segregants. Of them one strain $HD_4$ (4B) was selected for further study. Conidia of the above isolate were plated out in complete medium so that each plate had about 20-25 colonies and incubated at 28°C. The colonies developing were then transferred into minimal medium by the total transfer method and selected for the study of colour segregation and nutritional requirements (Table 4a and b).

TABLE 4(A)

FIRST ORDER SEGREGATION FROM THE DIPLOID $\frac{4\text{ A LYS}}{12\text{ G ISO}}$ ($HD_4$(4B)

| Total no colonies transferred on MM | Dark | Prototrophs Avallaneous | Green | Nutritional Deficient |
|---|---|---|---|---|
| 1022 | 992 | Nil | Nil | 28 |

TABL 4(B)

CLASSIFICATION OF NUTRITIONALLY DEFICIENT SEGREGANTS (ALL DARK)

| Total No. | Requiring Lysine | Requiring Isoleucine | Requiring Lysine and Isoleucine both |
|---|---|---|---|
| 28 | 11 | 15 | 2 |

It is clear from table 4 (a and b) that segregation occurred for nutritional markers. Missing types of segregants were for colour markers. Work is in progress with the second order segregants for finding out the missing segregants and analysis of several other heterokaryons is also in progress.

D.2.2. *Study of induced mutation in Streptomyces* $A_{21}$ (460), *a pink coloured strain*

Out of 184 strains of *Streptomyces* isolated from different soil samples, the strain $Ac_{21}$(460) was selected for detailed studies, for its pink colour, morphological variability and production of white secondary mycelium. The sporalution was better in straw infusion medium than that in the CD medium while in the latter the pink colour was more prominent. The pigment did not diffuse into any of the media.



Antibiotic property of the strain was noted by the cross-streak method but not so in Agar Cup method of assay. The strain was active against *Curvularia* sp., *Fusarium* sp. and *Alternaria* sp. in the beginning. Later on, however, it lost its antibiotic activity against *Fusarium*. In straw infusion broth growth with good antibiotic production was observed only when ammonium sulphate was added to the medium. The following media were tried to determine good growth and antibiotic yield as well. However, not much improvement in the production of antibiotic was found by shake culture method. The results given below were obtained by the stationary flask culture method after an incubation period of 7 days at 28°C.

| | Medium | Growth | Curvularia sp. | Alternaria sp. |
|---|---|---|---|---|
| | | | Zone of inhibition in mm | |
| (1) | Straw infusion | poor growth | 16 | 14 |
| (2) | C-D liquid + casein hydrolysate + yeast extract + vit. soln. | medium submerged growth | 18 | 27 |
| (3) | C-D liquid + casein hydrolysate | growth slightly less than medium (2) | 21 | 12 |
| (4) | C-D liquid + yeast extract | growth same as medium (3) | — | — |
| (5) | C-D liquid + vit. soln. | no growth | — | — |
| (6) | C-D liquid + malt ext + liquor | medium submerged growth | 22 | 18 |
| (7) | C-D liquid + .2% malt ext | no growth | — | — |
| (8) | C-D liquid + corn steep liquor | medium growth | 17 | 15 |
| (9) | C-D liquid + methionine | no growth | — | — |
| (10) | Nutrient Broth | poor growth | 17 | 23 |
| (11) | Ammonium sulphate medium (0.264%) (Gotleib and Pridham) | medium good growth | 22 | 24 |
| (12) | Ammonium Nitrate medium | medium growth | 19 | 16 |
| (13) | Ammonium sulphate medium (0.264%) + straw infusion (1:1) | medium good growth with aerial sporalation | 26 | 23 |
| (14) | Peptone + ammonium sulphate | poor growth | 14 | 14 |
| (15) | Peptone + ammonium sulphate + straw infusion | medium growth | 16 | 13 |

*Effect of Ultraviolet radiation.* UV radiation at 2537Å was used. The intensity of radiation was measured with a dosimeter and all experiments were done at a dose level 100 ergs/mm²/sec. for 20, 40, 60 and 80 seconds. The irradiated spore suspension was plated out in complete medium which had the following composition:

Casein hydrolysate—1.5 gm; yeast extract—1.0; peptone—1.5; Glucose—20.0; Hydrolysed nucleic acid—3.0 cc; Vitamin mixture soln.—1.0 cc; Distilled water 1000 cc; pH—6.8.

In the above complete medium most of the nutritional factors are present and the mutants are, therefore, expected to grow in it without showing any sign of deficiency. The plates were allowed to incubate at 28°C for 6 days. It was observed that the percentage survival of the colonies were:—9.7%, 1.3%, 0.21% and 0.03% at 20, 40, 60 and 80 secs., respectively.

(i) *Isolation of biochemical and morphological mutants.* The isolation of mutants were done satisfactorily by the Total isolation method of Fries. The survivors developing on complete medium after UV radiation were picked up, the inoculum was transferred into minimal agar plates and allowed to incubate at 28°C for 6 days.

Those having nutritional deficiency due to radiation usually failed to grow in minimal media and therefore all non-growing colonies of probable mutants were picked up and transferred again to complete media for further studies. In this experiment it was found that the percentage of biochemical mutants was 0.92%, 0.0%, 0.0% and 1.86% at 20, 40 and 80 secs. respectively.

The characterisation of these biochemical mutants is now under experiment.

(ii) *Isolation of morphological mutants.* By morphological mutants we mean those colonies that were different in size, shape, colour, form, texture, etc., from the original parent strain. Such changed mutants were isolated from the total isolation method plates and allowed to grow in CD slants. The percentage of morphological mutants was 11.1%, 10.7%, 6.9% and 6.5% at 20, 40, 60 and 80 secs. respectively. The study of the morphological mutants are now in progress.

(iii) *Effect of Radiation on antibiotic yield.* For this purpose 25 cc CD agar medium was plated out in each Petri dish and the irradiated colonies from the complete medium plates were transferred into it in a triangular way with 3 in each plate. About 75—80 colonies were isolated in this way from different lots of irradiation, and the plates were incubated at 28°C for 6 days.

Next 5 cc of CD agar medium inoculated with 0.2 cc of spore suspension of *Curvularia* was allowed to cover the surface of each plate and incubated again at room temperature for 48 hours. The zone of inhibition was noted in mm. It was found that in majority of the cases, the antibiotic property was increased as shown by the zone of inhibition from 35 to 43 mm., while in others the antibiotic property was decreased even down to 12mm.

D.2.3. *Study of mutants of citricacid-producing Aspergillus niger.*

Twenty five isolates of *Aspergillus niger* van Tiegham were made from soil and decomposed food materials and they were purified by making monosporus cultures.



In order to study the biochemical activities (yield of citric acid), isolates were grown in a special synthetic medium containing ammonium nitrate, sucrose and minerals, the pH being adjusted to 4.0. Conical flasks of 250 ml capacity each containg 50 c.c. of medium were inoculated with 0.5 c.c. of the spore suspensions and filtrates were titrated with standard N/10 NaOH soln. The cultures with 3 replicates of each were incubated at 28°—30°C and results were recorded at intervals of 48 hours upto the 8th day of incubation. The pH and total acidity (in terms of N/10 NaOH) were determined every day.

Preliminary observations showed the presence of 6 strains which are natural variants; each having higher acid titre as indicated by the values obtained. The selected strains, designated $A_1$, $A_2$, $A_3$, $A_4$, $A_5$, and $A_6$ were next grown in liquid culture, according to the method described above. Results were recorded every 24 hours upto the 7th day of incubation and are given in Table I.

TABLE 1

RESULTS SHOWING THE VALUES OF TITRATION OF THE FILTRATES OF 6 STRAINS OF A. NIGER OBTAINED IN RESPECT OF .9615 $\frac{N}{10}$ NaOH

| Days of incubation | $A_1$ | | $A_2$ | | $A_3$ | | $A_4$ | | $A_5$ | | $A_6$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *TV | **pH | T.V. | pH | T.V. | pH | T.V. | pH | T.V. | pH | T.V. | pH |
| 1 | 0.58 | 3.7 | 0.55 | 3.7 | 0.53 | 3.7 | 0.53 | 3.9 | 0.50 | 3.6 | 0.50 | 3.8 |
| 2 | 0.78 | 3.0 | 0.63 | 3.2 | 0.83 | 2.7 | 1.03 | 2.7 | 1.0 | 1.0 | 2.8 | 0.90 |
| 3 | 2.18 | 1.9 | 1.55 | 1.9 | 2.7 | 1.9 | 3.6 | 1.9 | 1.74 | 1.9 | 1.85 | 1.9 |
| 4 | 2.25 | 1.8 | 2.35 | 1.8 | 4.3 | 1.7 | 3.15 | 1.6 | 2.9 | 1.7 | 2.3 | 1.7 |
| 5 | 9.73 | 1.9 | 3.18 | 1.8 | 2.16 | 2.4 | 5.98 | 1.9 | 13.3 | 1.8 | 9.9 | 2.0 |
| 6 | 19.05 | 2.0 | 5.78 | 1.8 | 15.38 | 2.0 | 5.03 | 1.7 | 4.78 | 1.8 | 14.08 | 1.9 |
| 7 | 10.2 | 1.9 | 9.5 | 1.8 | 13.4 | 2.1 | 8.3 | 1.7 | 3.48 | 2.1 | 8.48 | 1.8 |

* Titre Value: N/10 NaOH solution required to neutralise total acidity.
** pH was determined colorimetrically.

*Effect of Ultraviolet radiation on strain $A_1$.* The strain was grown in C–D slants for 6 days. Spore suspension was then prepared in sterile tap water, thoroughly shaken and passed through sterile cotton wool so as to remove spore clumps. The strength of the suspension was finally made up to $12 \times 10^6$.

Five cc quantities of the suspension were taken in 9 cm petridishes and exposed to Ultraviolet rays of 2537Å from Phillip's Germicidal lamp. Radiation was given from a height of 26 cm. The dose administered was 100 ergs/mm²/sec. During irradiation, the lid of the petridish was taken off and the suspension was stirred continuously with a sterile glass rod. The duration of irradiation was 4, 8, 12, 16 and 20 minutes. Five replicates were set up in each case.

One cc of the irradiated spore suspension was then diluted with sterile water, plated out in CD medium and incubated at 28°C for 2–4 days. The number of colonies appearing were counted at the end of the experiment in each case. The experiment was repeated twice and from the number of colonies counted, average survival percentages of the spores were recorded (Table II).

TABLE II

PERCENTAGE OF SURVIVAL OF THE SPORES OF A. NIGER ON EXPOSURE TO ULTRAVIOLET RAYS

| Time of exposure (minutes) | Percentage of survival |
|---|---|
| 0 | 100 |
| 4 | 3.15 |
| 8 | 0.84 |
| 12 | 0.15 |
| 16 | 0.08 |
| 20 | 0.02 |

The table shows that the percentage of survival of the spores decreased with an increase in the time of exposure.

Morphological studies of the colonies, however, revealed the presence of the following types of mutants.

*Control*

Blackish brown, floccose colony with abundant long, uncrowded conidiophores bearing large conidial heads.

*Mutant*

Type 1—Pale-brown floccose colony with short conidiophores bearing small conidial heads, imparting dusty appearances to the colony.

Type 2—Chocolate-coloured compact colony with long crowded conidiophores.

Type 3—Puff-coloured colony with abundant sporulation.

Type 4—Dark brown floccose colony with numerous sclerotia.

Type 5—White cottony mycelia with scanty sporulation.

The selected isolates were transferred to CD agar and studies on their biochemical activities are in progress.

### D.3. Biochemical Genetics of Neurospora crassa and other organisms

Using the *Neurospora* back-mutation test as the model, attempts are being made to ascertain the mutagenic action of certain chemicals separately as also in combination with other chemicals having direct influence on the antimutagenic enzymes, such as, the catalase, in the biochemical system. Different strains of *Neurospora crassa* and organisms other than



Neurospora belonging to the class Ascomycetes have been collected for such studies; pecies which are being tried are:

1. Neurospora crassa—wild and mutant types
2. Ascobolus magnificus—wild type
3. " equinus "
4. " leveillei "
5. " virudulus "
6. Trichosphaeria pilosa "
7. Peziza nigrella
8. Chaetomium globosum
9. " indicum
10. " brasiliensis
11. Penicillium vermiculatum
12. " wortmani
13. Aspergillus chevalini
14. " nidulans

Monosporous cultures of several strains have been isolated in order to study their sexual behaviour and to find out through crossing experiments whether they are homothallic or heterothallic. Excepting in *Neurospora crassa* and *Aspergillus nidulans* no information with regard to this behaviour has yet been obtained in any of the types collected. Heterothallism in *Neurospora crassa* has been a great advantage for a thorough genetical study in this particular system. Sexual phases in the fungi, however, depend on nutritional factors and studies on this aspect have also been taken up.

### D.4. Isolation of bacteriophage from soil and preliminary action on some strains of root nodule bacteria

*Isolation of nodule and its culture in the media*

Nodules were collected from roots of *Vigna* and *Sesbania*. Isolation of *Rhizobium* sp. was done by sterilization of the nodule with alcohol 70% and mercuric chloride solution (0.01%) and subsequent washing with sterile distilled water (at least 10 times). Media selected for isolation of *Rhizobium* were yeast ext. agar medium.

*Purification of the bacterial strains*

Purification of the strains were done by serial dilution method. The strains were preserved in YWA. medium (nitrogen free). Comparative morphology of the strain grown on YWA. (with yeast extract) and the same with root extract instead of yeast extract. It was found that *Sesbania* strain grew well in root extract medium while *Vigna* strain failed to do so in such medium.

*Inoculation of Vigna and Sesbania plants in sand culture tubes with the corresponding strain and cross-inoculation study*

For this experiment seeds were sterilized with 90% alcohol and 0.01% mercuric chloride solution and subsequent washing with sterile distilled water (at least 20 times).

The seeds were then introduced into sand tubes (half filled with sterilized sand and seedling salt solution). One seed was put in each when they germinated and about 2–3 days old. Inoculation was done by making suspension of the young cultures of the *Rhizobium* strains. Visible nodules appeared in tube cultures on the 7th day after inoculation. After one month the number and size of the nodule was noted.

Mean number of nodule per plant — 3.4
Mean size of nodule per plant — 1.5 mm. ×2.0 mm.

In the control and cross-inoculated plants no nodule appeared.

*Isolation of Bacteriophage*

Soil was collected from the Sesbania beds of the Bose Institute garden containing decomposed root nodule. Medium used for soil culture was Laird's medium. Soil was air-dried, seived and the medium was inoculated. At the same time the soil suspension in Laird's medium was inoculated with a 24 hrs. old culture of *Vigna* strain. After 48 hrs. the soil suspension was first filtered successively through filter paper and finally by means of sterilized sintered glass-filter. Jena glass filter No. G5 (Bacteriological grade—porosity 1.1—1.5$\mu$) was used after sterilising its assemly in the autoclave. The bacterial cells are retained in the filter and the phage particles being smaller in size pass out into the filtrate. Its sterility test was performed by inoculating this filtrate in different culture media.

*Preliminary screening test to detect the action of phage materials—24 hrs. culture of Vigna and Sesbania strains*

Medium was cross-streaked with test organisms and phage. It was found that along the line of phage the whole bacterial colony was eaten up and became clear. So the nett result was that the meeting line of phage and bacteria was clear.

*Quantitative estimation of phage titre*

24–48 hrs. broth cultures of *Vigna* and *Sesbania* strains were inoculated with 1 cc. of the phage filtrate and incubated at 28°C for 24 hrs. Serial dilution was made and plated in YWA. medium. After incubation for 48 hrs. most of the colony were infected with phage. The following table shows the types of phage in four strains.

TABLE

| Strains | Types of phage | Size of the plaque |
|---|---|---|
| Vigna | Colony hyaline and flat, centre infected | 0.5 mm—6.0 mm |
| Sesbania | Whole colony infected and cleared | 0.5 mm×0.5 mm—3.0 mm×3.0 mm |
| Cicer | Centre infected outer ring-with like region | 0.5 mm×0.5 mm—3.0 mm×3.0 mm |
| Phaselous | Infection at the centre and also at the sides | 0.5 mm×0.5 mm—5.0 mm×5.0 mm |



*Lysogenic Host-phage reaction*

Lysogenicity test was performed with purified and aged culture of the strains. The strains were inoculated into Y.W.A. broth, incubated for 72 hrs. centrifuged at 3500 r.p.m. for 20 mins. and then filtered through sterilized sintered glass filter. These were tested by cross-streak method. It was found that all the strains are *Lysogenic* to some extent.

## D.5. Ecology of Soil fungi : Fungi on root regions of some crop plants

The association of soil microorganisms with the roots of plants has long been established but the conditions influencing this relationship are little understood. As a result, methods of crop protection undertaken do not always pay. It is proposed, in the present study to investigate the nature of soil fungi and amino acids present in the root regions and to determine how do they vary under different conditions of cultivation in the field.

The progress of the work may be considered under the following heads:—

1) Locality, nature and health of crop
2) Record of soil temperature
3) Record of soil moisture
4) Soil analysis (chemical & mechanical)
5) Free amino acids in soils and roots
6) Fungi isolated from the soils and the roots.

1. *Locality, nature and health of crop*

Four cultivated plots have been selected at Badkullah, a village about 60 miles away to the north of Calcutta for the study of association of fungal flora with the roots of crop plants. Gram (*Cicer arietinum*) was sown on 7-11-58 in all the four plots and monthly visits revaled the following facts. At first the results were recorded on 13-12-58 and no abnormality of crop health was noted. Subsequently on 18-1-59 it was found that plants in almost all the plots were showing disease symptoms. The plants were grouped into the following classes according to the severity of infection.

*Group I* (a) Plants already dead.
(b) Plants very much stunted in growth and almost dying.
(c) Plants stunted with lower leaves drying up.

*Group II* (d) Plants quite normal externally but showing a few discoloured patches on roots.
(e) Plants showing no such abnormality.

On the 13th, 14th and 15th February 1959, visits were paid to some gram growing localities of Badkullah, Dharmadah and Jalalkhali under the P.S. Haskhali (Nadia). The disease was found to be of common occurrence and was wide-spread, affecting 12 to 52.3% of plants in different plots.

*Symptoms and descriptions.* The yellowing of leaflets followed by their drying up is the general symptom beginning from the tip of lowermost leaves, proceeding upwards until the whole plant is dead. The dried leaves remain attached to the rachis indefinitely but occasionally shedding of top leaflets has been noted. The roots of diseased plants are dark-coloured and shrunken.

2. *Record of soil temperature*

The soil temperature was recorded weekly at 6 a.m., 10 a.m., 2 p.m. and 6 p.m. The maximum and the minimum ranges noted are given below:—

TABLE 1

SOIL TEMPERATURE° F

| 1959 | Maximum | Minimum |
|---|---|---|
| January | 88 | 57 |
| February | 88 | 57 |
| March | 102 | 62 |
| April | 111 | 76 |

3. *Record of soil moisture*

Soil samples were collected from 1″–3″ layers in well-dried specimen tubes which were stoppered and sealed with paraffin immediately. The moisture content was determined by drying (105°C) a weighed quantity of soil. The soil moisture varied between 6–18% calculated on dry weight basis or 14.2–42.6% of its moisture holding capacity.

4. *Soil analysis*

Mechanical and chemical analysis of the soils from plots selected revealed the following information.

| | W/W % |
|---|---|
| Coarse sand | 0.68— 1.54 |
| Fine sand | 32.07— 47.84 |
| Silt | 27.75— 42.75 |
| Clay | 13.75— 31.75 |
| Organic carbon | 0.384 —0.423 |
| Total nitrogen | 0.0588—0.00567 |



5. *Free amino acids in root region soils and roots*

TABLE 2

FREE AMINO ACIDS FROM SOIL AND ROOTS

| Names of amino acids | HB-plots | | | KB-plots | | | NG-plots | | |
|---|---|---|---|---|---|---|---|---|---|
| | S(I) | R(II) | S(II) | S(I) | R(II) | S(II) | S(I) | R(II) | S(II) |
| 1. Lysine | — | ± | — | — | ± | — | — | ± | — |
| 2. Cystine | Tr | — | — | Tr | — | — | Tr | — | — |
| 3. Aspartic acid | + | +++ | Tr | + | +++ | Tr | + | +++ | — |
| 4. Glutamic acid | — | ++ | — | — | +++ | — | — | ++ | — |
| 5. Alanine | — | ++ | — | — | +++ | — | — | +++ | — |
| 6. Proline | — | +++ | — | — | + | — | — | + | — |
| 7. Tyrosine | — | ± | — | — | ± | — | — | ± | — |
| 8. Tryptophan | — | ++ | — | — | ++ | — | — | ++ | — |
| 9. Valine | — | ++ | — | — | ++ | — | — | + | — |
| 10. Leucine | — | ++ | — | — | ++ | — | — | + | — |

R=Roots, S=Soil; Tr=Trace; +++=High; ++=Moderate; +=low; ±=doubtful; —=absent; S (I) & S (II), R (I) & R (II) indicates Soils & roots collected on 18.1.59 & 15.3.59 respectively

Roots and soil of root region were placed in 75% alcohol (V/V) kept as such for 24 hrs. with occasional shaking. The clear liquid was then decanted off and the amino acids were identified by Paper Chromatography. Ion-Exchange resin (IR-120) was used for desalting the soil extract.

6. *Fungi isolated from the roots and soils*

Dilution plate and Warcup's soil plate techniques were used for the isolation of fungi. Media used were:—1% malt agar fortified with 50 mg. of penicillin and 30 mg. of streptomycin per litre to prevent bacterial growth.

The following fungal species were isolated and preliminarily identified:

1. *Penicillium* spp.
2. *Aspergillus* spp.
3. *Curvularia* spp.
4. *Fusarium* spp.
5. *Cephalosporium* spp.

6. A fungus probably of *Verticilieae* group is occasionally found in mixed cultures but so far all attempts to isolate in pure culture have been unsuccessful.

Of the above fungi isolated from soil, *Cephalosporium* seems to be associated with soil organic matter or plant roots generally. The above fungi are quite common in all the four plots selected for experiment.

## D.6. Studies on Countercurrent distribution

D.6.1. *Poisson distribution—its scope and limitation in Countercurrent distribution*

It is known that the statistical consideration can be directly applied in the field of the countercurrent distribution from the tacit assumption of Bock [Bock, R. M., *J. Am. Chem. Soc.*, **72**, 4269, (1950)] that the fraction of the original solute distributed in the $r$th tube after $n$ transfers, i.e., $Tn,r$, is the same as the probability $Pn,r$, of a molecule under consideration will reach the $r$th tube from the $o$th tube after $n$ transfers.

From the statistical consideration binomial distribution can be approximated by the Gaussian distribution when $n$ approaches infinity and $p$ approaches $q$, i.e., $k$ approaches unity, where $n,p,q$ and $k$ are conventional terms used in countercurrent distribution technique. But when $p \ll q$ so that $q$ approaches unity ($\because p+q = 1$) the binomial form of distribution can be replaced by another approximate form, Poisson distribution which may be represented as

$$Tn,r = \frac{m^r e^{-m}}{r\,!} \qquad \ldots (1)$$

When $m = np = \frac{nk}{k+1}$ = mean of the distribution band;

and in terms of partition coefficient,

$$Tn,r = \frac{\left(\frac{nk}{k+1}\right)^r e^{-\frac{nk}{k+1}}}{r\,!} \qquad \ldots (2)$$

For the determination of $k$ from the countercurrent distribution band poisson in nature, an expression of $k$ relating to $r_{max}$ may be evaluated in the following way. Assuming Stirling's approximation to the logarithmic form of equation (2), it is differentiated with respect to $r$.

$$\frac{d(ln\,T_{n,r})}{dr} = ln\,\frac{nk}{k+1} - \frac{r+0.5}{r} - ln\ r+1 \qquad \ldots (3)$$

The derivative is set equal to zero, when $r = r_{max}$ and simplifying,

$$\frac{nk}{k+1} = r_{max}\ e\ 0.5/r_{max} \qquad \ldots (4)$$

and hence, $k = \frac{r_{max} e^{0.5/r_{max}}}{n - r_{max} \cdot e^{0.5/r_{max}}}$ ... (5)

Equations (4) and (5) give the value of the mean of the Poisson distribution and the partition coefficient respectively from the peak of the distribution band. $r_{max}$ in equations



(4) and (5) is the tube number where the maximum of the distribution band lies can be shown by differentiating equation (3) with respect to $r$,

$$\frac{d^2 ln\ T_{n,r}}{dr^2} = \frac{0{\cdot}5 - r_{max}}{r^2_{max}} \qquad \ldots (6)$$

It is seen from equation (6) that the value of $\frac{d^2(ln\ Tn,r)}{dr^2}$ would be negative if $r_{max}$ exceeds numerically 0.5. Therefore, it is clear that $r_{max}$ should have its own significance if the peak of the distribution band attains in a tube exceeding numerical value 0.5 (considering hypothetical fractional tubes). However at $r_{max} = 0.5$ there will an inflection. This limiting value of $r_{max}$ and its consequent mean and $k$ values are due to the assumption of Stirling's approximation.

But there is a difficulty in the case of a Poisson distribution that the value of $p$ and that of $K$ being very small, $r_{max}$ usually has values numerically very small and assumption of Stirling's approximation in the logarithmic form of equation (2) cannot give a perfect result. However, equations (4) and (5) are able to give quite satisfactory results when $r_{max}$ is comparatively large. This is shown by the close agreement of the $m$ values given in the Pearson's table (Pearson, *K*:—Tables for statisticians and Biometricians, Part I, 1930) for different Poisson distribution and the corresponding $m$ values calculated from the equation (4) from a knowledge of the corresponding $r_{max}$ values provided in the table.

It is known that in case of Poisson distribution the dispersion $\sigma^2$ approaches its mean $m$ which again approaches unity for $n$ approaching $\infty$. However, it is always numerically less than the dispersion of the Gaussian type of distribution where $p$ approaches $q$. As the dispersion $\sigma^2$ of a distribution band might be considered as a measure of the number of tubes amongst which the solute is distributed, there are two advantages of using Poisson type of distribution by adjusting the $K$ value of the distributed material. Firstly, the material is distributed amongst a less number of tubes with a sharp peak in comparison with that in Gaussian distribution and so the recovery of the distributed material is less troublesome. Secondly, the nature of the distribution band also provides more space for the distribution of impurities having partition coefficients higher than that of the solute of interest and thus they are efficiently separated. But there is a great disadvantage in case of separation of the impurities having partition coefficient lower than that of the solute of interest. The distribution band practically provides no space for their separation. For this reason symmetrical Gaussian type of distribution is generally preferred to the assymetric Poisson distribution, because the former type provides space to the both sides for the separation of both the types of impurities. But, however, in abasence of impurities having partition coefficients lower than that of solute of interest Poisson type of distribution band might be profitably used.

Poisson type of distribution band is occasionally found in case of a distribution of a solute by the salting out effect of the solvent system used.

D.6.2. *A further study of Rubidin, an Antibacterial Antibiotic*

An antibiotic, rubidin, obtained from a Streptomyces sp. $A_{12}$ reported earlier was found to be remarkably active against *Staph. aureus* and to a lesser extent to other test organism like *E. coli*, *E. typhosa* and *V. cholerae*.

The antibiotic, rubidin was isolated by growing the organism in Czapeck Dox medium of *p*H 6.4 and the final *p*H of the culture broth after 12 days attained at 7.9 to 8.3. The violet coloured broth was then filtered and acidified to *p*H 4 with 2(N) HCl whereby a dirty red ppt formed and the supernatant liquid was deep red in colour. The clear red filtrate was then shaken with ether and ethereal layer on evaporation produces dark red powder.

The tarry red ppt obtained above was found to possess even after thorough washing also an appreciable antibiotic activity chiefly against *Staph. aureus*. But this ppt was discarded by the previous workers.

As both the filtrate and the ppt have got the antibiotic activities somewhat similar in nature, it may be inferred that either these two are of the same character and the formation of tarry precipitate may be due to partial precipitation of the antibiotic present in the broth at the above mentioned *p*H, or they are completely different from one another.

To arrive at a decisive conclusion regarding the nature of both ppt and the red powder obtained from etheral extract the method of countercurrent distribution is resorted to.

50 mg. of each of the dried red precipitate and the red powder were distributed between the solvent system benzene and ethylene glycol using an all glass 24 tube countercurrent distribution apparatus. After the distribution, all the tubes were taken out and an aliquot part from each tube was estimated with Klett's colorimeter. Two distinct type of bands were obtained, of which one was concentrated mainly in the *o*th tube and to a much lesser extent in the 1st one and the other spreading from the 2nd tube to the 23rd tube having its highest concentration in the 6th tube. It was found by cup assay method that all these tube-content excepting that in *o*th and the 1st tube possessed some sort of antibiotic activity, having the highest activity in the 6th tube-content. The contents in the *o*th and the 1st tube do not possess any antibiotic activity. Thus the observation made before regarding the activity of the red ppt was found to be erroneous, owing to some amount of active material still adsorbed in the ppt even after thorough washing.

Now after washing twentythree times in the C.C.D. apparatus each time with sufficient number of shaking it was observed that the active material was completely removed from the red ppt leaving it in its original inactive form.

D.6.3. *Study of an acid-base indicating pigment from a Streptomyces sp.*

A deep purple coloured pigment was found to have striking acid-base indicating properties. This pigment was produced by a Streptomyces species $Ac_{2B}$ isolated from the garden-soil of the Bose Institute. The pigment was blue in alkaline solution and reddish pink in acid solution.



The culture was grown at 26°C in C–D medium supplemented with 0.2% malt and at *p*H 6.8 in stationary flasks. Neither the growth nor the production of pigment was observed in shake flasks.

*Method of purification.* The medium after ten days of incubation was filtered. The filtrate was acidified with 2N HCl to *p*H 1 to 2. The pigment was thus precipitated out, centrifuged and the mass left was washed with distilled water three times. Later on, it was dried in vacuo.

*Solubility.* Solubility of the pigment in different solvents was given in the following table.

| | | |
|---|---|---|
| Water | ... | Slightly soluble |
| Butanol | ... | ,, |
| Ethyl acetate | ... | ,, |
| Benzene | ... | Insoluble |
| Amyl alcohol | ... | ,, |
| Chloroform | ... | ,, |
| Butyl acetate | ... | ,, |
| N/10 HCl acid | ... | ,, |
| Methyl alcohol | ... | Highly soluble |
| Ethyl alcohol | ... | ,, |
| N/10 NaOH soln. | ... | ,, |
| Ethylene glycol | ... | ,, |

Elemental analysis of the pigment showed that it did not contain nitrogen, halogen and sulphur. Furthermore, phenolic reaction of the pigment was found to be positive.

*Countercurrent distribution of the pigment.* Before proceeding any step in characterising the pigment it was intended to distribute the pigment in the countercurrent distribution apparatus. As the result in column chromatography using cellulose powder column indicated that the substance was not homogeneous, it was intended that all the fractions of the pigment should be separated and isolated in pure form.

*Choice of suitable solvent system.* The key point in the countercurrent distribution technique is to have a suitable choice of the two immisible solvent phases, between which the substance is to be distributed, which may be exploited to its maximum extent for purification and separation purposes. This can only be achieved by no definite means but by a laborious process of trial and error.

From the examination of the solubility chart, it was seen that none of the solvents offered a good pair of immiscible solvent phases for this purpose. Both butanol and water had slight solubility of the pigment, but the resulting partition coefficient between the solvent phases was not at all satisfactory. If dilute alkali was added in the aqueous phase, the pigment was totally converted into blue alkali salt which was only soluble in the aqueous phase. The case was just the reverse when dilute HCl was added to the aqueous phase—all the pigment was transferred in the upper phase.

Now it was intended to try with a sodium salt of an organic acid which, due to its poor dissociation in the aqueous phase, might have a tendency to transfer in the organic solvent phase as undissociated molecule and thus, acting as a carrier, might transfer a portion of the pigment in the upper phase, unlike caustic soda solution. Both sodium acetate and sodium citrate were tried in different concentrations for this purpose and as the dissociation of sodium citrate in aqueous phase was much less than that of sodium acetate, it was observed that 1% aqueous solution of sodium citrate and butanol, previously mutually saturated, gave a satisfactory partition coefficient of the pigment.

But there arose a difficulty of quick phase separation due to emulsion formation. This difficulty was avoided by adding 5% sodium chloride in the 1% sodium citrate solution and this aqueous phase equilibrated with equal volume of butanol was taken as solvent system for countercurrent distribution.

The pigment was distributed between the aforesaid solvent system in a 24 tube all glass countercurrent distribution apparatus, set up and standardised in this laboratory, and estimated colorimetrically in a Klett Summerson photo-electric colorimeter using filter no. 42. From the nature of the distribution band, it was observed that the pigment was not homogeneous and there were at least two major and two minor fractions present in the sample. The first major band occupying the first 6 tubes, had sharp fall from its peak in the *o*-th tube which suggested that the band might proceed due to salting out effect without having any fractionation at all. The second major fraction occupying the last five tubes had its peak in the 22nd tube. The two minor fractions had their peaks of the bands at the 12th and the 17th tubes.

All the four coloured fractions were isolated. It was observed that the two major fractions only gave indicating property and so the two minor fractions were regarded as impurities and rejected.

As from the nature of the distribution band of the first fraction it was suspected that this band might be due to salting out effect, this fraction was again distributed in the countercurrent apparatus between butanol and water, mutually saturated, resulting two fractions. The first fraction showed the indicating property while the second did not.

So far from the study of countercurrent distribution technique it was concluded that the pigment is composed of five components of which only two components showed indicating properties.

Studies are now in progress to characterise these two components having indicating property obtained from the pigment produced by the culture $Ac_{2B}$ and the use of these compounds in analytical methods.

## E. ZOOLOGY

### E.1. Further studies on the effect of penicillin on the metamorphosis of tadpoles (Bufo melanostictus)

The effect of penicillin on the retardation of metamorphosis of tadpoles (*Bufo melanostictus*) was repeated this year. The tadpoles were kept in penicillin sol. (80 units/ml) for seven consecutive days, fresh treatment being made daily. It was observed that most of the tadpoles *remained in the larval stage for about* 8–10 *months*, after which spontaneous metamorphosis took place. The metamorphosis of a very few tadpoles, however, occurred within 3–4 months. It was also found that after penicillin treatment the black pigment of the skin disappeared to a considerable degree and they looked almost grey in colour. But no such change was noticed in the control batch. They looked healthy and were in normal appearance.

E.1.1. *Effect of sulpha-drugs on the metamorphosis of tadpoles* (*Bufo melanostictus*)

Studies on the effect of sulpha-drugs, viz., sulphaguanidine, sulphanilamide, sulphathiazole, sulphapyridine, sulphamezathine and sulphadiazine on the metamorphosis of tadpoles were also made this year. Some of the tadpoles in all groups including the control batch metamorphosed within about one month. At present conclusive results regarding the inhibitory or stimulating effect of the sulpha-drugs on the metamorphosis can not be reported. The detailed studies will be undertaken when the large number of tadpoles will be available in rainy season.

E.1.2. *Effect of Thiourea on the metamorphosis of tadpoles* (*Bufo melanostictus*)

Thiourea, an anti-thyroid drug, is known to be responsible for the inhibition of biosynthesis of thyroid hormone by interfering with the activity of the peroxidase enzyme system, which is mainly concerned with the thyroid gland in the formation of thyroxine. Naturally, if the production of thyroid hormone is largely blocked by the administration of thiourea in tadpoles, the metamorphosis of these larvae will be retarded in as much as the thyroid gland by thyroxine secretion initiates the metamorphosis and the thyroidectomized tadpoles do not metamorphose. With this idea attempts were made to stop the metamorphosis of tadpoles (Bufo melanostictus) by thiourea. It has been found that the metamorphosis of these larvae is stopped by thiourea, but they grow in size to some extent. It has also been observed that increase in size is not so marked or is absent when the tadpoles just after hatching are taken. The growth of the tadpoles of about 30 days old continues even after thiourea treatment although the metamorphosis is inhibited for about 4 months. Next attempts were made to study the effect of vitamin $B_{12}$ on the thiourea treated larvae, whether vitamin $B_{12}$ can initiate the transformation of these retarded tadpoles or not. The investigation is being continued.

E.1.3. *Effect of vitamin* $B_{12}$ *on the metamorphosis of normal tadpoles* (*Bufo melanostictus*)

It has been reported that vitamin $B_{12}$ is capable of initiating the metamorphosis of penicillin-treated (retarded) tadpoles (R. tigrina). Penicillin causes vitamin $B_{12}$ defi-

ciency in tadpoles by its inhibitory action on the growth of the microorganisms in the intestine and possibly due to this deficiency the metamorphosis is retarded. Attempts have been made to observe the effect of vitamin $B_{12}$ on the metamorphosis of normal tadpoles of B. melanostictus. It has been found that the number of tadpoles metamorphosed in vitamin $B_{12}$ treated group is equal to that of the control batch. In this case, therefore, vitamin $B_{12}$ is not effective in initiating or stimulating the metamorphosis of normal tadpoles. It might be that adequate amount of vitamin $B_{12}$ is produced in the intestine of these larvae by the microorganisms present and so an extra supply of very small amount of the vitamin (20$\mu$g per 1000 ml. water) is ineffective so far as the metamorphosis is concerned.

### E.2. Effect of penicillin and thyroxine on the body weight of albino rats

Antibiotics like penicillin and streptomycin are known to enhance the growth of animals and so does thyroxine, specially at the early stage of life.

It is known that thyroxine in very low doses causes protein anabolism whereas in high doses it causes profound protein catabolism and consequently increased nitrogen excretion. The mechanism of action of penicillin on the stimulation of growth of animals is not definitely established, although several theories have been advanced in connection with its action on the microorganisms of intestine. Attempts are being made to find out the mechanism of action of penicillin on the growth of rats and also the metabolic interrelations of penicillin, thyroxine and vitamin $B_{12}$ in rats. This work is the continuation on the same line of our previous work, viz., "effect of penicillin, thyroxine and vitamin $B_{12}$ on the metamorphosis of tadpoles". The tadpoles are very small animals and their organs are rather small in sizes and not well differentiated. So rats have been chosen as experimental animals for the purpose of investigating the effects of penicillin, thyroxine and vitamin $B_{12}$ on various organs and also their metabolic interrelations. Preliminary attempts are being made to study the phosphatase activity of the various organs in rats after penicillin, thyroxine and vitamin $B_{12}$ treatment and their correlation with growth recorded by the animals, because the phosphatases are intimately related to the growth of animals.

In our experiments the effect of daily injection of penicillin (200 units daily) and thyroxine (0.002 mg. daily) on the growth of albino rats were observed. Six rats were taken in each group and before the treatments started they were maintained on the laboratory stock diet for 21 days. Injections of penicillin and thyroxine were continued for 30 days. Penicillin and thyroxine treated rats grew in size and their body weight increased, the average increase in weight is greater than that of the control. The average gain in body weight of rats under experiment at the end of 30 days is given in the following table:

AVERAGE INCREASE IN BODY WEIGHT IN 30 DAYS OF SIX RATS

| Control | Daily penicillin injects 200 units | Daily thyroxine injects 0.002 mg |
|---|---|---|
| 30.6 gm | 40.9 gm | 33.5 gm |



It is evident from the above table that penicillin has a marked growth promoting effect on rats, thyroxine has the same effect, but in a smaller degree. Attempts are being made to study the effects of various doses of penicillin and thyroxine on the body weight of large number of these animals which would permit statistical analysis of the results obtained.

### E.3. Amoeba culture and micro-manipulation for nuclear transfer

Physiological studies on different types of amoebae, such as Amoeba proteus, A. descoides and A. limax, as reported previously, were continued. Proper skill in micro-manipulation technique and establishment of pure cultures of homogeneous clones of amoeba, are essentially required for the proposed work on nuclear transplantation from cell to cell. To be accustomed to micromanupulation techniques, a micro-manipulator of Peterfi type, constructed in this laboratory, is now being used. In the moist chamber of this apparatus both hanging drop and usual conventional methods of mounting specimens are being tried. After having acquired the required skill in micro-technique, the latest model of de Fonbrune apparatus, lately installed here, may be utilized.

For the establishment of homogeneous clones of these three types of amoebae by subculture, a new culture solution has been prepared*. In the mucilaginous culture media, prepared from the hydrilla leaves, the above mentioned amoebae and some other unknown types are grown. A drop of culture solution containing amoebae is placed over the glass slide and a single organism is sought through the microscope. A bent capillary tube is then brought in position directly over the organism, which is drawn into the tube with a small quantity of solution by the capillary attraction. Now the tube is emptied in the petri-dish of new culture solution. The first attempt was partially successful, as a few amoebae, though of miniature type, were found to be present in the culture. The first success seems to have been accidental, since subsequent attempts were not successful. However to insure success in raising a homogeneous clone of amoeba, a new culture solution is being prepared, the composition of which is given below. In the meantime heterogeneous colonies of amoebae are being raised in the culture media made of hydrilla plants for the preparatory work.

**Composition of medium (Musgrave & Clegg's medium*—1940)

| | | | | |
|---|---|---|---|---|
| (1) Lemco | ... | ... | ... | 0.05 gm. |
| (2) Sodium chloride | ... | ... | ... | 0.05 gm. |
| (3) Agar | ... | ... | ... | 2.5 gm. |
| (4) Water | ... | ... | ... | 100 c.c. |

## WORKSHOP

During the period under report the Workshop handled various types of works and only the major jobs are reported below:

**Mechanical sections**

1. Rectangular shaped Cloud Chamber with all its automatic control devices, and one camera with its film driving mechanism, flash tube reflectors, etc. etc.
2. Modifications of cylindrical Cloud Chamber.
3. Metal G.M. counters for I.G.Y. programme carried out at Darjeeling.
4. Different fittings and accessories for construction of one Neutron Generator.
5. Wave-guides, cell holders and other accessories of Micro-wave apparatus.
6. Apparatus for Photo-electric effect experiments.
7. Spherical symmetry apparatus.
8. Mechanical cell Disintegrator (Nossal shaker) having frequency of 5600 times/minute in average. A clutch system has been fixed up with it to enable the shaker to operate only when the motor attains a critical speed.
9. Flame Photometer.
10. A Gas-phase chromatography apparatus.
11. Remodelling of a Polaregraph.
12. Besides making of instruments the general servicing and repairing works on the following machines have been attended.

(1) Refrigerator centrifuge, (2) Warburg apparatus, (5) Fermentor, (4) Distilling apparatus, (5) Microtomes, (6) Vacuum pumps, (7) Centrifuge.

**Refrigeration section**

This section is busy with the maintenance work of running the refrigerators, air-cooling systems of different laboratories. They also handle the repairs and overhauling of these machines. The Refrigerators and Air-cooling units obtained from the Directorate of Disposals are being repaired. These units are being used to air-condition the following laboratories. (1) Micro-Manipulator room, (2) Ultra-centrifuge room, (3) Neutron Generator room. Another unit is ready to be used to cool one room of Nuclear Physics Dept. The arrangements of air-conditioning a glass house is in progress.

**Water Supply**

One new tube-well of 3″ diameter has been sunk to increase the supply of water. New tanks have been fitted up in all the buildings and the older pipes have been replaced with bigger diameter pipes to increase the pressure and quantity of water. The present consumption of water is about 50,000 gallons per day. One new tube well of 3″ diameter has been sunk at Shamnagar for the irrigation work. One reciprocating pump fitted with a 5 B.H.P. Diesel Engine is being fitted to pump water.



**J. C. Bose Centenary Exhibition**

An important feature of the workshop activity during the year under report was in connection with the Centenary Celebrations. The workshop undertook a special programme of renovating all the sensitive instruments used by Sir J. C. Bose for his fundamental experiments; these were demonstrated in working condition during the Centenary Exhibition.

## LIBRARY

The library consists of 4,806 volumes (2,400 books and 2,406 bound periodicals). During the year under report 431 volumes (224 books and 207 bound periodicals) were added to the stock against 219 during the previous year. The total number of periodicals received was 213 (inland 62 and foreign 151) out of which 112 were subscribed and the remaining 101 were received either in exchange of Institute publications or as gifts. The library also received a large number of technical pamphlets, circulars, reprints, annual reports, etc., from various research institutions and Government Departments, both inland and foreign.

Though the library is primarily meant for the use of Bose Institute staff the services of the library are by no means limited to it as is well attested by the many research workers and scientists from other institutions who make regular reference use of the library's resources.

For providing better reading facility to readers the library was kept open for 61 hours a week from 9 a.m. to 8 p.m. on weekdays and from 9 a.m. to 3 p.m. on Saturdays. As a result of the extension of library hours more readers not only from the Institute but also from outside institutions conveniently use the library particularly during evening hours.

The library grant for purchase of books and journals was reasonably increased to meet the demands of readers for more books and journals. This demand was partially met by the addition of a good number of periodicals in the current list of periodicals and the acquisition of important books. The library stock was further enriched by the addition of a valuable gift of books and journals from Dr. J. N. Ray of the Calcutta Chemical Co. Ltd. (147 books and 22 periodicals) to whom our thanks are due.

It is felt that further addition of important books and periodicals are extremely desirable to meet the pressing demands of readers and to build up an up-to-date research library. Efforts are also being made in a planned way to provide more money in addition to increased recurring grant for the purchase of very important back numbers of periodicals.

During the period under review a substantial addition to the library was a gift of 91 selected books worth about $ 1,000.00 by American authors from the Government of U.S.A. under the "India Wheat Loan Educational Exchange Programme".

## PUBLICATIONS

During the year under report Vol. 21 (1956–58) of the TRANSACTIONS OF THE BOSE RESEARCH INSTITUTE and ACHARYA JAGADISH CHANDRA BOSE BIRTH CENTENARY VOLUME being Vol. 22, 1958, of the TRANSACTIONS OF THE BOSE RESEARCH INSTITUTE were published.

Efforts are being made to reprint many important and popular works of Sir J. C. Bose which have been out of print for a long time. Other publication during the year under report was "Report of the Bose Institute for 1957-58." The Twentieth Acharya Jagadish Chandra Bose Memorial Lecture being the "Centenary Memorial Lecture" delivered on the occasion of the Acharya Jagadish Chandra Bose Birth Centenary Celebration by Dr. S. Radhakrishnan, Vice-President of India, is in press and will be published shortly.

## APPENDIX A

### I. Governing Body

Dr. Debendra Mohon Bose
Sri Sudhansu Mohon Bose
Sri Kedar Nath Chatterjee
Sri Sudhir Kumar Mandal
Prof. Sisir Kumar Mitra, F.R.S.
Sri Abani Nath Mitter
Sri S. C. Mukherjee, I.C.S. (Retd)
Dr. Basanti Dulal Nagchowdhury
Prof. Bhupati Mohan Sen

### II. The Council

Sri S. M. Bose
Sri K. N. Chatterjee
Sri S. K. Mandal
Prof. S. K. Mitra, F.R.S.
Sri A. N. Mitter
Dr. B. D. Nagchowdhury
Prof. B. M. Sen
} Nominee of the Governing Body

Dr. D. M. Bose, Director, Bose Institute (Ex-officio)
Prof. M. S. Thacker, Secretary to the Govt. of India, Ministry of Scientific Research & Cultural Affairs, (Nominee of the Central Govt.)
Shri A. K. Ghosh, I.C.S., Jt. Secretary, Ministry of Scientific Research & Cultural Affairs (Alternate).
Sri Jitendra Nath Lahiri, M.P., (Representative of the Lok Sabha).

Prof. P. Parija, Vice-Chancellor, Utkal University
Dr. K. Ahmed Chowdhury, Professor of Botany, Aligarh Muslim University
} Scientist from outside West Bengal, nominated by the Govt. of India.

Sri Nandadulal Sreemany (Representative of the Calcutta Corporation)

### III. Finance Committee

Prof. B. M. Sen, Chairman (Representative of the Council)
Sri A. K. Mukherjee, Accountant General, West Bengal
Dr. D. M. Bose, Director (Ex-officio)
Registrar—Secretary.

### IV. Building Committee

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

## APPENDIX B

*Director :* Dr. D. M. Bose, M.A., Ph.D., F.N.I.
*Registrar :* 1) Dr. S. K. Roy, M.Sc., Ph.D. (Lond.), D.I.C. Actg. upto 2-6-58.
2) Sri S. K. Das, M.Sc. (Lond.) from 3-6-58 to 31-12-58.
3) Sri U. K. Datta, M.Sc., from 2-1-59.

**Department of Physics :**

*Head of the Dept. :* The Director
*Research Fellow :* Dr. T. C. Bhadra, M.Sc., D.Phil.
*Research Scholar :* Sm. Anjali Chowdhury, M.Sc.
*Mary & Richard Keating student :* Sri Ajit Halder, M.Sc. (from 1-8-58).
*AEC Junior Research Fellow :* Sm. Nilima Basu, M.Sc. (upto 1-8-58).
*Govt. of India Senior Scholar :* Shri Amalendu Nath, M.Sc. (from 6-3-59)
*Attached worker :* Prof. H. P. Dey, M.Sc., P.R.S.

**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. D. P. Burma, M.Sc., D.Phil.
Dr. S. P. Sen, M.Sc., D.Phil.
Dr. A. K. Barua, M.Sc., D.Phil. (from 1-6-58)
*Research Scholars :* Sri S. P. Raman, M.Sc.
Sri Nirmal Kumar Sinha, M.Sc.
Sri K. Venkateswara Rao, M.Sc.
Sri A. K. Banerjee, M.Sc.
Sri M. N. Roychowdhuri, M.Sc. (from 11-3-59)
*Sir J. C. Bose Fellow (Calcutta University) :* Sri Jyotirmoy Dutta, M.Sc.
*N.I.S. Junior Research fellow :* Sri D. P. Chakravorty, M.Sc.
*Govt. of India Senior Scholar :* Sm. Archana Neogi, M.Sc. (from 20-6-58)
*Attached workers :* Dr. S. N. De, M.B., D.T.M., Ph.D. (Lond.)
Dr. P. N. Das, M.B. B. S.
Sri S. K. Chakravorty, M.Sc.
Dr. C. R. Raha, M.Sc., D.Phil.
Dr. K. K. Mukherjee, M.B.B.S.

**Department of Botany :**

*Head of the Dept. :* Dr. K. T. Jacob, M.A., Ph.D. (Lond.)
*Research Fellow :* Dr. S. K. Roy, M.Sc., Ph.D. (Lond.), D.I.C.
Dr. S. Bose, M.Sc., Filosofic Doctor (Lund.)
Sri R. K. Basu, M.Sc.
*Statistician :* Sri A. K. Roychoudhuri, M.Sc.



*Research Assistant :* Sri B. K. Datta
Sri A. T. Guhathakurtha
Sri A. K. Adhikari, M.Sc.
Dr. S. B. Sarkar, M.Sc., Dr.rer. Nat.
Sri V. S. Sarma, M.Sc.
*Research scholars :* Dr. B. B. Biswas, M.Sc. D.Phil. (upto 3-8-58)
Sri V. N. Gadgil, M.Sc.
Sm. Maharani Chakraborty, M.Sc. (upto 7-1-59)
Sri A. P. Mani, M.Sc., (from 30-10-58)
Sri Debdas Mukherjee, M.Sc. (from 2-6-58)
*Govt. of India scholar :* Sm. Mary Korah, M.Sc.
Sri Asoke Kumar Chatterjee, M.Sc. (upto 31-1-59)
*Attached workers :* Sri D. K. Chakraborty, M.Sc.
Dr. (Miss) Madhuri Dutta, M.Sc., D.Phil.
Mrs. Mridula Datta, M.Sc.
Prof. K. C. Basumallick, M.D., Ph.D. (Lond.)

**Department of Microbiology :**

*Head of the Dept. :* Dr. P. N. Nandi, M.Sc., Ph.D. (Lond.), D.I.C.
*Research Fellow :* Sri K. L. Chaudhuri, M.Sc.
*Research Assistant :* Sri A. K. Mishra, M.Sc.
Sri P. P. Mukherjee, M.Sc.
*Research Scholars :* Dr. (Miss) Mira Purkayastha, M.Sc., D.Phil
Sm. Ashalata Paul, M.Sc.
Sri Tikaram Sharma, M.Sc. (from 16-3-59)
Sri Sankar Lal Chakraborty, M.Sc. (from 1-4-58)
*Govt. of India Senior Scholar :* Sm. Itu Sanyal, M.Sc. (from 2-1-59)
*Attached worker :* Sri Narendra Nath Shee

**Department of Zoology :**

*Research Assistant :* Sri G. C. Bhattacherjee
*Attached worker :* Sri A. K. Medda, M.Sc.

**Workshop :**

*Superintendent :* Sri M. L. Chatterjee
*Superintendent of Electricals, Electronics, Refrigeration :* Shri S. R. Ghosh

**Library :**

*Librarian :* Shri P. K. Chakraborty
*Asstt. Librarian :* Sm. Asoka Dhar
*Accountant :* Sri A. N. Chowdhury
*Asstt. Accountant :* Sri M. L. Datta, B.Com.
Sri P. C. Chakraborty

## STAFF APPOINTED UNDER GRANT-IN-AID SCHEMES

**A. Board of Research on Atomic Energy :**

*Investigator-in-charge :* Dr. D. M. Bose

Scheme No. 1-A : *Cosmic Ray Investigation*:
Dr. M. S. Sinha, D.Sc. (*Research Officer*)
Sri I. L. Chakraborty, M.Sc. (*Junior Research Asstt.*)
Sri S. N. Sengupta, M.Sc. ( -do- )
Sri Nirmalendu Chowdhury, M.Sc. ( -do- )

Scheme No. 1-B : *Nuclear Physics Investigation* :
Sri A. M. Ghosh, M.Sc. (*Junior Research Asstt.*)
Sri N. K. Ganguli, M.Sc. ( -do- ) on study leave

Scheme No. II : *Neutron Generator Investigation*:
Sri Bimalendu Mitra, M.Sc. (*Junior Research Asstt.*)

Scheme No. III : *Investigation on the induced mutagenesis in crop plants using X-rays & Radioactive Isotope*:
Sri Amal Kumar Bose, M.Sc. (*Junior Research Asstt.*)
Sri Balaram Mazumdar, M.Sc. ( -do- )

**B. Indian Central Oilseeds Committee :**

Induced Mutants with economic characters in til, rape and mustard by X-ray
(*Investigator-in-charge :* Dr. K. T. Jacob)
Dr. U. K. Rai, M.Sc., D.Phil., *Botanist*
Sri G. Gopalan Nair, M.Sc., *Asstt. Botanist*
Sri Deb Kumar Basu, M.Sc., *Chemical Asstt.*

**C. Indian Council of Agricultural Research—Control of Weeds :**

(*Investigator-in-charge :* Dr. K. T. Jacob (upto 30-9-59)
Dr. M. G. Srivastava, M.Sc., D.Phil., *Asstt. Botanist*
Sri A. P. Mani, M.Sc., *Asstt. Botanist*

**D. Indian Central Jute Committee**

"Radiation Mutation in Jute"
(*Investigator-in-charge :* Dr. K. T. Jacob)
Sri Asoke Das, M.Sc., *Cytologist* (from 1-7-58)

**E. Indian Central Oilseeds Committee**

"Radiation Mutation & Improvement of groundnuts"
Dr. M. G. Srivastava, M.Sc., D.Phil. *Cytogeneticist* (upto 31-10-58)
Dr. J. G. Bhowal, M.Sc., D.Phil (from 22-11-58)



**F. Indian Central Oilseeds Committee & Indian Central Jute Committee**

Installation of Cobalt 60 source
(*Investigator-in-charge :* Dr. K. T. Jacob)
Sri D. N. Kumar, B.Sc., D.I.C. *Physicist* (from 1-9-58)

**G. C.S.I.R. Schemes :**

Sri Saroj Bandhu Ghosh, M.Sc., *Senior Research Fellow*
Dr. Atul Chandra Das, M.Sc., D.Phil -do-
Miss Maharani Chakraborty, M.Sc. -do-
Sri Rabindra Krishna Sinha, M.Sc., *Junior Research Asstt.*
Sri Pritindra Mohon Naha, M.Sc. -do-
Sm. Arati Sarkar, M.Sc., *Junior Research Fellow*

## LIST OF PUBLISHED PAPERS 1958--1959

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| 2. Anomalous scattering of low energy mumesons in copper | -do- | Proc. Nat. Inst. Sci. India, | **24,** 295, 1958 |
| 3. Low Energy spectrum of sea-level electrons and muons at 12°N | -do- | Ind. Jour. Physics (In press) | |
| 4. Construction of 14.Mev. Neutron Generator and Measurement of fast neutron flux | Bimalendu Mitra | Ind. Jour. of Phys. | April, 1959 |
| 5. On the making of electret and measurement of the changes of dielectric constant of a polarised electret forming material with time | T. C. Bhadra | Ind. Jour. of Phys. | **32,** No. 6, June 1958, pp. 281-296 |
| 6. Purification & properties of phosphoriboisomerase from *Spinacia oleracea* | M. Chakravorty | Science & Culture | **24,** 380, (1959) |
| 7. Purification and properties of a C-1 diphosphatase from *Spinacia oleracea* | M. Chakravorty, H. C. Chakrabortty & D. P. Burma | Archives Biochem. Biophys | **82,** 21 (1959) |
| 8. A stereospecific dihydrolipoic dehydrogenase from *Spinacia oleracea* | D. K. Basu & D. P. Burma | J. Am. Chem. Soc. | July 20 issue, 1959 |
| 9. Enzymes of the pentose phosphate pathway in the mung bean seedling | M. Chakravorty & D. P. Burma | Biochem. J. | July issue, 1959. |
| 10. Amino acid activation in the cell-free preparations of *Azotobacter vinelandii* | M. Chakravorty | Annals. Biochem. & Exp. Med. | In press |



| Title of the note | Authors | Where published | Reference |
|---|---|---|---|
| 11. Structure of Aegiceradienol, a new triterpene alcohol from *Aegiceras majus* Gaertn | K. V. Rao & P. K. Bose | Science & Culture | **24,** 486, 1959 |
| 12. Isolation of genin—A and isorhamnetin from the bark of *Aegiceras majus* Gaertn | K. V. Rao & P. K. Bose | J. Indian. Chem. Soc. | 1959 (in press) |
| 13. Synthesis of dl-malic acid –2'3–$C^{14}$. | C. R. Raha & S. P. Sen | J. Sci. Industr. Res. | **17B,** 236 (1958) |
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