

REPORT
OF
THE BOSE INSTITUTE
FOR
1959-60

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

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REPORT OF THE BOSE INSTITUTE

1959—60

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

The Institute has at present four main departments of research, Physics and Biophysics, Chemistry including Plant Chemistry, Physical and Biochemistry, Botany including Plant Physiology, Cytogenetics, Plant Breeding, Microbiology including Soil Microbiology and Genetics of Microorganisms. The income of the Institute is derived from endowment funds, from Government of India grants and from grant-in-aids for support of different research projects. The management of the properties and investments of the Institute is vested in a Governing Body, a Board of Life Trustees; and the management of the affairs of the Institute is vested in a Council, on which some representatives of the Governing Body, nominees of the Government of India, and some representative scientists are included.

Governing Body

Two meetings of the Governing Body were held during the year. Besides discharging the normal activities of the Governing Body, like approval of the budget estimates, the passing of audited accounts and the adoption of annual report, the Governing Body elected two additional members, Shri Saibal Kumar Gupta, I.C.S., Chairman, Calcutta Improvement Trust and Shri Amiya Nath Mitra, Bar-at-Law, Administrative Officer, Joint I. G. N. and R. S. N. Steamers Ltd., bringing up the total number of members of the Governing Body upto 11.

Council

Two meetings of the Council were held during the year.

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

The Council has approved the enhancement in the Institute contribution to the Provident Fund Account from $6\frac{1}{4}\%$ to $8\frac{1}{3}\%$ provided an equal contribution is made by the employees. It is now with Government of India for sanction.

The benefit of House Rent Allowance to all employees of the Bose Institute (research as well as non-research) drawing salaries up to Rs. 300.00 p.m. has been given effect to.

The Council approved the principle of extending the benefit of H. R. A. to all permanent employees of the Bose Institute (research as well as non-research) on the scale sanctioned by the Calcutta University for its employees, and has requested the Director to take up the matter with the Government of India.

A proposal for amalgamation of the grades of the Readers and Research Fellows was considered by the Council and a new grade of Research Fellows on the scale of Rs. 300-20-500/E.B.-25-625/E.B.-35-800/ was sanctioned for incorporation in the draft proposals for amendment of the Regulations and Bylaws of the Bose Institute, now pending with the Government of India.

Shri Upendra Kumar Datta, Registrar, was relieved of his duties with effect from April 6, 1959 and Dr. S. K. Roy, Research Fellow, who was appointed Actg. Registrar pending the appointment of a permanent Registrar, took over the charge. As per recommendation of the Selection Committee and accepted by the Council of the Bose Institute, Shri Sekhar Kumar Chatterjee, Secretary, Lady Victoria Dufferin Hospital, was appointed Registrar on probation for one year and joined on August 28, 1959.

The Development Programme of the Bose Institute under the Third Five Year Plan Period was approved by the Council and forwarded to the Government of India for consideration. For this plan period the total recurring and non-recurring expenditures of the Bose Institute were estimated at Rs. 52.82 lakhs and Rs. 32.52 lakhs respectively.

Grants

The following grants were received during the year 1959-60.

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|----------------------------------|--|
| (a) <i>Non-recurring grants</i> | <p>(i) Government of India, Ministry of Scientific Research and Cultural Affairs .. Rs. 2,00,000.00</p> <p>(ii) For erection of a 20 curie Cobalt 60 source at Nilgunge</p> <p>From Indian Central Oilseeds Committee Rs. 4,830.00</p> <p>From Indian Central Jute Committee .. Rs. 7,185.36</p> <p>(iii) Ministry of Scientific Research and Cultural Affairs, Government of India, grants to individual workers for Fundamental Research Rs. 38,200.00</p> |
| (b) <i>Recurring Grants</i> | <p>(i) Government of India, Ministry of Scientific Research and Cultural Affairs .. Rs. 5,41,500.00</p> <p>(ii) Government of India for Training in Scientific Research Rs. 12,754.38</p> <p>(iii) Governing Body, Bose Institute .. Rs. 30,210.55</p> |
| (c) <i>Grants-in-aid Schemes</i> | <p>The following grants-in-aid were received during the year.</p> |

Sponsoring Body	Schemes	Amount received
Indian Central Jute Committee	Radiation mutation in Jute	Rs. 15,471.50
Indian Central Oil-seeds Committee	X-ray irradiation of Oilseeds	Rs. 28,360.00
-do-	Radiation mutation and improvement of Ground-nuts	Rs. 19,470.00
Council of Scientific and Industrial Research	Antibiotic production	Rs. 9,129.00
-do-	Isolation of macromolecules by countercurrent method	Rs. 6,109.00
-do-	Induced mutation in <i>Aspergillus niger</i>	Rs. 4,490.00
-do-	Mycology and plant pathology	Rs. 6,300.00
-do-	Microbial synthesis of proteins	Rs. 6,300.00
Council of Scientific and Industrial Research	*Chemistry of Indian plant resins	Rs. 2,954.00
-do-	*Biosynthesis and metabolism of gibberelic acid	Rs. 3,700.00
-do-	*Application of vapour phase chromatography in the analysis of Indian natural oils etc.	Rs. 10,410.00
Department of Atomic Energy, Govt. of India.	(i) Cosmic ray, (ii) Nuclear Physics (iii) Neutron generator, (iv) Induced mutagenesis in crop plants.	Rs. 1,25,000.00
Sir J. C. Bose Trust Fund No. 1	Research scholarship, stipends and foreign scholarships	Rs. 14,000.00

Acharya Jagadis Chandra Bose Endowment Lectures

The first two of the Endowment lectures were delivered by (i) Professor R. Ruggles Gates, F.R.S., of the Harvard University, on 'Heredity in the Races of Man' on November 6, 1959 and (ii) by Professor George Gamow, Colorado University (recipient of the Unesco Kalinga Prize) on 'What is life?' on November 12, 1959 and on 'Origin of life on the earth' on November 13, 1959. The lectures are being published.

Acharya Jagadis Chandra Bose Memorial Lecture

The twentyfirst lecture of the series entitled 'Impact of Technology on the Indian Economy' was delivered by Sir Jehangir Ghandy, Director-in-charge, the Tata Iron and Steel Company, Ltd., on Monday, November 30, 1959. The lecture has been published along with the Director's report presented to the fortyfirst anniversary meeting of the Bose Institute.

* New Schemes.

New Constructions

Western Block : In the last annual report, the construction of a single storied building to the west of the Institute quadrangle was mentioned. It was used to house the Jagadis Chandra Centenary Exhibition. This year an additional floor (first floor) has been erected on it. The southern wing of the ground floor contains the chemical engineering units and the northern wing the electronics and refrigeration workshops. The first floor which is nearing completion will contain a well equipped small seminar room with seating accommodation for about one hundred people. The northern wing is being equipped as an enzyme laboratory, with two mezzazine floors, one of which will be air-conditioned.

Neutron Laboratory : To the north of this western block, the erection of a neutron laboratory is nearing completion. It consists of a hall ($22' \times 15' \times 18'$) which will house a small 320 kv Cockroft Walton generator. The insulated positive terminal of the generator will be connected with the upper terminal of the positive ion tube for neutron production. There is an opening $4' \times 4'$, on the floor of this hall through which the earthed target end of the neutron tube will enter a basement room ($18' \times 16' \times 9'$), in which all experiments with neutrons will be conducted. Further details on the construction and equipment of this laboratory is given in A.6.

Laboratory for tissue culture : The ground floor of the library building to the south of the quadrangle, has now been divided into two wings, one of which is being fitted up as a library stack room. It is proposed to air-condition a portion of this stack room to house the Jagadis Chandra Boses papers. The other wing has been partly air-conditioned and is being equipped as laboratory for maintenance of and for investigations on normal and neoplastic cultures of plant cells and tissues.

Cobalt 60 sources : The 20 curie Cobalt 60 gamma field at Nilgunge is working satisfactorily. A 2 curie Cobalt 60 source has been installed in a specially protected isolated room. The source when not in use is stored at the lower end of a steel tube reaching 20 ft. below the earth's surface.

Land purchase : The Institute's efforts to purchase a suitable plot of land of not less than 50 bighas on the Jessore Road area for extension of laboratories and for concentration in one area the field stations of the Institute, have not been so far successful.

The continued erosion of the embankment work protecting the Director's quarters of Falta, by the river Hooghly and the annual expenses involved in repair are causing concern to the Institute.

Instruments/Apparatus received against grant of \$10,000.00 from the Rockefeller Foundation, U.S.A. (1959)

1. Servall Superspeed Refrigerated Centrifuge with spares and accessories.
2. LKB Conductivity Bridge.
3. Towers Fraction Collector.
4. Cells for Electrophoresis Apparatus.
5. Coleman Spectrophotometer.

6. Cells for Beckman Spectrophotometer.
7. Spares and accessories for Spinco Model E Analytical Ultracentrifuge.
8. Spares and accessories for Beckman Spectrophotometer.
9. Servall Superspeed Angle Centrifuge.

Study abroad and Deputation

1. Dr. A. K. Barua, Research Fellow in Chemistry was awarded a post doctoral fellowship by the University of Rochester, New York.
2. Dr. S. B. Sarkar, Research Assistant, Department of Botany, was awarded a fellowship in the Max-Planck Institut fuer Biologie, Tuebingen, West Germany.
3. Dr. D. P. Burma, Research Fellow in Chemistry was awarded a fellowship in the laboratory of Prof. Severo Ochoa of the New York University College of Medicine, New York.
4. Shri H. C. Chakraborty, Senior Laboratory Assistant, Department of Chemistry, was awarded a French Government scholarship to work in the Institut du Radium, France.
5. Shri Debdas Mukherjee, research scholar, Department of Botany, was awarded a research assistantship by the University of Kentucky, U.S.A.
6. Sri A. K. Halder, Mary and Richard Keating student, Department of Physics, was awarded a scholarship by the Liverpool University, U.K.
7. Dr. S. P. Sen, Research Fellow in Radio-Chemistry, attended the Ninth International Botanical Congress in Montreal, Canada, to which he had been invited.

Grants for study abroad

Grants of Rs. 2000.00 each out of Sir J. C. Bose Trust Fund were made available to Dr. S. P. Sen, Dr. A. K. Barua and Shri H. C. Chakraborty in partial defrayal of their cost of passage abroad.

Degrees awarded

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

1. Shri Debi Prasad Chakraborty, Research Assistant, Department of Chemistry.
2. Sm. Mary Korah, Government of India Research Training Scholar, Department of Botany.
3. Sm. Nilima Basu, A.E.C. Junior Research Fellow in the Department of Physics.

Visitors

The following visited the Institute during 1959-60.

(1) Sir Harold Jeffreys, F.R.S., with Lady Jeffreys. (2) Dr. Erich Mosetting, National Institute of Health, Maryland, U.S.A. (3) A 5-member delegation of well-known journalists and editors from Malaya. (4) Dr. Pierre Baranger, Professor of Chemistry, Ecole Polytechnique de Paris. (5) Dr. George A. Rubossow and Mrs. Helen Rubossow of 420, Riverside Drive, New York. (6) Prof. David Slwenbey of Cambridge University.

B. Research Scholar

1. Sm. Latika Danda, M.Sc., in the Dept. of Botany with effect from 14.4.59.
2. Shri Rabindra Prasad, M.Sc., in the Dept. of Botany with effect from 14.4.59.
3. Shri Biswaranjan Banerjee, M.Sc., in the Dept. of Botany with effect from 21.3.60.
4. Shri B. K. Burman, M.Sc., in the Dept. of Chemistry with effect from 1.9.59.
5. Shri S. K. Chakraborty, M.Sc., in the Dept. of Chemistry with effect from 1.9.59.
6. Shri Radhakanta Mandal, M.Sc., in the Dept. of Chemistry with effect from 10.12.59.

C. Mary and Richard Keating Student

1. Shri Subhendu K. Datta, M.Sc., in the Dept. of Physics with effect from 1.12.59.

D. Sir J. C. Bose Fellowship of Calcutta University

1. Dr. K. K. Mukherjee, M.B.B.S., in the Dept. of Chemistry with effect from 1.10.59.

E. Govt. of India Research Training Scholar

1. Sm. Anima De, M.Sc., in the Dept. of Botany with effect from 2.4.59.
2. Shri S. D. Bhattacharjee, M.Sc., in the Dept. of Chemistry with effect from 1.4.59.

Appointment under grants-in-aid Schemes*C.S.I.R. Schemes—Senior Research Fellow*

1. Shri Jyotirmoy Datta, M.Sc., with effect from 16.7.59.
2. Shri S. P. Raman, M.Sc., with effect from 23.10.59.
3. Dr. A. N. Lahiri, M.Sc., Ph.D. with effect from 1.12.59.

Junior Research Assistant

1. Md. Mominal Haque, M.Sc., with effect from 14.8.59.

Indian Central Oilseeds Committee's Scheme

1. Shri G. Gopalan Nair, M.Sc., Botanist. with effect from 16.2.60.
(vice Dr. U. K. Rai resigned)
2. Shri Nirmal Sen, M.Sc. (Tech.), Chemical Assistant, with effect from 21.9.59.

Resignations

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

1. Shri A. P. Mani, Research Scholar, 31.8.59.
2. Sm. Mary Korah, Govt. of India Research Training Scholar, 30.7.59.
3. Shri D. Mukherjee, Research Scholar, 9.8.59.
4. Shri M. N. Roychowdhuri, Research Scholar, from 13.8.59.
5. Shri A. K. Halder, Mary and Richard Keating student, from 26.10.59.
6. Shri U. K. Datta, Registrar, from 6.4.59.
7. Dr. U. K. Rai, Botanist in I.C.O.C. scheme, from 30.10.59.
8. Shri Nirmal Sen, Chemical Assistant in the I.C.O.C. scheme, from 5.3.60.
9. Shri K. V. Rao, Research Scholar, from 30.4.59.

Seminars

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

Subject	Speaker
1. The miracle of an egg, experimental embryology	Shri R. L. Brahmachari of Indian Statistical Institute
2. Constant solubility test as a criterion of protein homogeneity	Shri Nirmal K Sen
3. Detoxication studies with coumarins	Shri D. P. Chakraborty
4. The chemistry of blood clotting	Dr. G. C. Chatterjee of Indian Inst. of Biochemistry and Experimental Medicine
5. How are enzymes synthesized?	Dr. D. P. Burma
6. A stereospecific dihydropolipoic from <i>Spinaicia abraeca</i>	Shri D. K. Basu
7. Role of essential fatty acids in nutrition	Shri D. Ganguly of Calcutta University
8. Structure and synthesis of D.N.A.	Sm. Maharani Chakraborty
9. Rhizosphere microflora	Dr. A. C. Das
10. In quest of the flowering hormone	Dr. S. P. Sen
11. Irradiation experiments on jute	Shri R. K. Basu
12. Some aspects of soil fungi and root infection	Dr. A. C. Das
13. Self-reproduction	Shri J. Datta
14. Microwave technique and paramagnetic resonance spectroscopy	Shri A. K. Halder
15. An anatomical approach to herbicidal action and selectivity of auxin herbicides	Shri A. P. Mani
16. Effects of irradiation on paddy	Shri B. Mazumdar
17. Radiation hazards and radiation dosimetry	Shri A. M. Ghose
18. Fast neutron dosimetry and effects of neutron doses on biological materials	Shri B. Mitra
19. Acute radiation syndrome and nitrogen metabolism	Dr. K. K. Mukherjee
20. Natural coumarins in therapy	Dr. D. P. Chakraborty
21. Trifiden A, an antigen from ragweed pollen	Dr. Anil Bhattacharjee of Calcutta University
22. Studies on microbial protein synthesis with <i>Azotobacter Vinelandii</i> as the test organism	Dr. D. P. Burma
23. Some recent studies on the behaviour of the motile pulvinus of <i>Mimosa pudica</i>	Shri B. K. Datta and A. Guhathakurtha
24. Bio-electric potential associated with plant movement etc.	Shri B. K. Datta and A. Guhathakurtha
25. Radiobiological studies at Brookhaven National Laboratory	Dr. James L. Brewbaker of Brookhaven National Laboratory, New York.
26. Root auxins in relation to aging and geotropism	Dr. A. N. Lahiri

RESEARCH ACTIVITIES—NON-TECHNICAL REPORT

Ultrasonic radiation

The anomalous absorption of ultrasonic radiation in benzene, carbon tetrachloride and carbon disulphide has so far been studied satisfactorily only by the pulse technique methods. No satisfactory explanation of the nature of this anomalous absorption has yet been given.

Investigations are in progress in this laboratory to obtain sharp diffraction patterns of monochromatic radiation traversing ultrasonic fields in these liquids. By cooling the liquid and using ultrasonic radiation of reduced intensity, it has been possible to obtain sharp diffraction photographs in benzene and in carbon tetrachloride; in carbon bisulphide only a visual observation of such patterns has been possible. It is expected that the methods being developed will enable the determination of the co-efficient of absorption as well as velocity of the ultrasonic waves at different field intensities.

Cosmic rays

The study of the assymetry in the direction of emission of electrons from stopped mu-mesons has been carried out at Darjeeling with a large rectangular Cloud chamber. Out of nearly twenty thousand tracks photographed in the chamber, 881 unambiguous cases of mu-meson decay electron could be scanned, out of which 482 electrons have been observed to be emitted in the upward direction and 399 in the downward direction. The magnitude of polarisation was found to be 27%. This is a little higher than the theoretical expectation. The cause for this discrepancy is being further investigated using a thickness of 1 meter of lead over the cloud chamber at Darjeeling. To obtain a large volume of data on mu-meson interaction, and assymetry in the direction of decay, it is proposed to carry on further study with a specially large cloud chamber recently assembled, which is to be set up in an underground tunnel with a cover of over 100 ft. of earth. Under such conditions the cosmic ray particles are expected to consist almost entirely of mu-mesons.

IGY Programme

During the year, the Institute has participated in the IGY Cosmic Ray Programme. Cosmic ray intensity measurements with two identical meson telescopes and one neutron monitor set up at Mayapuri, Darjeeling (7000 ft. altitude) have been tabulated for the IGY period (January to December 1958) and the IGC period (January to November 1959), and sent to the Indian Coordination Committee. Sudden decrease in cosmic ray intensity associated with solar flare and magnetic storms have been observed on the following dates : March 25, May 29, September 15, 1958 and May 11, July 17, 18 and September 2, 1959. Similar variations in cosmic ray intensity have also been observed by participating laboratories in other parts of the world.

Data obtained by means of combination of fast recording ionisation chamber and Gieger counter on interaction of high energy nuclear active particles with matter obtained at Darjeeling, have been analysed mainly to evaluate the following properties of the nuclear active particles (i) energy spectrum, (ii) charge to neutral particle ratio, (iii) interaction cross section with lead and aluminium.

Nuclear Physics

A gamma ray detector, the efficiency of which is approximately constant over a wide range of photon energy, is a useful tool in experimental nucleonics. It has been found that a combination of 3.5 cm of aluminium placed before a 2" NaI/Tl crystal has an efficiency of 35% for photons of energy lying in the range of 0.25 Mev to about 6 Mev. This type of counter is being used in experiments on Compton scattering and photoelectric absorption of Co-60 gamma rays. Two investigations are being carried out at present : (i) on the angular distribution of Compton scattered cobalt 60 photons and (ii) photoelectric absorption of cobalt 60 photons in copper, zinc and cadmium.

Radio Carbon dating

The Institute has been carrying out investigations extending over several years for the assembly of a reliable and sensitive apparatus for radio active carbon dating measurements. Different types of arrangements for counting the beta ray emission from C^{14} compounds including a proportional countertube containing radioactive CO_2 or C_2H_2 surrounded by a ring of Geiger counters for elimination of background counts were tried. These have been discarded in favour of a liquid scintillation counter saturated with radioactive acetylene and kept at a temperature of about $-78^\circ C$. The background count is reduced by coincidence counting with two photomultiplier tubes. Different methods of keeping the scintillation liquid at a steady temperature of about $-80^\circ C$ is being tried.

The other part of the investigation viz. for obtaining high percentage yield of CO_2 or C_2H_2 from wood materials contemporary or of prehistoric origin has been worked out satisfactorily.

Neutron generator

As has been described elsewhere a neutron investigation laboratory is under construction, containing a top floor room for the housing different types of Cockcroft Walton generators, which will accelerate through different voltages positively charged deuterons in a positive ray tube to an earthed target of T/Ti (tritium gas adsorbed in Titanium). The earthed lower end of the positive ion tube will terminate in a basement room where all investigations with neutrons will be carried out. The rooms are being air-conditioned and every precaution is being adopted to effectively shield the rooms so as to keep the intensity of stray neutron diffusion outside these rooms below the prescribed tolerance level.

Physical and Biochemistry

Vapour phase chromatography, an apparatus for separation of constituents of a mixture of organic vapours, has been constructed in the workshop, employing only a few imported components. Different types of supporting solids like C-22 fire bricks powders, and purified "cellite 545" powder have been tried. For resolving different types of vapour mixture, different liquid phases have been found to be specially suitable. Liquid phase columns containing squalene, tricresylphosphate, liquid silicone, didecyl phthalate have each special resolving power for mixtures containing hydro-carbons, alcohols, fatty acids, esters and ketones. By using these columns in suitable sequence it is possible to separate the constituents of a mixture containing all the above mentioned organic vapours. An apparatus for vapour

phase chromatography of such complex mixtures of organic vapours is being constructed in the workshop.

Studies on radiation damage metabolic effects due to total body radiation in small animals were continued during the past year. Urine from controls and from animals exposed to gamma radiation of intensities from 300r to 1200r from a cobalt 60 source were tested specially for the increase of free ammonia and of total sulphur.

Different methods were employed to estimate the ammonia present in the urine of irradiated rats. The possibility that a part of the ammonia might be derived from the nitrogenous constituents of the urine was examined. It was noted that urea, uric acid and creatinine did not break down during steam distillation in the presence of alkali; glutamine however breaks down under such treatment.

Physicochemical studies on large molecules of biological origin

The methods employed for such investigations are separation by electrophoresis, filter paper ionophoresis, sedimentation by ultra centrifuge and chemical precipitation.

The materials investigated during the year were (i) β -lactoglobulin isolated from buffalo's milk; previous observations made in western countries had shown that two genetically different β -lactoglobulins (A and B) occur in cow's milk. Similar investigations carried on buffalo's milk from different local herds here showed the presence of one (B) component only.

Filter paper ionophoresis showed that lactoalbumin of buffalo's milk moves faster than that of cow's milk. The observations may find practical application in detecting admixture of buffalo's milk with cow's milk.

Seed proteins

Studies on crystalline α -globulin from sesame seeds has been continued. The two components which were isolated last year now appear to be due to presence of monomer and dimer units.

Studies on toxic proteins of plant origin has been continued. The seeds of *Abus precatorium* contains a toxic albumin Abrin which has remarkable agglutinating properties with human blood. Filter paper electrophoresis and sedimentation studies with a solution of total protein precipitates showed the presence of four components. Of these there was only one agglutinating component. Further work on purification of different components and determination of their toxicity on animals is in progress.

Protein component of seed of *Croton tiglium* are being studied for the presence of a toxic agglutinating component. Agglutinating tests with human RBC and sheep RBC show that the 50 percent fraction of the soluble protein extract does not agglutinate human RBC but sheep RBC is rapidly agglutinated.

Toxins from *Vibrio cholerae*

A method was described last year for studying separately the endo-and exo-toxins from the bacteria *V. cholerae*. It was found that the bacteria free filtrate from saline washings of

V. cholerae grown on different culture media, showed the presence of different toxins. This year the work is being continued with a view to finding out the influence of factors like varying the nutrient constituents, effects of pH of the media, of temperature of incubation and of strains of *V. cholerae* on the production of enterotoxins.

Plant Chemistry

In continuation of the survey of coumarin bearing plants, last year paper chromatographic separation of natural coumarins was taken up. The biochemical effect of the coumarin *posralene* on the tyrosinase and *melanine* formation in toad was studied. A histochemical method has been developed for the detection of melanine in amphibian subjects which may find use in the bio-assay of drugs recommended for chemotherapy of leucoderma. The antibacterial activity of purified glycoside of *Centella asiatic* was further studied. The survey of saponin and alkaloid bearing plants were continued. Investigations on Indian plant resins specially of the non-volatile constituents are being undertaken.

A number of investigations have been undertaken on the constitution of mesuol, mesuone, and of the tri-terpene compounds isolated from the pods of *Albizia lebbek Benth.*

Synthesis of some mutagenic compounds

Ethylenimine and its derivatives are potent pharmacological as well as good alkylating agents. The reactive imines and epoxides have been found to show strong mutagenic action on microorganisms and plants. A number of imine and epoxy derivatives have been prepared in this laboratory; some of the imines are being tested on Barley, and on the microorganism *Neurospora*.

Enzyme Chemistry

Studies on the mechanism of protein synthesis in intact cells of *Azotobacter vinelandii* were reported last year. It has been followed this year by a more detailed study of protein synthesis in cell free preparation of *A. vinelandii*, using certain amino acids tagged with C^{14} . The particulate fraction containing mostly cell wall fragments and membranes, show that the incorporation of amino acids is independent of externally added ATP. It appears that these particulate preparations have all the enzymes and co-factors required for protein synthesis. Analysis of the distribution of the radioactive amino acids between the nucleic acid and the protein shows that the major part of the amino acid is associated with the proteins—the nucleic acid presumably plays the role of an intermediary.

A comparison with the pH 5 fraction of rat liver extract shows that the latter has a great deal of similarity with the pH 5 fraction prepared from *A. vinelandii*. The mechanism of protein synthesis appears to be similar in the two systems, but they differ in detail. The rate of synthesis in the two systems are most probably different.

Radio Chemistry

Using the radio active isotopes carbon 14, sulphur 35, and iron 59, investigations on different metabolic processes, like the role of iron in photosynthesis, the binding of indole acetic acid to protein molecule and its role in growth promotion, the production of ascorbic

acid as a breakdown product of sucrose in *Avena* and its growth promoting properties have been studied.

Of special interest has been a comparative study of the metabolites synthesized in apices of short day plants, due to photoperiodic induction effect on its leaves. The proteins formed in the apices of rice plant under photoperiodic induction using C-14 and S-35 tracer, have on hydrolysis been found to contain the same amino acids but in altered ratio. It is possible that protein of the same amino acid composition but with an altered sequence has been formed under photoperiodic treatment. It would be of considerable interest to find out whether nucleic acid synthesis in the same apices is similarly affected. The next step could then be to establish whether these macromolecules have florigenic properties.

Gibberlic acid

Investigations have recently been commenced to study the problem of gibberlic acid from the following standpoints: (i) isolation of the growth active compound formed in the culture filtrate of *Giberella fugikuroi*, (ii) physiological nature of the gibberlic acid action in plant tissues, (iii) determination of the possible pathway of biosynthesis of gibberlic acid.

Crown gall tumour

Investigations on the production of crown gall tumour in plants by inoculation with *Agrobacterium tumefaciens* in different host plants like *Bryophyllum*, *Kolonchoc*, *Nasturtium*, *Hollyhock* have been going on in the Institute for several years. Bacteria free cultures in synthetic media have been maintained with some gall tumour tissues for five years. Successful implanting of bacteria free tumour tissues on healthy plants is considered to be a strong proof of the autonomous nature of the cultivated tissues. This experiment was performed successfully during the year on *Hollyhock* plants; these were implanted with *Hollyhock* crown gall tissues cultured in synthetic media for several years.

Since last year attempt has been made successfully to induce crown gall tumour in certain plants with small chromosome numbers, possessing larger sized chromosomes, like *Vicia faba* ($2n=12$), *Pisum sativum* ($2n=14$) *Coreopsis lanceolate*, and to culture these gall tissues in synthetic culture media. It is expected that a comparison will be possible between the chromosome characteristics of normal callus tissues with gall tissues of the same plants grown in synthetic culture media.

Experiments are being conducted to find out proper concentration of different growth substances which will selectively cause cell division and result in durable cultures of normal callus tissues.

Glycolytic enzymes

Last year investigations were reported on glycolytic enzymes in higher plants, specially bearing on oxidative phosphorylation associated with the dehydrogenation of triose phosphate in *mung* seedlings. This year it has been possible to isolate and obtain in pure crystalline form the enzyme aldolase used for the cleavage of fructose-1,6-diphosphate. Microscopic examination of aldolase crystals obtained from *mung* seedlings reveals the same shape and size as reported by Taylor for crystalline rabbit skeletal muscle aldolase.

Reflex arc phenomenon in *Mimosa pudica*

When the tip of one of the leaflets of *Mimosa pudica* is lightly touched with a glowing match tip it is followed by successive closure of the pinules from the apical to the basal end of the leaflet touched. After some time the main pulvinus drops, followed by the closure of pinules of the remaining three leaflets attached to the petiole. The observation is interpreted as due to an excitation originating from the stimulated point and travelling through the petiole to the main pulvinus where it releases an efferent impulse originating from the pulvinus, through the petiole to the remaining subpetioles. To find out if any electrical correlate of the mechanical response could be detected and to measure the corresponding velocity of the transmission of excitation, two points along the petiole were connected to two electric penrecorders. Depending upon the intensity of stimulation, it has been possible to record electrically either the efferent or the afferent impulse. When the impulse is moderate the afferent impulse can be measured electrically and is between 50-180 mm per second, but not the efferent impulse from the pulvinus to the subpetiole which is estimated to be of the order 300 mm per second. When the stimulus is strong the afferent impulse cannot be measured electrically and vice versa the outgoing impulse can be electrically recorded. It is possible that the fast travelling impulse may not be of an electrical nature. The investigation is being continued.

Cytology

Observations were continued on a particular aspect of nuclear division in the endosperm nuclei of some of the Palmae species, cocos, areca nut, the formation and breakdown of the mitotic spindle mechanism. The balance of the spindle mechanism is found to specially sensitive to alterations in the environment, particularly in relation to light and temperature. The breakdown of the spindle mechanism following coumarin treatment follows essentially the same pattern as that with light, indicating the non-specific nature of the reaction.

Radiation genetics

Gamma field :

The radioactive cobalt 60 source at Nilgunge was ready for field operation since February, 1959. The first crop to be sown in the gamma field was sesamum (April 1959) and were followed by jute, ground nut and mustard. The results and some of the effects on the different crops are given below at appropriate places.

In the gamma field, stimulation of plants growth was observed in plants of all the varieties growing in a particular zone (Bed IV).

Sesamum

The white flowered and the small seeded mutants which were isolated in the X_3 and X_4 generations after irradiation with 20,000r and 14,000r respectively continued to give higher oil content (42.8% and 48.7% respectively) over the control (T.16=40.6%) in the year under report(X_{10} generation).

The multicoloured seed mutant continued to give higher oil percentage as in previous years.

Two white flowered plants were found in the G_1 (gamma 1) generation, after 662 r gamma radiation in the seedling stage.

Early flowering was observed (from 9-12 days) in the T-12 and T-16 types grown in the gamma field.

Brassica : The early flowering type isolated in previous years continued to breed true.

The appressed pod mutant reported in previous years, and also other selections were grown in the gamma field. Slightly increased seed and pod yield were noticed in some of the plants, as well as early flowering behaviour.

Jute : Fresh irradiation with X-ray and P-32 showed that D-154 is more resistant than other varieties (eg. R26, TM, CG) with respect to germination capacity after irradiation. There was a general decrease in basal diameter and height of treated plants of all varieties in the P_1 generation. Flowering was generally late in all treatments in all the varieties. Various abnormalities in the leaves and stems have been found in the X_1 generation. In G_1 plants crinkling of leaves was frequently observed.

Groundnut : Variegation and other routine changes have been frequently observed in the X_1 generation. Pollen sterility increased in all the treatment except S-35. In the 2nd generation however the sterility became normal.

Plants in the P_2 generation generally showed a tendency to early flowering by 2.5 to 1.5 days. Several morphological variants, eg. "bushy type" "small leafed type" and "dwarf type" were isolated in the X_1 and X_2 generation and the segregation behaviour of some of them have been studied.

A few treated plants have given indications of having higher oil content. These are being kept for observation for the next year.

Paddy : With P-32 treatment, no morphological variation of treated plants were found in the P_1 generation. In P_2 generation different kinds of chlorophyll mutants (e.g. albino, xantha and virescent types) were scored. They were more frequent in the variety *Rupsail* than in *Patnai*. An early maturing type was isolated (in var. *Rupsail*) in the P_2 generation which flowered 28 days earlier than the control. It had weak growth however and had poor seed setting.

Aus : Various types of chlorophyll mutants were found in the X_2 and P_2 generations. The maximum frequency occurred in the variety *Dular*.

In the var. *Marich bati*, segregation of colour of tillers and husks occurred in the P_2 generation.

Early flowering types (by 7 to 15 days) were also observed.

Boro : Doses of 15,000r or more, resulted in high sterility (90% vs. 23% in control). One dwarf plant showed floral abnormality.

Tomato : Fresh treatment with X-ray resulted in chimaeral plants (pale green region in leaves).

Some morphological variants were found in the X_2 and P_2 generations eg. potato leaf mutant and plants with a reduced number of locules per fruit.

Studies on the effects of Indole Acetic acid and Gibberllic acid on various types of the chief dwarf mutants showed only slight stimulation effects.

Hybridizing experiments with different varieties of tomatoes (e.g. Teziers Prim, Yellow Cherry and Red Currant) gave indications of heterotic effects with respect to some of the flowering and fruiting characters (eg. flowering period, fruit maturity, yield etc.).

Chemical mutagenesis : The effects of dimethyl sulphate treatment on jute seeds were observed in the C_1 generation. Significant differences were observed between the treated plants and the controls, with regard to such characters as pollen sterility, height of plants, and survivality. The C_2 generation are under observation.

Cotton : Cytological and cytotaxonomical studies of 8 species of *Gossypium* have been made during the current year. Most of the *Gossypium* species have been found to have the chromosome number $2n=26$, except *G. latifolium* and *G. darwinii* where the chromosome number is $2n=52$, *Urena lobata* has the chromosome number $2n=28$.

Some differences in the chromosome size of the complement in the different species seem to be present.

Chronic irradiation effects : Collection of seeds and plants were made of certain species growing in radioactive monazite sand and the adjacent non-radioactive soil (mostly laterite) at Manavala kurichy (a coastal village in the Kanyakumari district of Madras) with a view to find out the presence of any aberrant types in plants growing in monazite regions, and which could be ascribed to the effects of the radioactivity present in such sands. Several plants (of the two species *Crotalaria striata* and *Vinca rosea*) found growing on monazite sand showed morphological (including floral) abnormalities. Though none of the control plants (growing in non radioactive areas) showed such abnormalities, more evidence is necessary to establish any theory regarding the nature of their origin.

Microbiology

Investigations on the production of a broad spectrum antibiotic substance from one of the selected strains of *Streptomyces A₁₄* (475) was conducted on a semilarge scale and purification of the active principle was successfully achieved. The sulphate and acetate forms of the substance show remarkable activity *in vitro* and toxicity test is in progress. A bacterial antibiotic produced by a strain of *Pseudomonas sp.* was isolated and the pure substance was obtained as needle-shaped indigoblue crystals having a melting point 110-112°C. The substance possesses strong antifungal property and toxicity tests are being done.

The effect of gamma radiation from a powerful Co^{60} source at the World Agriculture Fair—1960, New Delhi on *Asp. niger* and other microorganism was studied and compared with that of UV radiation. Many new types of mutants appeared in the former case. Selected mutants of *A. niger* showed increased production (60% in excess of the parent) of citric acid and the activity of the mutants remained unaltered after subculture.

The effect of chemical mutagens on a methionineless strain of *Neurospora crassa* was studied using di-chloro-ethylene-imine, a chemical, synthesised in the Dept. of Chemistry of the Institute. A group of similar new chemicals are contemplated for the study of chemical mutagenesis in microorganisms and higher plants. Bacteriophages affecting nodule bacteria were isolated in pure form from soil and specificity of phage action was established against different strains bacteria by cross inoculation experiments.

In Transformation reaction, mutations have been known to be induced in bacteria eg. salmonella under the influence of isolated DNA fractions from a genetically dissimilar member of the same strain of bacteria. The specially-prepared cell-extract from a 'donor' strain was obtained and added to the culture medium of the 'receptor' strain which resulted in the acquisition of genetically active unit present in the cell-extract of the former. Experiments were carried out with *Staphylococcus aureus*, a golden yellow coloured pathogenic form of bacteria as the donor and *Staph-albus* a white form of mild pathogen as the receptor. There were definite indications of genetic alteration of the receptor as is evidenced from the appearance of colonies of different character on culture plates.

Zoology

Investigations are being continued for studying in detail the mechanism of retardation of metamorphosis in the tadpole *R. tigrina* and its counteraction by vitamin B-12. Attempts are being made to repeat on a large scale the results obtained in a previous year, that while the intestinal flora in normal tadpoles contain two vitamin B-12 producing micro-organisms, these micro-organisms are absent in microflora of penicillin treated tadpoles. During the year there has been an unusually heavy mortality amongst the collection of *R. tigrina* which can be collected during the rainy seasons only. The investigation will be continued during next year. Tadpoles were treated simultaneously with thyroxine and penicillin, which individually produce antagonistic effects were carried out during the year. From the rather complicated results obtained, it was thought probable that penicillin may not only counteract the metamorphosis producing effects of thyroxine, but may have some effects on other organs. It was therefore thought worthwhile to repeat the experiments on large animals like albino rats. The investigation is proceeding.

Amoeba : It is proposed to undertake some investigations on the respective roles of the nucleus and of the cytoplasm on the growth and multiplication in some unicellular organism like amoeba. During the year attempts were made to grow clones of amoeba from single individuals. Though some success has been obtained the problem of devising a suitable nutrient medium for growing uniformly large sized amoeba proteus in clones has not been completely solved.

Scan: Bl. Lib. Gm

A. PHYSICS

A.1. New Apparatus

A.1.1. *Microphotometer*

The microphotometer whose construction was reported last year in A.1.1. has been recently equipped with a G-10 Varian recorder, and a constant voltage transformer for the light source. Additional provision has been incorporated in the light aperture system, so as to take the microphotograph of the entire length of the spectral lines. In order to eliminate random fluctuations in the light source another photomultiplier which will receive light directly from the source is being appended to the system. The output of these two photomultipliers are combined in such a way as to give the difference of the two. Thus the accuracy of measurement is increased.

A.1.2. *Magnetic Field Strength Meter Using Proton Magnetic Moment*

Nuclear absorption method might be used as an accurate measure of field strength. The instrument described herein is based on this method.

A proton sample (distilled water contained in a glass cell) is mounted within a small coil which is inserted in the magnetic field to be measured, having the axis of the coil at right angles to the direction of the field. R. F. power is fed to this coil at a frequency determined by a heterodyne frequency meter or a communication receiver. When this frequency coincides with the Larmor precessional frequency of the protons, transitions occur which results in the absorption of energy from the r-f field. Absorption of energy changes the circuit impedance, and the resonance is observed as a change of the r-f voltage across the circuit. With a regenerative type detector this energy drop appears as a V-shaped form on the cathode ray oscillograph (C.R.O.) trace. The field strength is determined from the well known equation for the precessional frequency which in the case of proton reduces to

$$H = (234.8 \pm 0.2) \times 10^{-6} \times f$$

where H is in gauss and f in cycles per sec.

Different sets of interchangeable sample coils are used to cover a range 1000 to 3500 gauss. The maximum value of gauss measurable depends upon the characteristic of the electro-magnet used. In order to cover higher range another large electro-magnet is under construction. To determine the frequency a variable frequency signal generator (6 Mc/s to 14 Mc/s) is loosely coupled with the sample coil and varied to obtain the zero-beat, indicating that the two frequencies are the same. Thus the frequency and hence field strength corresponding to any dial setting can be determined. To cover a wide range of frequency (up to 50 Mc/s) we are trying to obtain (i) a heterodyne frequency meter and (ii) one General Radio 805A signal generator. These equipments will help us to extend the range of NMR work to different unknown samples.

The whole arrangement including the sample coil along with its proton sample, has to be shielded properly, otherwise stray pick-ups and noise mask the absorption signal beyond any recognition.

A.2. Ultrasonics

A.2.1. *Measurement of the impedance of a crystal transducer when loaded with different liquids as a function of intensity and frequency*

While carrying out investigations on ultrasonic waves in liquids by radiation pressure and optical methods, it has been qualitatively observed that the characteristics of the transducer are greatly influenced by the (i) load condition, (ii) intensity of ultrasonic energy and (iii) frequency.

Knowledge of the impedance of the transducer helps in understanding the propagation mechanism in liquids and in knowing the limits of validity of the theories so far proposed for the absorption of ultrasonic energy. Theories have been developed on the assumption that the ultrasonic waves surfaces are plane. In order to verify this, we carried out a series of measurements on the impedance of the transducers under varied conditions. Such measurements reveal that the characteristics of the transducer are greatly influenced by the above mentioned factors. Hence the assumption of the plane surface of the waves does not hold good under some of these conditions, the wave-form gets distorted. Fourier analysis of the distorted wave form shows the constituent frequency components; this may account for the large absorption in certain liquids.

In the present investigations, impedance of the transducer was determined from the measured values of the r.f. voltage across and the current through the transducer. The voltage was measured by means of a V.T.V.M. and the current by means of a thermammeter. A variac was used in the primary side of the power supply to vary the intensity of ultrasonic energy, which is taken to be proportional to the d.c. voltage applied to the plate of the oscillator valve. Details of the experiments and the results will soon be published in another place.

A.2.2. *Investigations on finite amplitudes ultrasonic wave propagation in liquids*

During the last two years, investigations on finite amplitudes on ultrasonics wave propagation in benzene have been being carried out by optical method in the frequency range 5 Mc/s to 15 Mc/s (only the odd harmonics were used). These frequencies are the harmonic frequencies of the quartz crystal blank, the fundamental frequency of which is 1 Mc/s. In consideration of the difficulties encountered in the measurements at lower frequencies, the fundamental and the 1st odd harmonic frequencies were not employed in the present set of investigations. About 150 photographs of the diffraction patterns of light formed by the passage of ultrasonics under the following conditions have been taken. (the temperature of liquid has been kept within the range 28–29°C by circulating ice cold water through the annular space of the jacket).

- (1) At different frequencies i.e. 5, 7, 9, 11, 13, 15 Mc/s.
- (2) At different electrical power input to the oscillator valve i.e. at different ultrasonic intensities.
- (3) Absorption measurements were carried out at different frequencies and intensities by shifting crystal position 0.5 cms forward and backward in the direction of propagation of ultrasonic waves about a fixed position of the crystal.
- (4) For velocity measurements : The distance of the centre of the quartz crystal and the screen on which the diffraction pattern is received was measured. The separation between the spectral lines being measured by means of a comparator.
- (5) The usual method of determining the pressure along the wave front of ultrasonic radiation by means of a disc or sphere probe introduces some distortion of the wave surface. An alternative method which is being tried now, is to measure the intensity variation in the diffraction pattern induced in a beam of monochromatic light traversing the liquid at right angles to the direction of the ultrasonic propagations in the liquid. The change in intensity in the diffraction patterns as function of the frequency and intensity of the ultrasonic wave is being measured. From such measurements it will be possible to calculate the pressure along the ultrasonic wave front.

Intensities of the spectral lines of the diffraction pattern are being microphotographed by means of microphotometer described in Sec. A.1.1.

Similar investigations have been carried out with carbon tetrachloride and carbon disulphide. No photographs of the diffraction pattern in these liquids could be taken; due to their large absorption coefficient and low boiling points, thermal equilibrium condition is difficult to attain.

By keeping the apparatus in a low temperature bath for a sufficient length of time, it is possible towards the evening to obtain sufficiently sharp diffraction pattern photographs in carbon tetrachloride. With carbon disulphide such condition of equilibrium is more difficult to realise. Visual observation of sharp diffraction patterns could be made in CS_2 at temperature between 28° — 30° using low intensity u.s. radiation. Alternatively such sharp patterns would be observed in ordinary u.s. field usually by keeping the temperature of CS_2 between 14° — 16°C . At these low temperatures however under the present warm humid conditions lens surfaces and the glass trough become coated with moisture. The investigations are being continued.

A.3. Microwaves

A.3.1. Development of millimeter wave length techniques

It was reported in last year's annual report (vide Sec. A.3.1. (ii)) that investigations on the development of various techniques in connection with the generation of millimeter wave-length radiation had been undertaken. Recently wave guides for 3 cms, and 1 cm

waves, detectors, harmonic generator crystals couplers, bends have been imported from U.S.A. The components received are being assembled, employing additional components made in the workshop.

A3.2. *Resonance Absorption Experiment*

It was reported in the last year's Annual Report (vide Sec. A.3.3.) that investigations (i) on the paramagnetic resonance due to ions in a crystalline lattice whose energy levels can be influenced by external magnetic fields (ii) on the free-radicals produced in living tissues due to radiation, would be taken up in this laboratory.

Accordingly, order has been placed for the following articles out of the special grant made by Government of India for this type of investigations.

- (1) Electromagnet capable of producing about 10,000 gauss field. The magnet core is being made by a local firm as per design supplied.
- (2) Complete stabilised d.c. power supply to feed the electromagnet. This unit consists of :
 - (i) constant voltage transformer
 - (ii) selenium rectifier with stabiliser unit
 - (iii) variac for varying current through the magnet coil so as to get a variable magnetic field.

Copper wires for winding the magnet coil have been purchased. Special type of varnish and coil formers are under purchase.

As reported earlier (A.1.2) proton resonance gauss meter has been developed in the laboratory to measure the magnetic field.

An oscilloscope Tektronic 545A has been ordered for observing and recording the absorption-dip.

A.4. **Cosmic Ray**

A.4.1. *Assymetry in mu-meson decay*

The study on the decay asymmetry of mu-mesons started last year at Darjeeling has yielded very fruitful results. Altogether twenty thousand pictures were taken with a triggering arrangement which photographed stopped particles inside the cloud chamber fitted with eleven $\frac{1}{4}$ " Cu-plates. The purpose of the experiment was to find out the anisotropy if any, in the emission of decay electrons from the stopped muons. The cosmic ray mu-mesons are expected to show a partial polarisation due to a steep fall in the parent pion spectrum at high altitudes. The amount of polarisation has been calculated by Hayakawa from the two component theory of the neutrino and the theoretically expected polarisation has been found to be 23%.

From the twenty thousand pictures taken up to December 1959 we could scan out 881 cases of unambiguous mu-electron decays of which 482 electrons have been emitted in

the upward direction and 399 in the downward direction. The anisotropy found in our case is 1.19 ± 0.07 giving a value of 27% for the magnitude of the polarisation. This is a little higher than that expected from the theory and indicates that a certain percentage of mu-mesons decaying in our chamber might have arisen out of K-e decays in the higher atmosphere. The mu-mesons photographed in our chamber had momentum between 320 to 360 Mev/c at Darjeeling corresponding to an average energy of 1.7 Bev when they were formed.

It has been reported at the last Moscow Conference that the anisotropy found in mu-meson decay electrons increases with the energy of the mu-mesons studied. We have therefore decided to continue this decay asymmetry experiment at Darjeeling with about a metre of lead absorber above our cloud chamber so as to photograph mu-mesons of higher energies. We shall then be able to say whether the decay electrons arising out of these higher energy mu-mesons show a higher degree of anisotropy.

A.4.2. *Mu-meson scattering*

The mu-mesons that we have photographed in our multiplate chamber have been found to be very suitable for the investigation of another aspect of mu-meson interaction, viz., scattering. It is well known that the experimental scattering distributions of mu-mesons at large angles exceed the distributions expected on the conventional scattering theory where the nuclear charge is assumed to be distributed over a sphere of radius $R = 1.2 \times 10^{-13}$ A^{1/3} cm. This effect has been found to be particularly conspicuous at higher energies; but the data at low energies are too meagre to draw a useful conclusion. We have therefore selected about 150 good mu-meson tracks passing through more than four plates and with decay electrons at the point of stopping. These mu-mesons had momenta in the region $40 < p\beta < 170$ Mev/c, and the individual momentum of each particle could be estimated from the range of the particle from the point of scattering to the point of stopping with an accuracy of 2%. Thus we have been able to remove the two serious difficulties encountered in mu-meson scattering experiments viz. (i) the ambiguity in the identity of the particle as a mu-meson and (ii) the difficulty in accurately measuring the momentum of each individual particle at the point of scattering.

There were altogether 903 scattering events and the projected angles of scattering were measured to an accuracy of 0.2° directly on the film with a goniometer eyepiece fitted in a microscope. From these measurements we have obtained a differential distribution of the quantity $p\beta\theta$ and compared this experimental distribution with the theory. The theoretical and experimental distributions agree well upto a value of $x=2$, where $x = 1.37 \times 10^{-3} p\beta\theta$, but shows an excess of scattering beyond $x=2$ the maximum anomalous scattering cross section for this excess has been found to be

$$\sigma_{an} = 9 \times 10^{-28} \text{ cm}^2/\text{nucleon}$$

It has however been felt that in order to collect a large volume of data on mu-meson interaction we have to continue our studies in some underground station with a very big

cloud chamber. We have already in successful operation a big cloud chamber at Calcutta with helium as the ionising gas. We are now attempting to secure some suitable underground station for continuing these important investigations.

The following paper has been published during 1959-60.

Low energy Spectrum of the Sea level electrons and muons at 12°N by Nilima Mishra and M. S. Sinha.

A.4.3. *The IGC Programme*

Cosmic ray intensity measurements with two identical cubical meson telescopes and one neutron monitor pile was continued till November 1959. The bi-hourly values of cubical meson telescope data for a part of the IGY period (Jan. to Dec. 1958) and IGC period (Jan. to Nov., 1959) have been tabulated. The tabulated meson intensity data along with bihourly pressure values have been sent to the Indian Co-ordination Committee.

A few typical variations in cosmic ray intensity have been observed during this period. Sudden decreases in intensity associated with solar flares and magnetic storms have been observed on the following dates : March 25, May 29, and September 15, 1958 and May 11, July 17, 18 and September 2, 1959. Similar variations in neutron intensity have also been observed by other participating laboratories in different parts of the world (private communication). Most of these variations seem to have world wide character, of which decreases on March 25, 1958 and July 17 and 18, 1959 exhibits interesting features. A paper describing these findings will be published soon.

A.4.4. *Investigations on High Energy Nuclear-active Particles*

We have completed the analysis of data obtained at Darjeeling (alt. 2200 metres) with a penetrating shower detector employing an ionisation chamber in combination with shielded counter trays and air shower trays. The energy band of nuclear active particles investigated extends from 12 Bev to 450 Bev. Some of the preliminary results on the absorption (L) and interaction (λ) mean free paths and exponent (γ) of the energy spectrum of nuclear active (*n.a.*) particles up to an energy limit of 200 Bev have been reported last year (vide Annual Report, Bose Institute, A.4. of 1958-59).

The data have been analysed mainly to evaluate the following properties of *n.a.* particles :

- (i) the energy spectrum
- (ii) the charged-to-neutral ratio
- (iii) the interaction cross section of lead and aluminium nuclei.

A.4.4.1. (I) *The Energy spectrum* : The energies of the primary *n-a*-particles inducing penetrating showers have been estimated on the assumption that ion chamber burst pulses associated with a shower event are entirely due to the cascade electrons generated by the decay photons from neutral pions produced in energetic nuclear collisions. The energy of the neutral pions (E_{π^0}) has been derived utilising the standard shower theory formulations. Assuming total inelasticity in nucleon nucleon collisions, the energy of the primary of a nuclear event has been taken to be $E_N = 3E_{\pi^0}$.

The energy frequency distributions of the nuclear events have been divided into two groups :

(a) LPB events or local penetrating bursts which are penetrating showers induced by primary n-a-particles not accompanying an air shower.

(b) EPB events or extensive penetrating bursts which are penetrating showers associated with extensive air showers. The energy frequency distribution of these events have been found to obey a power law with exponents.

$$\gamma = 1.44 \pm 0.15 \text{ for LPB events}$$

$$\gamma = 0.92 \pm 0.12 \text{ for EPB events}$$

The value of the exponent γ ($=1.55 \pm 0.14$) for LPB events reported last year when compared with the present value indicates that the same spectrum is maintained upto about 450 Bev. A single value of γ ($=1.44$) in fair agreement with the exponent of the primary spectrum ($\gamma=1.5$) of the corresponding energy range indicates that within the energy region investigated :

- (i) the shape of the primary spectrum is maintained by the secondaries indicating the same mode of propagation throughout the atmosphere.
- (ii) the absorption length of n-a-particles within the atmosphere is the same within the energy band under investigation,
- (iii) there is no appreciable change in elasticity (α) in high energy n-n collisions.

A comparatively flat distribution for EPB events indicates that n.a. particles associated with air showers are very rich in energy content.

A.4.4.2. II. *The Charged-to-Neutral Ratio* : The charged-to-neutral ratio of n.a. particles inducing penetrating showers has been ascertained from the same experiment by noting the presence or absence of response from a counter tray placed on top of the penetrating shower detector. The results show that the ratio (R) increases from 1.11 at the lowest energy band ($12 \leq E \leq 60$ Bev) to 2.04 at the highest energy band ($240 \leq E \leq 450$ Bev) indicating that pion contribution to the charged n.a. particles increases with the increase of energy up to 450 Bev. This observation is consistent with a relativistic increase in the mean lifetime of pions with increase of energy.

In the lowest energy band ($12 \leq E \leq 60$ Bev) the contribution of pions can be neglected because of the fact that the probability for decay is more than that for interaction in the top layers of the atmosphere. Hence the charged-to-neutral ratio is in effect the proton-neutron ratio. Experimental value in this energy band agrees fairly well with the theoretical value ($R=1.12$) calculated from the formula given by Olbert and Stora and reported by Altmann (Phys. Rev. 107, 1687, 1958). With the experimental value of R for the lowest energy band and using the theoretical formula a value of the elasticity parameter $\alpha = 0.5$ has been derived. This value of α is less than the value ($\alpha = 0.7$) usually assumed in n-n collisions at air shower energies ($E \geq 10^{13}$ ev).

A.4.4.3. *The Interaction Cross Sections* : The Interaction mean free path (λ) in lead and aluminium have been determined from attenuation measurements of the n.a-particles. The interaction cross sections σ_{Pb} and σ_{Al} have been evaluated from the experimental value of λ_{Pb} and λ_{Al} corrected for the non-vertical character of a part of the beam of n.a. particles. The results are shown in Table I.

TABLE I
INTERACTION CROSS SECTIONS (σ) OF LEAD AND ALUMINIUM

Energy Range (Bev)	σ_{Pb} (barns)	σ_{Al} (barns)
12—60	1.62 ± 0.13	0.35 ± 0.027
60—120	1.74 ± 0.16	0.415 ± 0.046
120—240	1.82 ± 0.19	0.423 ± 0.048
240—450	2.02 ± 0.28	0.498 ± 0.094

The purpose of evaluating σ values in different energy groups is to investigate energy dependence, if any, of the interaction cross section. Columns 2 and 3 of Table I show an apparent energy dependence; the cross section increases as the energy of the primary n.a. particle increases. A critical analysis of the experimental set-up and the energy spectrum and interaction characteristics of n.a. particles indicate that such an effect may be a characteristic feature of the method employed in deriving the interaction cross section from attenuation measurements. Further experiments with a more precise set of penetrating shower detector is expected to give a better and unambiguous estimate of the energy dependence of σ . A paper embodying these results is in course of publication.

A.5. Nuclear Physics

A.5.1. *Development of a photon counter with approximately constant special sensitivity*

A gamma ray detector, the counting efficiency of which is approximately constant for a wide range of variation of photon energy, is an useful tool in experimental nucleonics. Such a counter has been constructed in the laboratory by placing a suitable filter before a conventional NaI/Tl. scintillation detector. Using theoretical total cross section data for gamma rays, which have been well-established experimentally, and assuming paraxial incidence of gamma rays on the filter-crystal assembly, we have investigated the effect of variation of the atomic number as well as the thickness of the filter on the performance of the counter. We have found, for example, that a combination of 3.5 cms of aluminium filter before a 2 inch NaI/Tl crystal has an efficiency of $(35 \pm 2)\%$ for photons of energy lying in the range 0.25 Mev to about 6 Mev. This type of counter has been used in experiments on Compton scattering and photoelectric absorption of Co^{60} gamma rays described below.

A.5.2. *Investigations on the angular distribution of Compton scattered Co^{60} photons*

Although the Compton effect is one of the most fundamental interactions between matter and radiation and as such requires a very careful experimental investigation, the measurement of the angular distribution of Compton scattered photons has so far been a rather neglected subject. Using a photon counter of constant spectral sensitivity as described above, we have measured the relative differential cross-section for Compton scattering. The range of the angle of scattering used in the present experiment is 13° to 140° . To avoid the effect of multiple scattering, the experimental values were extrapolated for infinitesimal thickness of the scatterer. The experimental cross-sections agree with the Klein-Nishina distribution and deviate unambiguously from Dirac-Breit-Gordon-Schroedinger distribution.

A.5.3. *Investigations on the photoelectric absorption of Co^{60} photons*

Photoelectric cross-sections of Co^{60} photons in copper, zinc, cadmium, tin and lead have been measured by the sphere transmission technique. Development of the uniform spectral sensitivity photon counter has made this measurement possible. Experimental transmission data are corrected for multiple scattering by extrapolation. Final processing of the data and their comparison with relevant theory is under way.

A.5.4. *Radiocarbon Dating Investigations*

Since the last report, effort was concentrated on obtaining better yield of acetylene from SrCO_3 . The yield of acetylene as well as that of SrCO_3 is now satisfactory. The yield of SrCO_3 obtained from burning of sample wood is above 95% and that of acetylene from SrCO_3 about 85%. We have now started working with raw sample wood. This is mechanically cleaned and then crushed to fine pieces and subsequently treated by dil^(c) HCl and NaOH. After the acid and alkali treatment, the sample is dried in a laboratory oven and then burnt in a special type of electric furnace. Sufficient quantity of SrCO_3 has been prepared from samples wood to date the contemporary sample.

Our next procedure is to test the liquid scintillation technique for the counting of the prepared acetylene at a temperature of -78°C . The lowering of the temperature of the scintillation cell to a temperature of -78°C and maintaining it at a constant value for a period of about 12 hours have, however, proved to be a bottle-neck. The yield of solid carbon dioxide from commercially available carbon dioxide solidifier is very low. We are trying some other method for obtaining higher yield CO_2 snow, or alternately other methods of keeping the scintillating liquid at a steady temperature near -80°C .

All the mechanical and electronic accessories for the liquid scintillation counting of acetylene have been completed and it is hoped that routine operation will be possible in a few months.

A.6. Neutron Generator

A.6.1. The existing 14.1 Mev neutron generator is being remodelled and used as follows. A 4 π -scintillation β -counter has been developed for the study of β -particles from radioactive nucleus obtained by (n, 2n) reaction.

The (n, 2n) reaction of 14.1 mev. neutrons in copper has been studied. The data have been utilised for accurate determination of neutron yield from the generator. The yield obtained was 2×10^9 n/sec under the experimental condition. (Report in the 47th Session of Ind. Sc. Cong. 1960).

A change in the design of the present (t, d) generator has become necessary and work in this direction has already been undertaken. In the existing generator the target is kept at high negative potential and the ion-extractor probe is kept at earth potential. But this has got certain disadvantages which have become apparent when the machine has been put on operational basis. One of the disadvantages is that one cannot approach very near the target to utilise a larger flux of neutrons for experimental purposes.

Hence it is now proposed that the accelerator be remodelled so that

- (i) The target is earthed.
- (ii) The ion-source be kept at high positive potential. To achieve this the following alterations are being undertaken :
 - (i) High voltage Cockroft-Walton generator which gives -240 Kilovolts H.T. is now being changed in design to give + 320 K.V., nominal.
 - (ii) Design of the accelerator tube is being changed, so that it will be vertical and the ion-source remains very near to the high potential end, and the target near the ground.

A.6.2. Construction of a neutron laboratory

A large room ($22' \times 15' \times 18'$) with an underground basement ($18' \times 16' \times 9'$) is being constructed and very soon the generator will be shifted there. The proposed vertical construction of the accelerator tube will enable the grounded end of the discharge tube with the target being extended well inside the basement room, through an opening at the centre of the room ($4' \times 4'$). The ceiling of the basement and the walls of the upper room are being shielded with soil and reinforced concrete, so that when the maximum allowable flux that can be handled is more than 10^{11} n/sec, the neutron flux at the nearest residential quarters is less than 10 n-sec/cm². This has been calculated with a safety factor of 10 and this is much less than allowed radiation corresponding to tolerance, 0.3 rem per week 5 days, 24 hr/day) which is 22 fast neutrons/cm.² sec (the value used at Oak Ridge).

The upper room and the basement are being completely air-conditioned; humidity control will be introduced as far as possible.

This remodelled machine is expected to be put on regular operational schedule from October 1960. Physical experiments like (i) measurement of total cross section, (ii) non-elastic cross section by sphere-transmission method, (iii) measurement of angular distribution of protons from (n, p.) reaction, (iv) angular distribution of neutrons in (n, 2n) reaction—are proposed to be studied. The electronic circuits and other auxiliaries necessary for these investigations are expected to be ready within October.

Biophysical, chemical and genetic experiments are also planned to be conducted with neutrons from the generator. Preliminary biophysical experiments will be mainly on fast-neutron dosimetry in biological media.

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B. CHEMISTRY

B.1. Physical and Biochemistry

B.1.1. Vapour-phase chromatography

Chromatographic columns containing squalane, didecyl phthalate, tricresyl phosphate, and silicone oil were tested for their resolving power of mixtures of organic compounds with different functional groups. Two different supporting media, namely "C-22 fire-brick" powder (40-60 mesh) and purified "Celite 545" were used. It has been found that "Celite 545" is a better supporting medium than "C-22 fire-brick" powder. For the same liquid phase much shorter columns of "Celite 545" can be used with better resolution.

Of the liquid phases, pure didecyl phthalate and ortho-tricresyl phosphate have been prepared. It was observed that the resolving power of the squalane column was very high for saturated and unsaturated hydrocarbons when they were used separately. But when their mixture was used, the peaks overlapped.

The tricresyl phosphate column can separate a mixture of saturated and unsaturated hydrocarbons. It can also resolve a mixture of saturated and unsaturated monohydric alcohols. The liquid phase used in silicone oil column is "D.C. silicon fluid 550". The silicone column can separate the components of a mixture of lower fatty acids and is also useful in case of alcohols. Didecyl phthalate column has been proved to be, to some extent, an all-purpose one. In fact, it is very suitable for esters and ketones. By using these columns in suitable sequence in series it is possible to separate the constituents of a mixture of hydrocarbons, alcohols, lower fatty acids, esters and ketones.

B.1.2. Effect of ionizing radiation on albino rats

(a) Nitrogenous end-products of the urine were studied in a group of six albino rats from the same litter. Urea, uric acid, creatinine, free ammonia, neutral sulphur and individual amino acids including taurine were measured in 24-hour samples of urine. Readings were taken on alternate days. After control readings for more than 2 weeks, the rats were exposed to X-ray from a 200 KV therapeutic set ($\frac{1}{4}$ mm. Cu + 1 mm Al filters; target distance 36 cm). The dose given was 600 r at a rate of 30 r/min. Two rats were kept as controls.

After 12 hr. blood examination was conducted. The total and differential WBC and RBC counts were found to fall steeply. The animals showed marked epilation with anorexia and general muscular weakness.

In urine, urea and uric acid showed marked increase. Free ammonia was increased but to a small extent. Creatinine excretion remained unchanged. Large increase in neutral sulphur output and in taurine was noteworthy. Amongst the amino-acids, the excretions of lysine, leucine, tyrosine and glycine increased. The excretions of α -alanine, valine and phenylalanine remained unchanged.

(b) Thirty female rats, weighing between 120 g. and 180 g., were exposed to gamma-radiation from a Co^{60} source and the urinary excretion products were analysed. The rats were divided into 5 groups of six rats in each group. They were kept in individual metabolic cages. The 24-hr. urine samples were analysed.

Urea, uric acid, free ammonia, creatinine, total and inorganic sulphates were estimated. The urinary amino-acids were also individually estimated after desalting the urine by means of ion-exchange resin.

Of the 5 groups, 2 were kept as controls while the other three were subjected to 300 r, 600 r and 1200 r respectively. These dosages were chosen because they are known to be moderate, lethal and supralethal for rats. The purpose was to find out the correlation if any, between the responses, as evidenced from urinary excretory products of the organism, and the degrees of radiation injury.

In the groups exposed to 300 and 600 r, urea, uric acid, creatinine and free ammonia increased after irradiation, but came down to normal levels between 2nd and 3rd weeks. In the group exposed to 1200 r a secondary rise in free ammonia occurred and all the animals succumbed within the first 2 weeks. The amino-acid increase was significant only in the 1200 r group, where the excretion of glutamic acid, glycine, alanine, valine and phenylalanine was raised. Taurine was not found to increase but marked increase occurred in the excretion of total sulphur. The increase of free ammonia and of total sulphur was the most interesting.

(c) In order to confirm the earlier observation of increase in free ammonia in rat urine following irradiation, experiment was conducted to measure ammonia in urine by employing different methods. Normal urinary pH is acidic, increase in ammonia makes the urine alkaline. So, the pH values of 24-hours' urine samples were recorded before and after irradiation. The urinary ammonia was estimated by micro-Kjeldahl method without and with alkali and also with Nessler's reagent.

Six albino rats were used in the experiment. Two were kept as controls and four were exposed to gamma-radiation from a Co^{60} source. The dosage employed was in the vicinity of 400 r. The urinary pH after irradiation became markedly alkaline and after 3-4 days again became acidic. The controls showed acidic pH throughout the experiment.

The urinary ammonia, estimated by the above methods, showed increase after irradiation. However, the dosage employed being rather low, the rise was not very high.

(d) The possibility that part of the ammonia might be derived from the nitrogenous constituents of the urine has not been overlooked. It was noticed that urea, uric acid and creatinine did not break down during steam distillation.

Glutamine however breaks down to ammonia by steam distillation in presence of alkali or by passage through the resin "IR 120" column.

B.1.3.1 *Preparation and physico-chemical studies on crystalline β -lactoglobulin of buffalo milk*

This work was taken up with a view to making a comparative study of the physico-chemical properties of crystalline β -lactoglobulin of buffalo milk with that of cow's milk. The β -lactoglobulin of cow's milk, first crystallized by Palmer in 1934 (J. Biol. Chem. 1934,

104, 359) has been extensively investigated with the assumption that it is homogeneous. The demonstration by Li (J. Am. Chem. Soc. 1946, **68**, 2746) of its electrophoretic heterogeneity and the discovery of Aschaffenberg and Drewry (Nature, 1955, **176**, 218) that two genetically different β -lactoglobulins occur in cow's milk (some producing two β -lactoglobulins A and B, some only A and some B) rendered questionable much of the earlier data which were in most cases obtained from β -lactoglobulin prepared from pooled milk. Since this discovery, work on A and B β -lactoglobulins of cow's milk (occasionally described as bovine β -lactoglobulin) obtained from individual cows, after typing them according to the method of Aschaffenberg and Drewry by filter paper electrophoresis of whey proteins at pH 8.6, has been taken up with renewed interest in many laboratories.

These observations led to the present work on the whey proteins of buffalo's milk which is widely consumed in this and other eastern countries, to see whether such genetically controlled β -lactoglobulins as in cow's milk occurred also in the milk of buffalo which belongs to the sub-family *Bovinae* of the family *Bovidae*.

Preparation of β -lactoglobulin : Crystallization of β -lactoglobulin of buffalo's milk was accomplished by following the method of Aschaffenberg and Drewry (Biochem. J., 1957, **65**, 273). After lowering the pH of the final concentrated solution of lactoglobulin to 5.2, it was dialysed against glass-distilled water saturated with toluene. Large diamond shaped crystals appeared from the oily layer within 12-16 hours after its separation at the bottom of the bag. Only one type of crystals has so far been observed.

Typing of buffaloes : All attempts hitherto made to type buffaloes according to the method of Aschaffenberg and Drewry indicate that buffalo's milk contains only one type of β -lactoglobulin which corresponds to the β -lactoglobulin B of cow's milk. This was carried out by paper electrophoresis at pH 8.6, both by horizontal and hanging strip techniques, of whey proteins of individual buffalo's milk, running with each experiment a sample of whey protein of cow's milk as standard containing almost equal proportions of β -lactoglobulins A and B. Milk from about 20 buffaloes and a sample of pooled milk collected from 6 buffaloes have been so far examined. Further work is in progress to confirm this observation.

Electrophoresis : Moving boundary electrophoresis of β -lactoglobulin of individual buffalo's milk was carried out in a Tiselius apparatus with 11 ml. cell at 0°C in the pH range 4.00-7.70 at 0.1 ionic strength and different protein concentrations. The isoelectric point is close to pH 5.2, almost the same as in the case of cow's milk. The descending boundary shows partial resolution at pH 4.66 and 4.82 at higher concentrations of about 3%. At other pH values, the descending boundaries appeared somewhat skew. The ascending boundaries were very sharp. The appearance of the descending boundary is similar to that of the β -lactoglobulin B of cow's milk.

Sedimentation : Sedimentation experiments were carried out in a Spinco model E analytical ultracentrifuge at pH 4.8 with protein concentration ranging from 1.5 to 2.9% at 20°C and 1 to 6.8% at 8°C. The value of the sedimentation coefficient, $S_{20,w}$, was found to

be 2.92. Single symmetrical peaks were obtained both at 20° and 8°C indicating correspondence of the material with the β -lactoglobulin B of cow's milk.

The results hitherto obtained seem to indicate that the occurrence of β -lactoglobulin in buffalo's milk is not genetically determined as in the case of cow's milk. Its properties are close to those of β -lactoglobulin B of cow's milk, although further detailed studies which are in progress may reveal differences. It also appears, in view of these observations, that designating β -lactoglobulin of cow as bovine β -lactoglobulin is not warranted.

Further work on typing of buffaloes and other physico-chemical measurements are in progress.

B.1.3.2. *Preparation of crystalline lactalbumin of buffalo's milk*

The crystallization of lactalbumin from the precipitate obtained by lowering the pH of the whey to 4 was carried out by Aschaffenberg and Drewry's method with slight modification. Instead of allowing the ammonium sulphate concentration in the protein solution to increase by slow evaporation, the method of Theorell and Akesson (Arch. Biochem. Biophys. 1956, **65**, 439) for the crystallization of old yellow enzyme was adopted by dialysing the protein solution at 50% ammonium sulphate saturation against a solution of ammonium sulphate, the concentration of which was progressively increased to 70-73% over a period of several days. Long needle-shaped crystals were formed, which did not show any variation in crystal form as in the case of cow's milk.

Filter paper electrophoresis : Paper electrophoresis at pH 8.6 in veronal buffer of ionic strength 0.05 shows that the lactalbumin of buffalo's milk moves faster than that of the cow's milk. There is no band corresponding to the α -lactalbumin.

Further work on this material is in progress.

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

(a) *Studies on crystalline α -globulin from sesame seeds (Sesamum indicum)*:

Preliminary results of sedimentation measurements were reported in the last Annual report (1958-59, p. 29). These measurements have been further extended at different protein concentrations at constant pH and at constant protein concentration at different ionic strengths. The protein concentration has been varied between 0.47% and 1.73%. Proportions of the two components appear to remain unchanged although the value of the sedimentation co-efficients, as expected, increases with decreasing protein concentration. The sedimentation co-efficients $S_{20,10}$ of the slow and fast moving components were determined respectively as 14.4, and 21.1. The effect of variation of ionic strength from 0.35 to 1 at pH 7.62 and with protein concentration of 0.34% also does not indicate any change in proportion of the components present. The material, appears to be a mixture of monomer and dimer units. Another series of experiments at different pH values at constant protein concentrations have been carried out. The material is stable and the two components exist in constant proportions between pH 5 and 9.

(b) *Study of mustard seed protein:*

The work on proteins from mustard seeds has been extended. It has been possible to purify the fraction A by ammonium sulphate precipitation and alcohol fractionation at -5°C . By the later procedure most of the colouring matter and the minor component were eliminated as found from the ultracentrifugal patterns. Similar methods are being adopted to purify the fraction B.

B.1.5. *Studies on toxic proteins of plant origin*

(a) *Isolation and characterization of the toxic protein of Abrus precatorius:*

Seeds of *Abrus precatorius* have been reported by various workers to contain a toxic albumin "abrin", which was found to be quite fatal in small doses and also remarkably haemagglutinating with human cells. The present work was undertaken with a view to isolating the toxic and haemagglutinating fractions in as pure a state as possible and determining their physico-chemical and biological properties.

Abrus seeds after decortication and crushing in a grinding mill were extracted by shaking the ground meal in a mechanical shaker with six times its volume of phosphate buffer containing NaCl (Na_2HPO_4 0.06M, NaH_2PO_4 0.04M, NaCl 1M) at pH 6.4 in the cold room for 10-12 hours. The extractable nitrogenous material in the meal was found to be 51-54% of the total. Of this approximately 37-40% represented non-dialysable products. The extract after filtration and centrifugation was saturated to 80% with ammonium sulphate to precipitate the total proteins. This was further fractionated according to Danielsson's method (Acta Chem. Scand. 1950, 4, 672) of isoelectric precipitation applied to proteins of leguminous seeds. The precipitate of total proteins was dissolved in phosphate buffer containing Na_2HPO_4 0.03M, NaH_2PO_4 0.02M and NaCl 0.2M and the solution was dialysed exhaustively in the cold against water. The residue (R) and supernatant (S) both showed strong haemagglutinating activity.

Treatment of the residue: The solution of the residue in 2% NaCl on dialysis against acetate buffer of pH 5.2 for several days, gave a precipitate which on further purification from contaminants from the supernatant (SR) showed no haemagglutinating activity. On the other hand the proteins of the supernate (SR) showed strong haemagglutinating activity in dilutions of 1 in 25000 approximately.

Treatment of the supernatant (S): After precipitating the protein of this fraction with 80% ammonium sulphate, it was treated with acetate buffer of pH 5.2 to remove the non-haemagglutinating fraction insoluble at this pH. From the solution, after discarding the undissolved residue by centrifugation another haemagglutinating fraction was obtained by dialysing it against a buffer of pH 4.6. This fraction showed haemagglutinating activity in dilutions of 1 in 10000.

Filter-paper electrophoresis: Purification of different fractions was followed by paper electrophoresis at pH 8.6 in veronal buffer of 0.05 ionic strength. The supernatant (SR) showed three bands one of which corresponded to the haemagglutinating component of the supernatant (S). Very high haemagglutinating activity of the supernatant (SR) would

suggest that the other two components make a substantial contribution to the activity, part of which, of course, is due to the presence of the component from supernatant (S) as an impurity which is very difficult to remove.

The fraction obtained from the supernatant (S) by treatment as described gave a single band.

Sedimentation : Sedimentation experiments with a solution of the total protein precipitate showed the presence of four components. The non-agglutinating component precipitated by dialysis of the solution of residue (R) against pH 5.2 showed the presence of two components of $S_{20,w}$ 7.65 and 11.85. They are likely to be monomeric and dimeric forms of the protein. The agglutinating component obtained from the supernatant (S) by dialysing against pH 4.6 showed a single sedimenting component of $S_{20,w}$ 4.67.

Further work on purification of different components and determination of their toxicity on animals is in progress.

(b) *Isolation of toxic proteins of Croton tiglium*:

Seeds of *Croton tiglium* after decortication were freed from oil (40% oil content) first by pressing under a hydraulic press and subsequently by extraction with petroleum ether (40-60°C) for 12 hours. The oil-free seed meal prepared from different lots of seeds was found to contain 6-8% N. The meal was extracted with 10% NaCl both in the cold and at room temperature. The extractable nitrogen is about 34% at room temperature. The NaCl extract after filtration and centrifugation was fractionally precipitated by ammonium sulphate at 50% and 80% saturation.

Preliminary observations on toxicity showed that the 50% fraction is toxic to mice in doses of 10 mg. of protein dissolved in phosphate -NaCl buffer. Death ensues 20-22 hours after intraperitoneal injection. The 50-80% fraction is non-toxic and non-agglutinating. Agglutinating tests with human RBC and sheep RBC showed that the 50% fraction does not agglutinate human RBC but sheep RBC is rapidly agglutinated by it, the activity being retained in dilutions of 1 in 6000.

Further work is in progress.

B.1.6. *Activities of bacteria-free preparations from Vibrio cholerae*

The work reported in the last Annual Report (1958-59, p. 30), is being continued with special reference to the factors in the production of filtrable enterotoxin. The effects of varying the constituents, pH etc. of the media, the temperature of incubation and the strains of *V. cholerae* are being studied.

B.1.7. *Biochemical properties of natural coumarins*

In continuation of the previous work (this Report 1958-59) on the effect of psoralene on the tyrosinase activity and melanine formation, it has been observed that after feeding toads (*Bufo melanostictus*) with psoralene, melanine formation in the skin and liver tissues was enhanced. An increase of tyrosinase in the liver and in the dermal layer of the skin

was also observed, the increase being remarkable in the case of the dermal layer. In *in vitro* experiments, tyrosinase in the dermal layers of the skin and in the liver was slightly increased but no significant change in the melanine pigmentation of the liver or skin was noticed. The enhanced melanine formation has been estimated histochemically as well as spectrophotometrically. After feeding psoralene, liver and kidney did not show any neutral lipid in it but fat was present in both capsules and tubules of testis.

A histochemical method for the detection of melanine in amphibian subject has been developed. The method may also be used for bio-assay of drugs which may be expected to be useful in the chemotherapy of leucoderma. This method was employed to test some coumarins for their ability to augment melanine formation.

Xanthotoxin, psoralene, iso-psoralene and seselin were tested for their tyrosinase activity. Psoralene was found to be most active of all the compounds.

B.2. Plant Chemistry

B.2.1. Investigation of *Centella asiatica*

The methyl ester of the triterpene acid, obtained from the alcoholic extract of the defatted plant (*vide* previous Report), has been identified as methyl ester of asiatic acid, $C_{31}H_{50}O_5$, m.p. 220-21°, $[\alpha]_D^{32} + 54.5^\circ$ ($CHCl_3$) by comparison of its mixed m.p. with an authentic sample of methyl ester of asiatic acid. Further work on other constituents of the plant is being continued.

The antibacterial activity of the purified glycoside of *C. asiatica* was further studied and no activity has been found against *Eberthella typhosa*, *Bacillus anthracis*, *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas pyocyanea* and *Vibrio cholerae*.

B.2.2. Chemical investigation of *Helichrysum* flowers

Further examination of the yellow pigment obtained from the flowers of *Helichrysum annum* revealed that luteolin (previous Report), presumed to be present in the flower as glycoside, occurred also in the free state. Two other non-glycosidic flavonoid constituents were also found to be present in traces by means of paper chromatography.

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

(a) Further investigation of the fruits of *Barringtonia acutangula*.

Isolation of three new neutral sapogenins, namely, acutagenol A, B, C and two acid sapogenins was reported from the fruits of *B. acutangula* (*vide* previous Reports). One of the acid sapogenins was identified as barringtogenic acid, present in *B. racemosa* (Pillai and Anantaraman, *J. Chem. Soc.*, 1956, 4369). Results of further investigations and additional data on the sapogenins previously mentioned are now presented.

It is felt desirable that acutagenol A, B, and C should be re-named as barringtogenol B, C, and D respectively, barringtogenol from *B. racemosa* being regarded as barringtogenol A.

Barringtogenol C, $C_{30}H_{50}O_5$, m.p. 315-20°, $[\alpha]_D^{28} + 33.8^\circ$ (dioxan) gave a pentaacetate, $C_{40}H_{60}O_{10}$, m.p. 222-24°, $[\alpha]_D^{30} + 18^\circ$ (ethanol) and a pentabenzoate, $C_{65}H_{70}O_{10}$,

m.p. 315-17°, $[\alpha]_D^{20} + 30.9$ (dioxan). It contained one hindered double bond (perbenzoic acid titration) which could not be hydrogenated over Adams' catalyst. It has an α -glycol system but it did not form any isopropylidene derivative with acetone. The elimination of HCHO, characterized by dimedone derivative, in the Cu-bronze oxidation of barringtogenol C suggests the presence of $\begin{array}{c} | \\ -C(OH)-C-CH_2OH \\ | \end{array}$ group in the molecule. The formation of a 11-keto derivative with CrO_3 in boiling acetic acid, having a characteristic UV absorption (λ_{max} 245 m μ) indicates barringtogenol C to be a triterpene alcohol of the β -amyrin group. Further, it yielded a 12-keto derivative (saturated to tetranitromethane) with H_2O_2 in acetic acid. It formed a crystalline DNP derivative of m.p. 265-67°.

Partial benzylation of barringtogenol C afforded a tribenzoate, m.p. 274-75°, which did not consume any periodic acid. The tribenzoate on chromic acid oxidation gave a colourless crystalline diketone, m.p. 270° (saturated to tetranitromethane), λ_{max} 232 m μ .

Barringtogenol D, $C_{30}H_{48-50}O_4$, m.p. 305-10° furnished a crystalline triacetate, $C_{36}H_{54-56}O_7$, m.p. 230-31° $[\alpha]_D^{20} + 74^\circ$ ($CHCl_3$), but on benzylation it gave a glassy product. It contained a hindered double bond as it slowly consumed approx. 1 mole perbenzoic acid and could not be hydrogenated over Adams' catalyst. It may be presumed on biogenetic grounds and also from IR spectrum, that barringtogenol D might also belong to the β -amyrin group. There is no evidence of the presence of a carbonyl group in the molecule from the IR spectrum or from its reaction with sodium borohydride or lithium aluminium hydride and with other carbonyl reagents under standard conditions. Three of the four oxygen atoms in the molecule are part of three hydroxyl groups. The triacetate was indifferent to CrO_3 -pyridine complex, but in boiling acetic acid CrO_3 oxidized it to a colourless product, m.p. 254-58° (λ_{max} 241 m μ and saturated towards tetranitromethane) which formed no DNP derivative. Attempted dehydration with $POCl_3$ also failed. The IR spectrum of the triacetate does not show any band for hydroxyl group. The inert oxygen is probably present in an oxide ring. This was supported by the infrared band at 9 μ , characteristic of $-C-O-C-$ of an oxide ring. The oxide ring could not be opened by boiling the triacetate in acetic acid in presence of hydrochloric acid. Hydrogen chloride in Ac_2O converted the substance into a colourless crystalline tetra-acetate, m.p. 282-85°, $[\alpha]_D^{30} - 12.5^\circ$ ($CHCl_3$).

Barringtogenol D did not consume periodic acid. When oxidised with CrO_3 in pyridine it yielded a colourless crystalline product, m.p. 285-92° (DNP, m.p. 225-30°) which showed positive Zimmermann's test for a keto-group in position 3. It gave no colour with $FeCl_3$ indicating the absence of diosphenol. Since the molecule contains no α -glycol system, positions 1 and 2 in ring A may be assumed to be unsubstituted.

Further elucidation of the structures of barringtogenol C and D is being attempted.

(b) *Albizzia lebbek* Benth

From the neutral fraction of the sapogenin mixture, obtained from the pods of *A. lebbek* Benth., the isolation of a new triterpene named albigenin $C_{29}H_{46}O_2$, m.p. 226-28° $[\alpha]_D - 114^\circ$, has been reported (*vide* previous Report). It was shown to contain one hydroxyl

group and a carbonyl group. Modified isolation methods gave a larger yield of the substance, the constitution of which it has now been possible to elucidate.

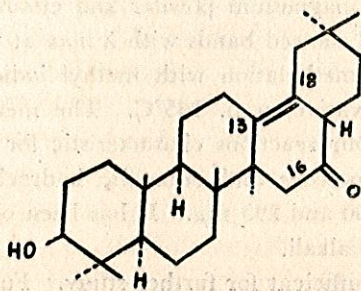
The infra-red spectrum of albigenin taken in a Baird-IR spectrophotometer showed bands at 3655 cm^{-1} and 1695 cm^{-1} characteristic of hydroxyl and carbonyl group respectively. Further, the infra-red spectrum of the sapogenin showed it to be a triterpene rather than a steroid.

Albigenin formed an acetate, and a benzoate $\text{C}_{36}\text{H}_{50}\text{O}_3$, m.p. $312-15^\circ$, $[\alpha]_D -72^\circ$ (CHCl_3). Albigenin when oxidised with chromium trioxide-pyridine complex afforded a colourless compound (albigenone) $\text{C}_{29}\text{H}_{44}\text{O}_2$, m.p. $200-204^\circ$, $[\alpha]_D -98^\circ$ (CHCl_3). Albigenone responded positively to Zimmermann colour reaction while the parent compound failed to do so. This demonstrated that the hydroxyl group in albigenin occupied the C_3 position of the triterpene nucleus.

Albigenin or its acetate could not be hydrogenated under standard conditions and resisted osmic acid attack. In its rate of consumption of perbenzoic acid (*vide* previous Report) and inertness towards hydrogenation and osmic acid attack, the double bond in albigenin resembled that of albigenic acid in which it is at the 13:18 position.

The epoxide obtained from albigenin acetate on treatment with hot acetic acid containing mineral acid gave two compounds which have been indentified as 3-acetyl-16-oxo-28-nor-olean-11:13-diene, and 3-acetyl-16-oxo-28-nor-olean-12:17-diene respectively on the basis of their characteristic ultra-violet absorption spectra. The formation of these compounds necessitates albigenin to be a nor-compound and this also accounts for the C_{29} formulation of albigenin. The occurrence of such a nor-compound in nature can be easily visualized as being the result of a decarboxylation of the corresponding β -keto-acid which seems highly probable in view of the close association of albigenin with albigenic acid and echinocystic acid both of which are β -hydroxy acids.

A direct correlation between albigenin and albigenic acid has been achieved on the basis of an unambiguous partial synthesis of albigenin from albigenic acid on the following lines. The epoxide obtained from methyl 3-acetyl-16-keto albigenate on saponification under mild alkaline conditions, followed by reacylation, furnished the epoxide obtained from albigenin acetate by the action of perbenzoic acid. On the basis of the above experiments albigenin is represented as follows :



Incidentally albigenin happens to be the first example of a naturally occurring triterpenoid of the oleanane series in which the keto group is in position 16.

From the petroleum ether extract of the pods of *A. lebbeck* Benth. a sterol mixture was obtained from which γ -sitosterol was isolated and characterised.

(c) *Albizzia procera* Benth

Since a number of triterpene constituents have been obtained from the beans of *Albizzia lebbeck* Benth (this Report), a re-investigation of *Albizzia procera* Benth. from which machaerinic acid has been isolated by Farooq *et al.* (*Compt. rend Acad. Sciences*, 1958 **246**, 3261, was thought desirable. From the alcoholic extract of the defatted beans of *A. procera* we have isolated a good amount of neutral sapogenin in addition to acid sapogenins. The neutral fraction on acetylation and chromatography could be separated into two fractions : (1) colourless crystals, m.p. 300-305° and (2) a glassy low-melting fraction giving a pink Liebermann-Burchardt test and a yellow colour with tetranitromethane. The work to characterize the constituents is being continued.

(d) *Studies on the structure and stereochemistry of entagenic acid*

Evidences for the formulation of entagenic acid (*Naturwiss.* **43**, 250, (1956) as 21:22-dihydroxy oleanolic acid have been obtained on the basis of synthetic experiments.

B.2.4. *On the constitution of mesuol*

Further work on the constitution of mesuol (this Report 1957-58 and 1958-59) has been done. It yielded acetophenone and acetone on chromic acid oxidation. It was previously observed that dimethyl mesuol gave on similar degradation acetone while tetrahydromesuol gave only acetophenone. The present observation further substantiates the presence of an isopropylidene group in the side-chain of mesuol.

It has been observed that mesuol could be cleaved with 10% aqueous caustic potash at room temperature. The reaction products isolated were :

(a) an oily neutral product, (b) an acidic crystalline constituent, m.p. 160° and (c) a phenolic product, m.p. 171°.

The oily product (a) gave a 2:4-DNP derivative, m.p. 166° identical with that of acetaldehyde.

The phenolic constituent gave an olive green colour with ferric chloride. It did not show any colour change with magnesium powder and ethanolic hydrochloric acid. The UV spectrum of the compound showed bands with λ max at 225, 234, 260, 275, 285 and 338 m μ . The compound on methylation with methyl iodide in presence of potassium carbonate gave a methyl derivative, m.p. 135°C. The methyl derivative on treatment with alcoholic alkali gave colour reactions characteristic for coumarins. It did not give any colour with magnesium powder and ethanolic hydrochloric acid. The compound showed UV maxima at 260, 290 and 295 m μ . It has been observed that dimethyl mesuol is stable towards 40% aqueous alkali.

The acidic product was insufficient for further study. Further work is in progress.

B.2.5. *On the constitution of mesuone*

Further studies on the constitution of mesuone (this Report 1957-58) are reported.

It was observed that mesuone can only be acetylated in the cold with pyridine and acetic anhydride. The crystalline colourless acetate melted at 128°. The UV spectrum of the compound showed a maximum at 248 m μ . Attempted acetylation of mesuone in hot condition using pyridine-acetic anhydride and acetic anhydride-sodium acetate resulted in the formation of a tarry mass from which no crystalline product could be isolated.

Mesuone on hydrogenation in presence of palladised charcoal yielded an oily product which could not be crystallised. The hydrogenated product was treated with 40% aqueous caustic potash. From the reaction mixture an alkali-soluble colourless compound, m.p. 260° was obtained. The compound did not respond to ferric reaction.

The UV spectrum of the compound showed absorption maxima at 230, 250, 280, 325 and 340 m μ . IR spectrum of the compound showed bands characteristic for hydroxyl, carbonyl and gem-dimethyl groups.

On alkaline hydrolysis with 50% aqueous caustic potash mesuone yielded formaldehyde (identified as its 2:4 DNP derivative) and a crystalline phenolic compound, m.p. 210°. The compound shows UV maxima at 265 and 335 m μ . Further work is in progress.

B.2.6. *Chemical examination of Murraya kœnigii*

From the stem-bark of *M. kœnigii*, a sterol m.p. 136° and two other neutral constituents melting at 280° and 189° respectively were isolated.

The compound melting at 189° (C, 71.28; H, 6.9; methoxyl 15.7%) gave characteristic reactions for coumarins. With conc. H₂SO₄, it gave a yellow colour. The product was found paper chromatographically homogeneous. The UV spectrum of the compound showed bands with $\lambda_{\max}$ at 240, 275 and 330 m μ , suggesting coumarinic character of the compound.

The product melting at 280° (C, 82.81; H, 7.70%) gave no characteristic colour reactions for steroids or triterpenes. Neither did it give any reaction for coumarins. The UV spectrum of the compound showed bands with $\lambda_{\max}$ at 245, 270 and 305 m μ .

Further studies are in progress.

B.2.7. *Chemical examination of Glycosmis pentaphylla*

From the root-bark of *Glycosmis pentaphylla* some chemical constituents have been isolated. One of them was γ -sitosterol. The second neutral product, m.p. 130° was obtained in low yield. The UV absorption spectrum of this pale yellow compound showed bands with $\lambda_{\max}$ at 225, 272, 295 and 325 m μ . The IR spectrum of the compound showed the presence of bands characteristic for hydroxyl and lactone groups in conjugation with a double bond.

The phenolic constituent melting at 260° yielded a colourless crystalline acetate melting at 281°.

B.2.8. Chemical examination of *Swietenia mahogani*

From the seeds of *Swietenia mahogani*, a microcrystalline non-bitter substance, m.p. 220° was isolated. It appears to be different from the one reported by Guha Sarkar and Latif (*J. Indian Chem. Soc.* 1951, **28**, 485). Studies on the structures of this compound and associated products have been taken up.

B.2.9. Alkaloid Survey

From the root-bark of *Glycosmis pentaphylla* three alkaloids have been isolated. Two of them were identified as skimmianine, and dictamnine respectively. The third compound, m.p. 140°, is probably γ -fagarine, $C_{13}H_{11}O_3N$, as it was found to give a UV spectrum characteristic for furoquinolines.

Alkaloids in traces were also detected in the stem-bark of the plant.

From the stem-bark of *Hunteria corymbosa* an amorphous base was isolated as its picrate (decomp. at 200°). Further work is in progress.

B.2.10. Paper chromatographic separation of natural coumarins

In continuation of our previous investigation further studies with following compounds were undertaken. Psoralene, iso-psoralene, psoralidine, psoralidine dimethyl ether, wedelolactone, tri-O-methyl wedelolactone, dalbergin, nor-dalbergin, mesuol, and 5:7-dihydroxy-4-phenylcoumarin.

It was observed that ethanol : water mixture is the most suitable solvent for 3-phenylcoumarin derivatives like wedelolactone, tri-O-methylwedolactone, psoralidine and psoralidine dimethyl ether. Further work is in progress.

B.2.11. Preparation of some mutagenic compounds

Ethylenimine and its derivatives are potent pharmacological and good alkylating agents. The reactive imines and epoxides have been found to show strong mutagenic action on micro-organisms and plants. The following imine and epoxide derivatives have been prepared to study their mutagenic activity and also to find out a correlation, if any, between mutagenic activity and structure of the chemical agents.

- (a) 2:2-Dimethyl ethylenimine
- (b) 2-Phenyl-2-propyl ethylenimine
- (c) 2-Phenyl-2-ethyl ethylenimine
- (d) Epoxide of mesityl oxide.

The intermediates for the synthesis of the following compounds have been prepared : 2-phenyl-2-ethyl-3-methyl-ethylenimine; 2:2-diphenyl-3-methyl-ethylenimine; glycidyl ether of resorcinol and glycidic nitrile. The work is being continued.

B.2.12 Chemical investigation of Indian plant resins

Systematic investigations of the chemical nature of the plant resins, especially of the nonvolatile portions, have been undertaken.

The petroleum ether extract of *Hunteria corymbosa* yielded a thick resin, which on steam distillation gave a small amount of volatile oil. The non-volatile portion was found to contain some acidic fraction.

The neutral fraction was chromatographed over alumina when petroleum ether eluted almost the whole of the resin as a glassy mass which could not be crystallized.

The glassy mass was found to be transparent in UV light. It was directly acetylated and attempts for its crystallization by chromatography over alumina were of no avail.

Benzoylation of the resin yielded an amorphous mass m.p. 126-214°, which showed characteristic absorption [λ max 232m μ] for Ph-CO group in the ultraviolet spectrum indicating the presence of the free OH group in the compound. On hydrolysis this yielded a material m.p. 132-170° showing no tendency to crystallise. It gave a blue-green colouration in the Liebermann-Burchardt reaction indicative of the presence of sterols.

The acidic fraction which was obtained in low yield was directly esterified with diazomethane and chromatography over alumina yielded small amount of solid m.p. 182-210°. It gave no colouration in the Liebermann-Burchardt test. Attempts to isolate this fraction in larger amounts are being made.

The resins obtained from the petroleum ether extract of the bark of *Jatropha curcas* and *Pterocarpus marsupium* are under investigation.

B.3. Radiochemistry

B.3.1. Photosynthesis

The investigation on the role of iron-containing compounds in the primary CO₂ fixation reactions in photosynthesis has been continued. The occurrence of an iron-containing compound in the 60% acetone precipitable substances of *Antigonum leptopus* leaves has been confirmed by using Fe⁵⁹ as the tracer. Owing to its radiation characteristics (β , 0.46, 0.26 MeV; γ , 1.3, 1.1 MeV) Fe⁵⁹ is a very convenient tool for this type of investigation. Leaf discs 1 cm in diameter were allowed to metabolise Fe⁵⁹Cl₃ at pH 6.0 in high intensity light for 4 hr. and thoroughly washed with water. The discs were then divided into two parts and one part was suspended in a solution of NaHC¹⁴O₃ for 1 min. The acetone precipitates of the extracts of leaves allowed to fix C¹⁴O₂ were found to contain both Fe⁵⁹ and C¹⁴. Paper chromatographic analysis using butanol-acetic acid-water as the solvent system revealed both C¹⁴ and Fe⁵⁹ activity in a spot near the origin.

B.3.2. The action of plant growth hormones

(i) *Ascorbic acid and growth.* It has been suggested by Chinoy that the growth response of *Avena* coleoptiles floated on sucrose solutions is due at least in part to the formation of

ascorbic acid in the incubation mixtures, which itself has growth-promoting properties. Since ascorbic acid is known to be produced from glucose it would be expected that the known intermediates of ascorbic acid synthesis, namely, glucuronolactone, gulonolactone etc. should also elicit comparable growth response by gradual conversion to ascorbic acid. This was found to be so. Other sugars like galactose, lactose etc. were inactive. Since ascorbic acid decomposes in aqueous solution, fresh ascorbic acid had to be added once every 2 hr. to obtain the growth response; the same procedure had also to be followed for the lactones. When however C^{14} -labelled sucrose (prepared biosynthetically through photosynthesis by green leaves) was supplied to the coleoptiles for 3 hr. no C^{14} -activity was detectable in ascorbic acid. This could be due to breakdown during experimental manipulations or due to conversion to some other compound the identity of which is not known at present.

(ii) *The binding of auxins to protein.* It is generally believed that for an auxin molecule to be effective in growth, it has to be bound to protein. This possibility has been investigated using naphthalene acetic acid -1- C^{14} and indole acetic acid -2- C^{14} . Ten mm. sections of the 2nd or 3rd internode of etiolated pea (var. Little Marvel) were floated on solutions containing NAA-1- C^{14} and IAA-2- C^{14} for 3 hr. in darkness at 23°C. The coleoptiles were then extracted in phosphate buffer of pH 6.0, centrifuged and 0.1 M cold trichloroacetic acid added to the supernatant to precipitate the proteins. The residue obtained by centrifugation was washed several times by resuspending in TCA followed by centrifugation, dried and counted. The C^{14} -activity was detectable in either case. IAA- C^{14} was also found to be bound to the root proteins. The IAA-protein could also be separated with acetone and $(NH_4)_2SO_4$ between 40 and 60% saturation.

To investigate the possibility that the beneficial effect of CO_2 on auxin-induced growth could be exercised through its effect on the binding of auxins to proteins, the internode sections were incubated with the C^{14} -labelled auxin in presence of different concentrations of CO_2 provided through the incorporation of $NaHCO_3$ in the experimental solutions. It was observed that such a low concentration as 625 $m\mu g/ml$. did stimulate the binding of auxins to proteins, but higher concentrations were inhibitory. Thus only low concentrations of CO_2 are beneficial for the binding of auxins to protein.

An investigation has also been undertaken to study the relationship of the binding of auxin to protein synthesis in the tissues. Of the amino-acids tried so far, only aspartic and glutamic acids have been found to be stimulatory. It may be mentioned here that aspartic acid is known to conjugate with IAA forming indoleacetyl aspartic acid. The contribution of this type of IAA conjugates to the synthesis of IAA-bound proteins still remains to be investigated.

B.3.3. *Tracer studies on photoperiodism*

In an earlier investigation it was observed that apices of induced rice plants when supplied with $C^{14}O_2$ during the light period did not contain any new C^{14} -labelled compound as compared to the 'control' which would be expected according to the hormonal concept of, and CO_2 requirement for, the flowering of short-day plants. It could be argued however

that the pool size of such a 'hormone' is very small and a much longer period of equilibration is required for such a compound to be tagged. This time could further be shortened by increasing the specific activity of the $C^{14}O_2$ used. Rice plants were therefore allowed to photosynthesise in 15 mc of $C^{14}O_2$ for several days during the induction period and the buds analysed for C^{14} -labelled compounds by two dimensional paper chromatography. The earlier finding that there is quantitative but no qualitative difference was confirmed.

The above observation, however, applied only to the 80% alcohol-soluble fraction. Hydrolysis of the proteins of these plants, of plants treated with $Na_2S^{35}O_4$ to tag the sulphur amino-acids and of apices of rice plants allowed to metabolise acetate - $1-C^{14}$ has revealed the occurrence of the same amino-acids but in altered ratios. It is possible that proteins of similar amino-acid composition but with an altered sequence of occurrence are responsible for this observation. This would imply that the apices of induced plants synthesise new proteins from the amino-acids supplied from the leaves. It would be of considerable interest to see whether nucleic acid synthesis in the shoot apices is also similarly affected. Unless the florigenic property of such macromolecules is demonstrated, any suggestion in this connection must be considered as highly speculative.

B.3.4. *Gibberellic acid*

Investigations on the biosynthesis and metabolism of gibberellic acid have been started in this laboratory from December, 1959.

The problem is being attacked from three different standpoints : (i) the physiological nature of gibberellin action within the plant tissue, (ii) isolation of the growth-active compounds formed in the culture filtrate of *Gibberella fujikuroi* and (iii) determination of the possible pathway of biosynthesis of gibberellic acid.

In the study of growth-active substances, a consistent and reproducible bio-assay method is a prerequisite. Two bio-assay methods were formulated for routine work. In one method the elongation of the first leaf and first leaf sheath of rice seedlings grown in gibberellic acid solutions was taken as a criterion of growth activity. Subsequently, gibberellic acid purified from culture filtrates of the fungus *Gibberella fujikuroi* was tested by this assay method and positive results were obtained. The other assay was carried out with dwarf maize according to the method of Phinney. Eluates of the gibberellic acid zone of the chromatograms with known amounts of the acid, as well as the eluates of the gibberellic acid zone of purified culture filtrates produced growth effects permitting quantitative estimation.

In order to study the physiological action of gibberellic acid a series of experiments was carried out with rice (var. Rupsail) seedlings. Almost a linear relationship between growth and concentration was observed over the concentration range 0.1—10 ppm. A concentration of 100 ppm. showed a definite inhibitory effect on shoot growth. The growth of the shoot as reflected in the growth of the first leaf, however, was restricted only to the elongation of the leaf sheath. The growth of the leaf-blade was very little affected. It was further observed that growth effects due to gibberellic acid treatment become clearly discernible 12-14 days after treatment. In the initial stages no significant difference in growth could be

detected. Some of the possible interpretations of this delayed action are : (a) Gibberellic acid itself may not be growth active but it is broken down to some other substance(s) within the tissue and (b) it leads to an alteration in the endogenous auxin level of the plant which brings about increased extension growth. It was also observed that gibberellic acid was readily absorbed by the root and the optimum period of absorption was 24 hours.

In very young seedlings the base of the first leaf enclosed within the coleoptile is responsive to the action of gibberellic acid in extremely low concentrations (e.g. 10^{-13} — 10^{-12} M). The different regions of the leaf base respond in different manners.

The relationship between light and gibberellic acid treatment has been studied in some detail. In var. Rupsail it was observed that the growth effects in seedlings due to gibberellic acid treatment were much more magnified in the light than in the darkness. This study on light effects was further extended to four dwarf varieties of rice, viz., Mitsuryuto 832-Japan Dwarf, Nejureba 803-Japan Dwarf, T432-Madras Dwarf and T433-Madras Dwarf. From this investigation it was inferred that gibberellic acid promotes growth in all the varieties in light as well as in darkness. Light (from fluorescent lamps) inhibited growth and this inhibition was removed by gibberellic acid in varying degrees in different varieties. A gradation has also been observed in the inhibitory effect of red light—from none at all to pronounced action. This inhibitory effect was also eliminated by gibberellic acid in different degrees. Infra-red reversed the red-light effect only in one instance and gibberellic acid counteracted the action of infra-red in all cases. These observations on the interaction of gibberellic acid and red and infra-red light in relation to leaf growth should be considered in a proper understanding of the mechanism of action of gibberellic acid.

Growth-active compounds formed in the culture filtrate of *Gibberella fujikuroi* have also been studied. Three different strains obtained from the Northern Regional Research Laboratory, Peoria, Illinois, U.S.A., Bedford College, London, and Indian Agricultural Research Institute, New Delhi, have been used. All the three cultures have been found to produce gibberellic acid which was identified both by bio-assay and chromatographic methods. C^{14} -labelled acetate - $2-C^{14}$ has now been incorporated in culture media and the isolation of gibberellic acid - C^{14} is in progress.

B.4. Enzyme Chemistry

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

Part of this work has already been reported (Trans. Bose Institute, 1960); and part has been presented in the April, 1960 meetings of the Federation of the American Societies for Experimental Biology, Chicago, U.S.A. (cf. Fed. Proc., **119**, 348, 1960). A preliminary communication is in press (*Nature*).

In continuation of the studies already reported on the mechanism of protein synthesis in the intact cells of *Azotobacter vinelandii*, a more detailed investigation has been made with the cell-free preparations of *A. vinelandii*. C^{14} -glutamate and C^{14} -glycine have been used to study their incorporation into the particulate preparations. Of the various fractions

tested the fractions sedimenting between 2,000—10,000 *g* and 10,000—30,000 *g* are found to have maximum activities. With the careful preparation of the particulate fractions which represent mainly the cell-wall fragments and membranes, the incorporation of the amino-acids becomes independent of externally added ATP as the particles themselves are able to generate ATP by oxidative phosphorylation. The incorporating system is found to be susceptible to the actions of 2:4-dinitrophenol, KCN and anaerobiosis. The rate of incorporation of the amino-acids is linear with time up to about 1½ hours and the incorporation reaction is found to have a broad pH optimum between 6 and 8. Glutamate incorporation is stimulated in the presence of phosphate.

Analysis of the distribution of the radioactive amino-acids between nucleic acid and protein shows that the major part of the radioactivity is associated with the protein. Small amounts of radioactivity associated with the nucleic acid indicate its intermediary role in the protein synthesis. That the amino-acid is incorporated within the protein chain and is not merely attached to the end has been shown by the dinitrofluorobenzene method of Sanger.

The susceptibility of the incorporating system to the antibiotics and antimetabolites used in the investigations with the intact cells (already reported) has been tested and precisely the same results have been obtained. The suspension of incorporation in the presence of chloramphenicol and the insensitivity of the system to penicillin rule out the possibility of cell-wall synthesis in these preparations. Surprisingly enough, a mixture of 17 amino-acids, either at low or high concentrations, has inhibitory effect on the incorporation of amino-acids. Mixtures of nucleoside mono-, di- and tri-phosphates have no effect on the rate of incorporation.

It appears from these studies that the particulate preparations have all the enzymatic and cofactor components required for protein synthesis. An attempt was made, without success, to wash out and dialyze out some of these components. Treatment with NaCl, however, which extracts mostly nucleic acids as well as some proteins, reduces the activity to one-third. The supernatant cytoplasmic fraction of *A. vinelandii* was found to be incapable of restoring the reduced activity. Rat liver pH-5 fractions, however, restore the activity to a considerable extent. The pH-5 fraction prepared from *A. vinelandii* by the same method as applied to the mammalian system is as good as, and even better than, mammalian enzymes in this respect. These results unequivocally point out certain inherent similarities between the two entirely different incorporating systems—one from a mammal and the other from a micro-organism. This is also strongly supported by the fact that *A. vinelandii* pH 5-fraction can be substituted for rat liver pH-5 fraction in its capacity of incorporating amino-acids into microsomes of rat liver.

Since the mammalian pH-5 fraction is known to have a number of enzymes as well as soluble nucleic acid, the presence of these in the bacterial pH-5 fraction has been looked for. The amino-acid activating enzymes are found to be present in the latter preparation. Comparatively small amounts of RNA are present in the bacterial pH-5 fraction though the transfer of the activated amino-acids to the soluble RNA can be demonstrated as in the case of

mammalian preparations. The mechanism of protein synthesis appears to be similar in the two systems though there are differences in detail. The sites of synthesis in the two systems are most probably different.

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

Preliminary experiments have been carried out on the rates of incorporation of radioactive phosphate into the different cellular fractions of *A. vinelandii*. The particulate RNA is found to be more rapidly saturated with tracers than the soluble RNA. Cell-free fractions are now being used to study the mechanism of synthesis of soluble and particulate RNA. The role of the soluble RNA present in *A. vinelandii* pH-5 fraction (as reported under A) in the protein synthesis is also being investigated in detail.

B.4.3. *Stereospecific dihydrolipoic dehydrogenase from Spinacia oleracea*

As previously reported a preliminary communication regarding the isolation of this enzyme appeared in *J. Am. Chem. Soc.*, 1959, **81**, 3478. The properties of the enzyme were investigated in detail. Activation of the enzyme in presence of phosphate and arsenate and irreversible inhibition of the enzyme in presence of arsenite indicating the involvement of a di-thiol compound are worth mentioning. The partially purified enzyme was found to have a considerable amount of diaphorase activity. A full report of this work has been published in *J. Biol. Chem.*, 1960, **235**, 509.

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C. BOTANY

C.1. Plant Biochemistry and Physiology

C.1.1. Studies on Crown-Gall Problem

C.1.1.1. *Complex tumours.* Complex tumours or teratoma are known to develop when inoculations are made with mildly virulent strain of the causal organism—*Agrobacterium tumefaciens*—on cut stem surface of plants having no apical functional bud. These overgrowths characterised by differentiation into malformed leaves and buds generally occur in plant species whose cells possess the potentiality of regeneration. Experiments were undertaken to find out whether such tumours are formed with the strain of the causal organism used extensively in this laboratory for gall production. Inoculations were made on cut stem surface of both the lower and upper portions of Bryophyllum, Kalanchoe, Nasturtium and Hollyhock plants. Even after 90 days of gall development no differentiation was observed in any of the galls produced on all the four plant species. This confirmed virulent nature of the bacterial strain. Attempts to isolate bacteria-free tumour tissue from galls produced on cut stem surface of both the lower and upper plant portions have not yet met with success. Formation of galls on cut stem surface of the lower plant portion of Bryophyllum has been reported by some workers to suppress axillary bud and shoot development. In the present study no regularity in the inhibitory effect of the gall formed on bud development was observed. Secondary shoots developed in number of cases in the first leaf axil of one or both sides. Bacterial inoculations in the leaf axils and growing tips of Bryophyllum generally produced leafy galls from which normal shoots and leaves were found to develop. Results of these experiments appear to favour Smith's view according to which leaves and buds originate from the normal cells rather than from the tumour cells.

C.1.1.2. *Graft implantation of crown-gall tumour tissue culture in healthy plants.* Successful grafting of bacteria-free tumour tissue on healthy plant is considered as a strong proof of the autonomous nature of the cultivated tissue. Hollyhock crown-gall tissue maintained in culture on synthetic medium for over a period of 5 years was grafted on healthy Hollyhock plants using single slit method, double slit method (Braun and Morel, 1950) and horizontal slit method. Single slit method with 1.5 to 2.0 cm slit was most successful. Implanted gall tissues formed graft unions with normal tissues of healthy plants and gave rise to typical unorganised gall tumours which were indistinguishable from the galls produced on bacterial inoculation. That the implanted tissues were bacteria and virus free was further confirmed from the fact that no galls developed from the contiguous normal cells near the graft unions. Bacteria-free tissues isolated from these galls retained the capacity to grow in synthetic nutrient medium devoid of any growth substance, without any differentiation.

C.1.1.3. *Growth characteristics of Hollyhock crown-gall tumour tissue.* (i) *Carbon sources :* Crown-gall tumour tissues of Hollyhock grown in liquid medium can tolerate sucrose upto a concentration of 8%, when the medium is changed after every 14 days, for a growth period of 42 days. Optimum growth was obtained with 2% sucrose and gradually decreased

thereafter. Of the various sugars and polysaccharides tested the tissue could utilise laevulose, sucrose, maltose, trehalose, glucose, starch, melibiose and dextrin. Best growth was obtained with laevulose as the sole source of carbon followed by sucrose, maltose, trehalose and glucose. Though laevulose and glucose were utilised, the tissue growth on a mixture of laevulose and glucose in equimolar quantities was even poorer than that on glucose alone. Since maltose and trehalose gave far better growth than glucose, the above observation may be explained by a possible antagonism between glucose and laevulose. Laevulose has been reported to be a good source of carbon for other tissues of crown-gall origin but it was found to be even a better source than sucrose for Hollyhock gall tissue. Experiments are being undertaken to demonstrate interconversion of different sugars by crown-gall tissues growing in synthetic medium. Another important observation was the utilisation of starch by Hollyhock gall tissue. Starch utilisation has been successfully demonstrated only in case of *Rumex virus-tumour* tissue. Isolation and identification of extracellular amylase secreted in the medium by the Hollyhock gall tissue is being contemplated.

(ii) *Accessory growth factors and vitamins.* Experiments were undertaken to determine the necessity of substances such as thiamine, pyridoxine, glycine, pantothenic acid, and nicotinic acid by growing the Hollyhock gall tissues in medium with and without these substances and subculturing the tissues after every 14 days growth period through a total of 10 passages. Pantothenic acid and nicotinic acid were neither essential nor stimulatory for the tissue growth. Pyridoxine, though not essential, slightly stimulated tissue growth at low concentrations. Thiamine was found to be an essential growth factor and had definite stimulatory effect. In continued absence of glycine the tissue growth was considerably affected. Absence of glycine or any other organic nitrogen source could be overcome by addition of pyridoxine which is considered to mediate protein synthesis from inorganic nitrate present in the medium. Glycine could be replaced by other amino acid such as cysteine.

(iii) *Growth curve.* In tissue culture work it is of utmost importance to find out the period over which the tissues retain high rate of proliferation as the experiments are necessarily to be carried out where the effect observed can be attributable to the treatment only and not to any other factor. To determine this, growth of individual tissue pieces of Hollyhock tissue was studied for a period of 60 days, with and without change of medium. The growth curve in both the cases was typical 'S' shaped logistic type. Maximum rate of absolute growth, when the medium was changed was reached in 39 days; but when the medium was not changed it was reached in 27 days. Thus experiments could be conducted for a period of about 42 days and about 30 days in the two cases respectively. From the relative rates of growth it was found that the tissue obeys the law of exponential growth for a period of about 28 days and 18 days in the two cases respectively. Change of medium was found to be highly beneficial for tissue growth. Changing the medium at regular intervals almost doubled the total weight attained in a period of 60 days. From the available data on nutritional requirements for this tissue the beneficial effect appears to be more due to the removal of toxic metabolites than due to supplementing the nutrient constituents which are less likely to become deficient or depleted during the growth period.

(iv) *Explant weight and tissue growth.* Variability of tissue explants is another important factor in tissue culture work. Inherent variations were minimised by careful selection of mother tissues from which the explants are derived, method of cutting these tissues, and uniform distribution of the pieces within and between different treatments. Influence of explant weight on tissue growth was determined as variations due to differences in their weights are likely to affect the rate of growth and consequently the results. In these experiments the growth of individual tissue pieces at 3 days interval for a period of 30 days was studied under aseptic conditions. The results showed that tissue growth is influenced by explant weight; smaller explants (10 mg.) grow at a faster rate than larger ones (50-60 mg.). Tissue obeys the law of exponential growth for a period of 30 days though there is a chance of deviation towards the later stage of the growth period and the deviation is likely to be more pronounced when the explants are comparatively large. Considering the variation in the rate of growth and trend of growth for explants of different weight and the possibility of deviation from the growth trend with progress of incubation, it was found that explants weighing 20 mg. $\pm$ 10 mg. each could be most satisfactorily used without affecting the results of an experiment. No such detailed study has been reported for other tissues of crown-gall origin cultivated by different authors for the last 15 years.

C.1.1.4. *Bacteria-free crown-gall tumour tissue of Vicia faba and Coreopsis lanceolata.* Bacteria-free gall tissue of *Vicia faba* and *Coreopsis lanceolata* isolated last year was transferred to liquid medium in shake-culture after maintaining the tissues for more than 9 months on agar slants. Various bacteriological tests employed routinely, failed to reveal the presence of bacteria or fungi inside the isolated tissues. This confirmed bacteriological sterility of the tissues derived from crown-gall produced on inoculation with the causal organism. The tissues since their isolation were maintained on synthetic medium devoid of any exogenous growth substance. *Vicia faba* gall tissue in culture consists of irregular brownish cellular mass characterised by indefinite form and loosely cohering knobs or nodules which are readily separable, while gall tissue of *Coreopsis* is whitish compact cellular mass and is far less friable in texture.

(i) *Anatomical study.* Anatomical study of these tissues along with Hollyhock gall tissue grown in synthetic medium was undertaken. Histological examination revealed that the scalariform cells or tracheal elements were least and more scattered in Hollyhock gall tissue than in *Vicia* and *Coreopsis* gall tissue. In all the tissues three main types of cells were observed viz., (i) the larger hypertrophic cells, (ii) the small hyperplastic cells and (iii) thick-walled scalariform cells or tracheal elements. Various intermediates between the first two kinds were also observed. The tracheal elements were generally found in the hyperplastic regions. The cells around the small groups of tracheal elements appeared to be laid down in rows in *Vicia* and *Coreopsis*. The degree of organisation was more evident in *Vicia* than that in *Coreopsis*. In spite of this there was an obvious lack of cell and tissue organisation in normal fashion.

(ii) *Nutrient medium.* In order to select the best suited medium for growing *Vicia* and *Coreopsis* gall tissues experiments are in progress using six different media proposed by different

authors for growing plant tissues. Results so far indicate that Gautheret's medium may be a better nutrient than the 'tobacco medium' of Hildebrandt *et al.* (1946) used for growing Hollyhock gall tissue. As compared to the growth rate of Hollyhock gall tissue the growth rates of both *Vicia* and *Coreopsis* tissues in all the media tried were poorer. The continued culture of *Vicia* and *Coreopsis* gall tissues in liquid medium using shake-culture method which was standardised with Hollyhock gall tissue further supported the use of this simple method for tissue culture work. The shake-culture method which has eliminated the presence of agar in the nutrient medium, considered objectionable for various reasons, is being used in this laboratory for both experimental and routine tissue culture work.

C.1.1.5. *Normal callus tissue.* Having established bacteria-free crown-gall tumour tissues from three plant species belonging to different plant families attention was concentrated on cultivation of normal plant callus tissues. Normal tissues respond at both high and low concentration of growth substances by proliferation of cell and root formation. Different growth substances give different reactions and the concentration of these substances is a critical factor for a given species in growing callus tissues for an indefinite period. In order to find out proper concentration of different growth substances which will selectively cause cell division and thus result in durable cultures, experiments were undertaken using Hollyhock, *Coreopsis* and *Nasturtium* plant tissues. Growth substances viz., indoleacetic acid (IAA), naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) at varying concentrations from 10^{-8} to 5×10^{-6} gm. per ml. were used. In addition, coconut milk (15%) alone and in combination with 2,4-D (5×10^{-7} gm. per ml.), was also used. Best callus formation was obtained in case of *Nasturtium* with 2,4-D (5×10^{-7}), in case of *Coreopsis* with coconut milk, and in case of Hollyhock with a mixture of coconut milk and 2,4-D. In all the three plant tissues rooting was poorest with NAA both at low and high concentrations. In *Nasturtium* the roots developed at high concentration were enormously thickened throughout their length. Attempts are being made to cultivate swollen gall-like structures on these roots. IAA produced only poor callus, with heavy rooting in all the three cases. The callus tissues are being subcultured and maintained on tobacco medium agar slants supplemented with the growth substances giving best callus formation in each case.

C.1.2. *Glycolytic enzymes in higher plants*

Certain interesting observations relating to the mechanism of oxidative phosphorylation associated with the dehydrogenation of triosephosphate in mung roots were reported earlier (Annual Report 1957-58). For further elucidation of the process it was necessary to study the system with purified enzymes isolated from mung roots. The enzyme necessary for the study is "triosephosphate dehydrogenase" which may be a complex system. Since the substrate of the dehydrogenation reaction, glyceraldehyde-3-phosphate, is not readily available, common practice is to generate it *in situ* by the enzymic cleavage of fructose-1, 6-diphosphate by the enzyme aldolase. This requires a pure preparation of aldolase as well.

C.1.2.1. *Isolation of aldolase.* During the year under review, an attempt was made to prepare the enzyme 'aldolase' in a high state of purity from mung seedlings. The method

of Herbert *et al.* (1940) was adopted to assay the enzyme in germinating mung beans and it was found that maximum activity, as determined by $\mu\text{g.}$ of hexosediphosphate (HDP) cleaved per gm. of seedling, could be extracted by 0.1% potassium carbonate solution from three or four day old seedlings.

The conventional ammonium sulphate fractional precipitation method was followed to concentrate the enzyme in the alkaline extract. It was observed that though the maximum activity was obtained in the fraction precipitating between 50% and 60% saturation of ammonium sulphate, the fractions precipitated at other concentrations of the salt also retained a fairly high activity. The precipitated protein in each case was completely soluble in Tris buffer pH 8.6, giving a bright clear solution.

Obviously, ammonium sulphate precipitation did not produce satisfactory result. The next attempt was to find out if the enzyme could be purified by the method of adsorption and elution using calcium phosphate gel. The enzyme was adsorbed by the gel in the positive adsorption method, but appeared to be firmly held by the colloid and elution proved to be difficult. Several methods were tried to elute the enzyme, but in no case a satisfactory activity could be recovered.

The next effort was to extract the enzyme from an acetone powder of the seedlings. The starting material was an acetone powder of 3 day old whole seedlings prepared in the usual way by homogenising the seedlings with chilled acetone (-18°C). The prepared powder was stored at 2°C in a vacuum desiccator.

The powder was extracted with 10 volumes of 0.1% potassium carbonate solution and the extract was cleared by centrifugation. The enzyme in the clear supernatant was separated by the method of fractional precipitation with ammonium sulphate using a saturated solution of the salt adjusted to pH 7.2, or 8.6 with ammonia. The precipitation was not satisfactory at pH 7.2, and hence precipitation at pH 8.6 was followed. The fractions obtained at 30%, 30%-40%, 40%-50%, 50%-60% and 60%-75% were tested for enzyme activity. It was observed that the 50%-60% fraction yielded the maximum activity. Further experiments with the 50%-60% fraction established that the protein precipitating between 50% and 55% saturation of ammonium sulphate had the optimum activity.

Along with the determination of enzyme activity the homogeneity of the different fractions was also examined by paper electrophoresis and the 50%-55% fraction appeared to be electrophoretically homogeneous.

The yield of the enzyme under the conditions of preparation is rather low, only 1.0 mg. of active enzyme protein could be obtained from 900 gm. of fresh seedlings (70 gm. of acetone powder). The 50%-55% fraction had an activity equivalent to about 100-120 μmole hexosediphosphate cleaved in 60 mins. per mg. of protein. It could thus be claimed that the enzyme was purified about 50 folds.

C.1.2.2. *Crystallisation of aldolase.* An attempt was made to find out if the enzyme aldolase could be obtained in crystalline form. For this purpose acetone powder of the mung seedlings was extracted with 10 times its volume of cold 0.1% potassium carbonate

solution by thorough grinding with sand. To the clear carbonate extract of the powder obtained after centrifugation, calculated amount of saturated ammonium sulphate solution (adjusted to pH 8.6 with ammonia) was added to provide a 60% saturation of the salt. The system was allowed to stand for 45 mins. at 2°C. The precipitate was collected by centrifugation and the supernatant was discarded. The collected precipitate was dissolved in Tris buffer pH 8.60 and the resulting clear solution was made 50% saturated with respect to ammonium sulphate and allowed to stand for 45 mins. at 2°C. The precipitate so obtained was discarded by centrifugation and further ammonium sulphate solution was added to the clear supernatant to make it 55% saturated. The precipitate resulting after 45 mins. at 2°C. was collected by centrifugation and dissolved in minimum quantity of Veronal buffer, pH 8.64. To this solution saturated ammonium sulphate solution at the same pH was added dropwise till a faint turbidity appeared, care being taken not to disturb the system. The solution was left overnight when "crystalline aldolase" settled down. On microscopic examination the crystals revealed the same size and shape as reported by Taylor for crystalline rabbit skeletal-muscle aldolase.

At the time of writing, biochemical and physical properties of the crystalline material are under investigation.

C.1.3. *Respiratory activity of mung-root mitochondria*

In an earlier report from this laboratory (Annual Report 1957-58) results on the respiratory activity of mitochondria, isolated from 3 day old whole seedlings of mung-bean (*Phaseolus aureus*) were reported. While the young seedlings yield mitochondrial preparations possessing the capacity to oxidise the intermediates of the TCA cycle at a fairly high rate, a comparative study of the activity of particulate preparations from different organs of the seedlings may as well be interesting. The respiratory activity of the mitochondria in the roots is of special interest, specially in view of the fact that root systems are in demand of a constant supply of energy for their physiological activity. A measure of respiratory activity of these particulate bodies and the amount of high energy phosphate bond generated by way of oxidative phosphorylation can indicate how far the overall energy demand of the roots is satisfied by the energy liberated during respiration. During the year under review work was started to undertake an examination of this question.

It is well-known that respiratory activity of mitochondria isolated from plant tissues depends to a large extent on the method of preparation, because this factor often brings about solubilisation of different enzymes and co-factors in different degrees. Hence it was decided to establish first a method of preparation that would yield mitochondrial particulates from mung-root with reasonably consistent and reproducible activity.

C.1.3.1. *Methods of preparation.* The general method of isolation followed in this study is as described below. Mung-beans (*Phaseolus aureus*, variety N.P. 28 of the Indian Agricultural Research Institute, New Delhi) were germinated with usual care in large petri-dishes at 25°C in the dark. On the third day of growth, the roots were collected into ice-cold phosphate buffer. They were mopped, weighed and macerated with six volumes of suspending solution either manually in a glass mortar and pestle or mechanically in a blender. The

macerate was strained through two layers of cheese-cloth to free it from coarse fibrous materials. The filtrate was centrifuged at $500\times g$ for 5 minutes to sediment cell debris, unbroken cells and nuclei. The supernatant was collected and preserved and the residue was washed once with the suspending solution by centrifugation and the washings were added to the first supernatant. The mixed supernatant was centrifuged at $19,000\times g$ for 15 minutes. The supernatant was discarded and the sedimented mitochondria were washed by thorough dispersion in 6 ml. of the washing solution with the help of a Potter-elvehjem homogeniser and recentrifugation at $19,000\times g$ for 15 mins. When more than one washing was desired the process was repeated for the required number of times. The washed particles were finally suspended in ice-cold sucrose or KCl solution and used as the source of enzyme as quickly as possible. All operations were carried out in the zero-degree room at a temperature of $0^{\circ}\text{--}2^{\circ}\text{C}$.

Sucrose-phosphate-versene solution at concentrations of 0.4M, 0.1M and 0.01M respectively and sucrose-versene solution at the above concentration were used as suspending and washing solutions respectively in experiments using sucrose-salt medium, while potassium-chloride-phosphate solution at concentrations of 0.25M and 0.1M respectively and potassium chloride solution at 0.25M were used respectively for the same purposes in experiments using a salt medium. In the former case the final suspension was made in 0.4M sucrose solution, while in the latter case this was done in 0.2M KCl solution.

The nitrogen content per ml. of the final suspension was determined in the usual micro-Kjeldahl method.

C.1.3.2. *Preliminary studies using triphenyltetrazolium chloride (TTC) as hydrogen acceptor.* For preliminary studies, succinic acid, malic acid and α -ketoglutaric acid were used as substrates, and the reduction of TTC by the particulate preparation at pH 7.0, in presence of the different substrates was followed to study the oxidation of the substrates. The basic incubation system consisted of phosphate buffer pH 7.0, the substrate at 2×10^{-2} M, TTC, 0.3 mg. and 0.5 ml. of the particulate suspension (equivalent to about 0.8-1.0 mg. of N_2). The co-factors under test were generally used at a concentration of 0.2×10^{-3} M. The total volume in each case was 2.0 ml. At the end of one hour's incubation at 30°C the reaction was stopped by adding 6.0 ml. of rectified spirit and mixing thoroughly by vigorous shaking. In this treatment the protein was precipitated and the reduction product, red coloured formazan, was brought into solution in the 80% alcohol. The precipitated protein was removed by centrifugation and the optical density of the clear supernatant was determined in the "Spekkar" colorimeter. The respiratory activity was directly proportional with the optical density.

It was observed that the use of 0.4M sucrose solution with 0.1M phosphate buffer generally did not yield a very active preparation; oxidation of succinate, malate or α -ketoglutarate, even in presence of added DPN or cytochrome c, by such preparations proceeded at a very slow rate. However, when versene, at a concentration of 0.01M, was included in the suspending medium fairly active preparations were obtained. Even in these cases the rate of oxidation was slow unless DPN and cytochrome c was included in the system.

With added co-factors, the oxidation proceeded at a fairly rapid rate with any of the substrates, but the rate was maximum for succinate followed by α -ketoglutarate and malate successively. The difference in the rate of oxidation in presence or absence of added DPN was much more significant in cases of α -ketoglutarate and malate than it was with succinate as substrate.

Omission of phosphate from the sucrose-phosphate-versene suspending medium or use of KCl-phosphate as suspending medium produced similar results.

The endogenous activity of the preparations in absence of added DPN was practically nil. The preparation with the sucrose-medium appreciably reduced TTC in presence of DPN only indicating the presence of a DPN-linked oxidisable substrate or a DPN-reducing enzyme which could transfer the electron to TTC. The significant improvement in the rate of oxidation in presence of added DPN or cytochrome c indicates that most probably these co-factors are lost under the method of preparation. Further, the relatively low rate of α -ketoglutarate oxidation in presence of DPN and cytochrome c suggests a deficiency of other co-factors like Co-enzyme A in the preparation.

C.1.3.3. *Manometric studies.* The preliminary results obtained in the TTC-reduction method were studied further by following the rate of oxidation of succinate and α -ketoglutarate as determined by the manometric determination of the rate of oxygen uptake in Warburg apparatus.

It was confirmed that homogenisation in the KCl-phosphate solution followed by washing the sedimented particles with 0.25M KCl generally yielded a preparation of consistently good activity, whereas the preparation with the sucrose-medium, with or without versene, displayed considerable variation in activity. In the former method, double washing produced a very low endogenous activity and oxygen uptake in presence or absence of the co-factors (but in absence of any substrate) was equally low. The sucrose-medium preparation however displayed a relatively higher endogenous activity and in this case the oxygen uptake in absence of any substrate was considerably stimulated by added co-factors. It could thus be confirmed that use of potassium chloride-phosphate solution for suspension followed by washing with potassium chloride consistently yielded a better preparation.

The oxygen uptake in presence of succinate was independent of added DPN but required the presence of externally added cytochrome c, whereas the oxygen uptake in presence of α -ketoglutarate required the presence of both DPN and cytochrome c. This confirmed the earlier observation that plant root mitochondria, prepared in the way described, require these two co-factors. Further, optimal activity could be obtained only when a small quantity of an adenosine di- or triphosphate, glucose, and hexokinase were included in the respiring medium. This is but to be expected, as such a system would maintain a constant supply of acceptor to trap the high-energy phosphate liberated in course of oxidative phosphorylation.

At the time of writing this report, other methods of preparation, using other protective agents are being tried to find out a system that would retain most of the co-factors in the particulate bodies and also would yield a better active preparation. These will be followed by an attempt to solubilise the mitochondrial enzymes.

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MICROBIOLOGY

D.1. Production of antibiotic substances by microorganisms

D.1.1. Biological studies, chemical purification and in vitro test of antibiotic products by *Streptomyces* sp. A₁₄ (475)

The antibiotic developed by *Streptomyces* strain A₁₄ (475) was highly active against Gram-positive, Gram-negative and acid-fast organisms *in vitro*. It is a broad-spectrum antibiotic and equally active against fungi. The best growth in medium was shown at 28°-30°C and at pH 7.4. Fifteen different culture media were used for fermentation with an idea to select the best one for large-scale production as also easier extraction. The highest titre of the antibiotic rose on the 7th and 8th day of fermentation in stationary cultures (Table 1).

In vitro test

TABLE 1

ANTIBIOTIC SPECTRA OF A₁₄ (475) ON THE 8TH DAY OF INCUBATION IN VARIOUS
FERMENTATION MEDIA AGAINST DIFFERENT TEST ORGANISMS
Media (H. B. Warren) Anti & Chemo 5, 6, 1955

Test organism	A ₁₃	A ₇	A ₁₆	A _{5a}	A _{20a}	M ₁₂	A _c
Zone of inhibition							
<i>Bacteria</i>							
1. <i>S. aureus</i>	20	20	19	19	17	16	16
2. <i>B. subtilis</i>	21	20	20	19	18	17	18
3. <i>E. coli</i>	20	20	19	19	17	17	18
4. <i>Eb. typhosa</i>	14	12	10	12	+	12	10
5. <i>Myco. tuberculosis</i>	22	20	20	18	12	22	16
<i>Fungi</i>							
6. <i>Curvularia</i> sp.	37	37	32	37	35	32	28
7. <i>Alternaria solani</i>	33	32	29	30	30	27	21
8. <i>Fusarium vasinfectum</i>	17	18	18	18	16	17	16
9. <i>H. sativum</i>	22	17	20	20	14	18	18

Studies on carbon and nitrogen sources

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 aminoacids as the nitrogen source, two nitrogen sources in each case were given in the media. The amounts 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 rather restricted. Of the 24 aminoacids added to the media as nitrogen source, antibiotic production is limited to the utilisation of only 4 aminoacids—asparagine, glycine, histidine and arginine. However, the growth of the organism takes place in the presence of all the aminoacids used. Increase in the concentration of dextrose in the media up to 3-fold induces higher titre value of antibiotics but there is a depression on further increase of the sugar.

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Studies on the effect of trace elements (Fe, Cu, Zn and Mn)

It was shown that Fe present alone as trace element, helps growth and antibiotic production even when increased upto a concentration of 0.2 $\mu\text{g/ml}$. However, Zn and Mn have no pronounced action, and Cu inhibits growth of the organism. Experiments on mineral requirements show that absence of K, Ca, Mg and Al retarded the growth of the organism.

The growth and antibiotic production of the *Streptomyces spp.* A₁₄ (475) was investigated in a large number of fermentation media. Of these, the medium M₁₂ (composition in gms. Dextrose 100, Asparagin 5.0, K₂HPO₄ 5.0, Peptone 5.0, Distilled water 1000 ml.) was selected on account of its simple composition which consequently ensured easier purification of antibiotics.

D.1.2. *Isolation and chemical purification of antibiotic A₁₄ (475). Extraction and purification of the antibiotic A₁₄ (475)-hydrochloride form*

The active substance was found to be completely adsorbed in 2gm. Norit (SBX) charcoal, per litre at neutral pH and was later on eluted with HCl (pH 2). The acidic eluate, almost a colourless one, was passed through a column of Amberlite IR-4B(OH) concentrated to 1/50th of its original volume under vacuo and poured into 10 times its original volume of acetone. A white ppt. was obtained but the yield was too poor for further work.

However, the yield was increased by using a 50% ethanolic HCl (pH 2) and the eluate was deep yellow in colour. It was reduced to a small volume under vacuo and poured into a large volume of absolute alcohol. A complete precipitation of the impure A₁₄ (475) in the form of hydrochloride was formed in presence of (1:1) ethanol-ether mixture. A light brown precipitate was obtained, centrifuged and dried in vacuo.

Large amount of impurities, deep brown in colour that was found to be associated with the antibiotic created a baffling problem in the earlier part of the investigation. However, this has now been solved by shaking the aqueous solution of the impure antibiotic with 1/4th its original volume of phenol. Only one transfer was observed to be sufficient to remove 90% of the coloured impurities. The aqueous solution was worked out in the similar way as mentioned before to obtain the solid sample in a purer form.

The final purification was performed by the method of adsorption chromatography using acid-washed alumina as the adsorbing agent. Crude antibiotic was dissolved in water and poured into the column. The column was eluted with methanol and successively with dilute methanol and collected in fractions. The fractions, having the antibiotic property were combined together, reduced to a small volume under vacuum and poured into a large volume of a mixture alcohol-ether (1:1). A white ppt. was obtained, centrifuged and dried in vacuo.

Purification by precipitation with Picric acid

It was found that a yellow gummy precipitate was obtained when aqueous solutions of A₁₄ (475) were treated with picric acid. It was cooled to 4°C and kept for several hours. The sludge of A₁₄ (475)-picrate and free picric acid was separated from the supernatant solution by decantation and dissolved in warm methanol containing an excess of 2.5(N)HCl.

The hydrochloride was precipitated by pouring the solution into 10 volumes of ether. The precipitate was dissolved in methanol and reprecipitated with ether. After drying in vacuo, it was observed that the activity was increased in this case. Further purification was carried out by adsorption chromatography in an alumina column, as mentioned before.

A₁₄ (475)-Helianthate form

The antibiotic in the form of hydrochloride was dissolved in methanol and a hot solution of methyl orange in water was added with continuous stirring. After stirring for a considerable period of time it was kept for an hour at 4°C. The orange coloured ppt. of the helianthate was removed by centrifuging and dissolved in hot methanol. The solution was filtered while hot and it was then added to hot water. After keeping for few hours at 4°C, the crystals were removed by filtration and finally washed with water.

A₁₄ (475)-Sulphate form

An aqueous solution of A₁₄ (475)- hydrochloride was poured drop by drop in a 4% (2N) sulphuric acid in acetone, with constant stirring. After keeping for half an hour it was centrifuged, again suspended in warm water, centrifuged again and dried in vacuo to yield a white amorphous powder.

A₁₄ (475)-Acetate form

For the preparation of A₁₄ (475)- acetate, the form of the antibiotic method of procedure followed was the same as that of hydrochloride excepting that the acetic acid was used in place of the hydrochloric acid.

Properties

The substance is stable when boiled in aq. solution at (100°C) for 10 mins. It is also stable in the acidic pH but loses its properties gradually in the presence of alkali. The pH of the solution of the hydrochloride form is 6.6. The substance gave negative tests with Fehling's solution, 2:4 dinitro-phenyl hydrazine and FeCl₃. The substance decomposes without melting at 280°C. The UV spectrophotometric tests showed no absorption band within the UV range. Bio-autographic tests were done with 5 solvent-systems and their respective R_f values were determined (Table 2).

TABLE 2

R_f VALUES OF THE ANTIBIOTIC A₁₄ (475) IN VARIOUS SOLVENT-SYSTEMS

Solvent-system	R _f value
(1) Methanol system (3:1)	0.46
(2) Ethanol-water (3:1)	0.55
(3) Iso-propyl alcohol (7:3)	0.66
(4) tert-butyl alcohol-water (7:3)	0.63
(5) n-butyl alcohol ethanol-water (1:1:1)	0.53

The antibiotic titre of the substance was compared *in vitro* with that of streptomycin and result showed (Table 3) that the potency of the purified antibiotic substance is quite comparable with that of streptomycin.

TABLE 3

COMPARATIVE ASSAY RESULTS. (PURIFIED ANTIBIOTIC)

	A ₁₄ (475) (1mg/ml)	Streptomycin (1 mg/ml)
	Zone of inhibition in mm	
Staph. aureus	15	18
Bacillus subtilis	18	25
Bacillus anthracis	14	25
Proteus vulgaris	12	—
E. coli	15	22
V. cholerae	16	27
Eb. typhosa	20	30
Myco. tuberculosis	22	40
Curvularia sp.	26	—
Alternaria solani	26	—
Fusarium vasinfectum	18	—
Aspergillus niger	17	—
Helminthosporium sativum	20	—

D.1.3. Extraction of antifungal antibiotics from *Streptomyces* strains (Ac₂432, Ac₂435 and Ac₂450)

An attempt was made to extract the antibiotics from 3 selected strains of *Streptomyces*. 12 solvents were used to extract the antibiotics both from the culture broth and mycelial mat at different pH level with ether, chloroform, carbon tetrachloride, benzene, petroleum ether, ethyl acetate, amyl acetate, butyl acetate, butyl alcohol, amyl alcohol, benzyl alcohol, and ethyl alcohol. It was noted that the antibiotic yield from the culture broth of the strain Ac₂432 with most of the solvents was satisfactory in acidic pH, and extraction with ethyl alcohol was suitable. The strain Ac₂435 gave satisfactory results with n-butyl alcohol and amyl alcohol when the broth was extracted with these alcohols at neutral pH. The mycelial mat also yielded some antibiotics when extracted with these alcohols. However, since mycelial growth in this strain was very scanty extraction from the broth was considered favourable. The remaining strain Ac₂450 gave good results when the broth was extracted with ether, chloroform, benzene and also when the mycelial cake was extracted with the same series of solvents. A fairly good quantity of the active substance was extracted from the broth and the potency was determined by antibiotic assay.

D.1.4. Antifungal antibiotics of *Actinomycetes*

A number of strains of actinomycetes with antifungal properties were obtained. Out of these, 3 strains were finally selected. These were tested against fungi—saprophytic 2, plant pathogenic 5 and human pathogenic 1, so far. Screening of these strains against many other test organisms, remains yet to be done.

The following table shows the results obtained in the agar-cup method of assay in which 0.1 ml. of the culture broth was used in all cases. The culture broths were harvested after 6 days of incubation at 28°C, and the medium used for plating the *Candida* spp. (yeast-like human pathogenic fungi), was Sabouraud's agar medium and for the rest of the fungi it was Czapek-Dox agar.

TABLE 4

Strain No.	Test organism (fungi)							
	A. niger	A. oryzae	Alt. solani	Curvul- aria spp.	Fusar- ium spp.	P. nota- tum	P. chryso- genum	Condi- da spp.
Ac ₂ 432	15mm	17	30	35	20	12	20	—
Ac ₂ 435	25	20	30	30	22	25	27	22
Ac ₁ 450	12	15	30	30	15	15	22	—

D.1.5. *Effect of Media on the increased production of antibiotic*

Actinomycetes showing antagonistic properties against bacteria and fungi were selected to study the effect of various media with a view to determine the increase in the antibiotic potencies. The procedure for studying the effect of 12 different media in 250 ml. Erlenmeyer flasks and autoclaving for 30 minutes at 15 lbs. pressure. The flasks were inoculated and incubated at 28°C. The fermentation liquor was assayed daily from the 3rd to the 10th day of incubation using the agar-cup method, against bacteria and fungi. The test organisms used were—*Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli* and *Eberthella typhosa* among bacteria and *Aspergillus niger*, *Alternaria solani*, *Curvularia sp* and *Fusarium spp.* among fungi.

Out of the 12 media used, 4 were suitable for production of antibiotics. Three of them contained soyabean meal, NaCl and CaCO₃, in varying proportions with one or more of the following as sources of nutrients: cornsteep (liquid), glucose, dextrin, curbay, glycol—in variable quantities and the remaining one contained straw infusion, peptone, glucose, MgSO₄, K₂HPO₄, NaCl and FeSO₄.

D.1.6. *Broad-spectrum antibiotic production from a Pseudomonas sp.*

The work on an isolate of a *Pseudomonas sp.* was continued in this laboratory. It showed strong antifungal and antibacterial activities. The bacterium is strictly aerobic, Gram-negative in character and rod-shaped in structure. The rods occur singly, seldom in pairs, but never in chains. Cultural and physiological properties were studied at 25°C, since at that temperature pigmentation of the culture filtrate occurred. Nutrient and plain gelatin are rapidly liquefied and the medium becomes bluish green. The organism utilizes many other proteins or polypeptides such as casein, peptone, tryptone, soyabean protein etc. showing that it is strongly proteolytic in behaviour.

Antibiotic production in shake cultures

Since the yield of the antibiotic production is rather low in nutrient broth under static condition, shake flask cultures were employed. Here the rate of production was diminished.

Of all the media used for the production of antibiotic pigment, peptone-glycerol broth is the best. In addition to peptone, it contains—1% KNO_3 , 0.1% NaCl , 0.5% glycerol, 4% tap water 1 litre, pH 7.4.

The following table will clearly indicate the production of antibiotics in the various media.

TABLE 5

Composition of the medium	Temp.°C & Time of incubation	Colorimeter reading (using filter no. 42)
1. Nutrient + broth Glycerol (4%)	25 5-day old broth	266
2. " "	37 "	100
3. " "	37 "	75
4. " "	25 "	147
5. " (in shake flasks)	25 "	20
6. Potato-peptone broth	25 "	20
7. Peptone-glycerol broth	25 "	550

Thus the above data indicate the use of peptone-glycerol broth for the production of antibiotic from the *Pseudomonas* sp. isolated in the laboratory, was quite useful.

The production of the antibiotic was increased in peptone-glycerol broth in shake flask cultures.

TABLE 6

EFFECT OF SHAKING ON THE PRODUCTION OF ANTIBIOTIC IN PEPTONE GLYCEROL BROTH (5-DAY OLD)

Shaking flask culture	pH	Colorimeter reading (using filter no. 42 Klett-Summerson colorimeter)
(Reciprocating shaker 80 strokes/min.)	7.80	440
Stationary culture	8.2	93

Purification and isolation of the active principle

Several ion exchange resins such IRC-50, IRA-400 etc. were employed to extract the active principle, but celite, an adsorbing agent of various grades, was proved of no value.

Solvent extraction with chloroform was found most efficient in the extraction process. The chloroform extract was treated with 2N HCl until it was acidic and from the acidic solution the active principle was re-extracted with chloroform after adjusting the pH of the solution of 8.6-8.8. By this method the antibiotic was concentrated to a small volume in chloroform and on evaporation a blue mass was left. Further, purification of the blue

mass on alumina gave needle-shaped indigo blue crystals m.p. 110-112°C. Further studies are now being carried out to characterize the antibiotic and its use in chemotherapy.

D.1.7. *Effect of antibiotic in soil and its role against soil-borne plant pathogens*

The microorganisms producing inhibitory substances (e.g. antibiotics) normally live in soil and other natural habitats. Their antibiotic potentiality has been studied intensely during the last 2 decades. The disease organisms when introduced into unamended soil do not survive long and the conclusion was that introduced microbes were killed by antagonistic organisms. In this laboratory a strain of *Streptomyces* A₁₄ (475) was studied in detail for antibiotic production under laboratory conditions. The following experiments on the activity of the antibiotic elaborated by A₁₄ (475) in soil were done and the results are given below :

Expt.—1. Soil (sterilised) + A₁₄ (475) culture + *Curvularia lunata*

Expt.—2. Soil (sterilised) + Purified antibiotic + *Curvularia lunata* A₁₄ (475)
Dose (500 µg/gm)

Expt.—3. Soil (sterilised) + *Curvularia lunata*

EXPT.—2. Soil (sterilised) + A₁₄ (475) culture

It was shown that in Experiment 2 the purified antibiotic got adsorbed in the soil and there was very little or no antibiotic property left to inhibit the soil-borne plant pathogen, *Curvularia lunata* whereas in Experiment 1 the antagonistic *Streptomyces* A₁₄ (475) inhibited the growth of *Curvularia* to a great extent. The results are given in the table below :

TABLE 7
NUMBER OF COLONIES X 10

Days of incubation	Soil sterile + <i>Curvularia lunata</i>	Soil sterile + A (475) <i>Curvularia lunata</i>	Antibiotics extracted from soil ($\mu\text{g/gm}$)
0	.02	.02	0
7	0.12	0.28	0
14	0.30	0.023	0
28	0.38	0.0011	0.15
35	0.32	0.0011	0.20

The above results show that the presence of small amount of free antibiotic in soil inhibited *Curvularia lunata* to a certain extent.

D.2. Radiation-induced mutation in microorganisms

D.2.1. Ultraviolet-induced mutation in *A. niger* (Strains A, C, & D)

Biochemical mutants were obtained by conducting experiments in the following way : after irradiation with UV rays, the spores were plated in proper dilution in complete medium which contains casamino acid 1.5 gm; yeast extract 1 gm; peptone 1 gm; vitamin

B-complex solution 3 ml; nucleic acid solution 1 ml; glucose 20 gms; agar 20 gms; distilled water 1 litre pH 6, so as to give rise to separate colonies. After 3-4 days of incubation at 30°C, the colonies were transferred to agar slants of minimal medium (NaNO₃ 3 gm; KCl 0.5 gm; MgSO₄ 7 H₂O, 0.01 gm; KH₂PO₄ 1 gm; glucose 40 gm; agar 20 gm; water 1 liter, pH 6. Strains unable to grow on this medium were picked up as nutritional or deficient mutants. For testing nutritional requirements, the mutants were grown in dilution plates of minimal medium and each of the plates, when solidified, was enriched at 3 spots of the agar surface with stock solutions of (1) amino acid (hydrolysed casein (5 mg./cc), (2) vitamin solution and (3) nucleic acid solution. The plates were incubated at 30°C for 4 days when the fungus growth was observed in the region of one of the stock solution, thus indicating that the organism has lost the capacity of synthesising its growth factor as a result of irradiation. Final determination of the growth factor requirements of the mutants was made by following the testing methods adapted elsewhere.

TABLE 8

BIOCHEMICAL MUTANTS OF *A. NIGER*

Strain Index	Growth factor requirement of mutants
A ₁₂	Guanine, Hypoxanthine
C ₈	Adenine, Hypoxanthine
C ₁₆	Arginine
C ₂₄	Adenine, Hypoxanthine
C ₃₂	Aneurine HCl
C _{10,000(1)}	Methionine, Cystine, Cysteine
C _{10,000(2)}	Methionine, Cystine, Cysteine
C _{30,000}	Methionine, Cystine, Cysteine
D ₁₂	Nicotinic acid
D ₂₀	Methionine, Cysteine
E ₁₆	Methionine, Cystine, Cysteine

D.2.2. *Effect of Gamma radiation Co⁶⁰ source at the World Agriculture Fair Exhibition, U.S. Pavilion, 1960, New Delhi*

Effect of Gamma radiation was studied with the mutant strain C and at 3 dosage levels viz., 10,000; 20,000 and 30,000 r/min. The post-irradiation method was the same as was in the case of UV irradiation experiment. Percentage of survival of the spores at different dosages are given in Table II.

TABLE 9
PERCENTAGE OF SURVIVAL OF THE SPORES OF *A. NIGER* STRAIN C ON
EXPOSURE TO GAMMA RADIATION

Dose of gamma radiation (r/min.)	Percentage of survival
0	100
10,000	33.7
20,000	14.5
30,000	6.0

It will appear from the table that percentage survival of spores as a result of gamma radiation follows the same pattern as in the case of UV radiation. The rate of killing increases with the increase in the dose of gamma radiation.

Biochemical activities of mutant strains

Survivors of the parent strains D and E, obtained after UV radiation, were grown in 100 ml. flasks in the fermentation medium and the filtrates were titrated on the 8th day of incubation against standard N/10 NaOH. Details of the process have been described in the preceding report. The results of titration revealed that yield of citric acid diminished in most of the cases and only a few survivors showed higher yield. The number of mutants showing higher yield than the parent are given in table 10. Data represent the results of titration of derived mutants 200 each of the two strains.

TABLE 10
PERCENTAGE OF MUTANTS SHOWING HIGHER YIELD OF CITRIC ACID AS COMPARED
WITH THE PARENT

Parent strain	Yield of citric acid (%)	High-yielding mutants (%)
D	1	3.5
E	1	1.5

D.2.3. *UV-induced mutants of A. niger and their increased biochemical activity (citric acid production)*

In continuation of the experiments for the study of biochemical activities of mutants of *Aspergillus niger* van Teighem, 3 selected wild strains designated as A, B and C were irradiated with ultraviolet rays varying the dose rate from 0-20 mins.

The fungus was grown in Czapek Dox agar medium and spore suspension was made to the concentration of 14×10^6 . 5 ml. of the suspension was exposed to ultraviolet rays of wave length $2537\text{\AA}$ from a Phillips Germicidal Lamp. The rays were focused into the material and the suspension thus received doses measuring $100 \text{ ergs/mm}^2/\text{sec}$. During irradiation, the top of the petridish were taken off and the suspension was stirred continuously with a sterile glass rod. Five replicates were set up with each of the four strains and the suspensions were irradiated for 4, 8, 12, 16 and 20 minutes. Percentage of survival of the spores of 3 strains are given below.

TABLE 11

PERCENTAGE OF SURVIVAL OF THE SPORES OF A,B, AND C AS
OBTAINED ON EXPOSURE TO UV RAYS

Time of exposure (minutes)	Percentage of survival		
	A	B	C
0	100	100	100
4	46.4	7.8	5.3
8	16.4	1.7	1.3
12	3.7	1.38	0.76
16	2.62	0.59	0.27
20	1.2	0.11	0.15

The above table indicates that the percentage of survival of the spores decreased with the increase in the time of exposure.

TABLE 12

Strain	Titre value (N/10 NaOH)
A (Parent)	2.78
A ₂₀ ₄	3.1
A ₂₀ ₆	3.0
A ₁₆ ₁	2.83
A ₁₆ ₂	3.8
A ₁₆ ₅	3.5
A ₁₆ ₁₀	3.53

Strain	Titre value (N/10 NaOH)
C (Parent)	3.78
C ₈ ₄	4.0
C ₁₂ ₁₁	4.83
C ₁₂ ₁₂	6.9
C ₁₆	6.0

Strain	Titre value (N/10 NaOH)
B (Parent)	5.43
B ₄ ₄	5.6
B ₄ ₆	6.28
B ₄ ₇	6.2
B ₄ ₈	7.2
B ₈ ₃	8.38
B ₁₂ ₈	6.5
B ₁₆ ₃	7.0
B ₄	6.93
B ₅	8.2
B ₆	6.2
B ₇	8.3
B ₈	5.9
B ₂₀	5.8
B ₂ ₁	7.5
B ₃	8.4
B ₄	5.48
B ₇	6.38
B ₁₀	5.03
B ₁₁	6.03

Biochemical activities of survivors—Survivors after irradiation were grown in 100 ml flasks in a special synthetic medium containing ammonium nitrate, sucrose and minerals at pH 3.8. Filtrates are titrated against N/10 NaOH and results were recorded from the 3rd day to the 8th day of incubation after every 24 hours. Eighty strains each of the three parent types were studied and it was observed that yields of *citric acid* decreased amongst the mutants in most cases. There were only few mutants of *A. niger* among the whole lot which showed significant increase of citric acid production. The strains showing titre values higher than the parent type are given above.

Acid toleration—Acid toleration capacities of the parent types were determined by growing them at different pH adjusted to 1,1.5, 2,2.5, 3 and 3.5. Twentyfive ml. quantities of the medium were taken in each flask (cap. 100 ml.) and was inoculated with 0.5 cc. of the spore suspension. 3 sets of flasks were set up at each pH and they were incubated at 30° C for 9 days. The mycelial mat was then removed, dried and weighed. The average of 3 readings are finally recorded in table 13.

TABLE 13
GROWTH OF *A. NIGER* (WILD) AT DIFFERENT pH, OF THE MEDIUM

pH of the medium	Mycelial weights (dry) gm		
	A	B	C
1	0	0	0
1.5	0.1124	0.12	0.1745
2	0.3548	0.3501	0.345
2.5	0.3760	0.3772	0.3476
3	0.383	0.402	0.3996
3.5	0.4026	0.4397	0.4149

Detailed nutritional requirement of the above 30 biochemical mutants isolated in course of this work was studied (Table 14). The frequency of aminoacid-requiring mutants was larger than others. There were mostly single deficient mutants and it seems those with unknown factors might have complex requirement e.g. deficiency in 2 or 3 essential metabolites. The next step was taken to determine the exact requirement of the substances, the synthesis of which are blocked in the genetic system of the mutant. In most cases mutants show stability and do not change their nutritional requirement.

TABLE 14
THE FOLLOWING MUTANTS WERE ISOLATED AND CHARACTERISED

(1) Aminoacid-requiring	15 strains
(2) Nucleic acid-requiring	6 "
(3) Either vitamins or aminoacids requiring	5 "
(4) Unknown (?)	4 "

D.2.4. *Effect of Gamma radiation on broad-spectrum antibiotic-yielding Streptomyces spp*

Two species of *Streptomyces*—Ac₂₁ (460) and Ac₁₄ (475) were selected—the former because of its characteristic pink colour and antibiotic activity against fungi and the latter colourless one for its broad spectrum antibiotic activity against bacteria. The spore suspension was (made in 1% calsolene solution and Ringer's fluid 1:1) irradiated by Gamma rays at the U.S. Pavilion, World Agriculture Fair, 1960, New Delhi, the doses administered being 5000, 10,000 and 20,000 r/min respectively. The percentage survival of the colonies at different doses were the following :

TABLE 15

Organism	Dose Gamma radiation r/min	% Survival
Ac ₂₁ (460)	5,000	17.12
	10,000	12.41
	20,000	10.79
Ac ₁₄ (457)	5,000	27.59
	10,000	21.11
	20,000	10.61

Isolation of Morphological and Biochemical mutants of Ac₂₁ (460)

The isolation of mutants was done by the filtration techniques in which germinated spores are passed through the sintered glass filter. The irradiated spore suspension (1 cc in each case) was allowed to grow in 20 cc M.M. medium and after 24 hours of germination at 28°C, (the normal period of germination was about 6 hours)—24 hours' time was given with a view to allow the germination of non-mutated spores. The spore suspension was then filtered through the sintered glass filter no. 4 and the filtrate was then plated out in complete medium. All the colonies were picked up by total isolation method of Fries by transferring all the colonies into minimal agar plates and allowed to incubate at 28°C for 6 days. It was found that the percentage of biochemical mutants recorded here was—0.0%, 0.0% and 1.2% at 5000, 10,000, and 20,000 r/min respectively.

For determining the characterisation of the biochemical mutants, the well-known auxanographic technique was adopted and the following results were obtained. Out of a total 704 colonies picked up from filtered irradiated spore suspension, after preliminary auxanographic test, 4 strains showed nutritional deficiency. Of the deficient mutants, 3 showed requirement of amino acids and nucleic acid. The deficiency of a particular compound was demonstrated on further analysis with amino acids and pure chemical constituents of nucleic acids. The results obtained showing nutritional deficiency in biochemical groups are given below.

TABLE 16

(Biochemical mutants) Growth requirements	No. of strains isolated
Aspartic acid	1
Glycine	1
Histidine, Threonine	1
Guanine, Xanthine	1

Isolation of Morphological mutants

Colonies different in size, shape, colour, form, texture etc. from the original parent strain were scored as morphological mutants. They were isolated from the total isolation method plates and allowed to grow in C-D slants. The percentage of morphological mutants was 3.1%, 5.1% and 6.2% at 5,000 10,000 and 20,000 r/min. respectively.

Isolation of Morphological and Biochemical mutants of Ac₁₄ (475)

The procedure followed in case of this strain was the same as in Ac₂₁(460). However, in this organism no morphological mutants were obtained. The characterisation of biochemical mutants is now under progress.

Effect of Gamma radiation on antibiotic yield of Streptomyces

The effect of Gamma radiation on the development of high-yielding strains had been thoroughly studied both with Ac₂₁(460) and Ac₁₄(475). In the former, 950 colonies were tested for antibiotic activity against *Curvularia* sp. as a fungal organism and in the latter 1260 colonies were examined against *Staph. aureus* as bacterial test organism. In both cases, the antibiotic property was increased for various mutants. The following table shows increase or decrease of activity (in per cent) at different doses.

TABLE 17

Streptomyces strain	Dose of irradiation r/min	Antibiotic activity (in per cent)		
		Increase	Decrease	No change (± 5)
Ac ₂₁ (460)	5,000	1.6	3.1	95.3
	10,000	1.8	2.7	95.5
	20,000	2.1	2.3	95.6
Ac ₁₄ (475)	5,000	2.6	4.3	93.1
	10,000	0.0	8.0	92.0
	20,000	0.0	11.0	89.0

D.3. Microbial Genetics

D.3.1. Isolation and characterisation of *Ascobolus* sp. for mutation study

The study of Ascomycetous fungi has been taken up particularly with a view to look into the genetics of such lower organisms. The *Ascobolus* species commonly growing in and around Calcutta during the monsoon was collected accordingly. The pale yellow discs of apothecia were crushed, to the crushed mass about 10.0 ml. of distilled water was added and the suspension was centrifuged. The spores were treated with dilute NaOH solution (1.0%) washed with water and allowed to germinate in hanging drop cultures. The germinated spores were grown in a medium containing some growth promoting substances. The cultures were exposed to some amount of sunlight every day for about 1 hour or so during the incubating period. The formation of apothecia was observed in 10 days. Monosporous cultures were then isolated and their sexuality tested. No formation of apothecia has been observed in any of the monosporous cultures. This shows that they are heterothallic in nature.

D.3.2. Effect of chemical mutagens on the methionineless strain of *Neurospora crassa*

Work on chemical mutagenesis with *Neurospora crassa* has been taken up. A methionineless strain (S4894) obtained through kind courtesy of Prof. M. Westergaard of Copenhagen University is being used for the testing of the mutagenic action of Di-methyl-ethylene-imine synthesised in the Chemistry Laboratory of the Bose Institute. For the detection of mutation through chemical action on the marker gene, the reverse mutation technique has been employed. The experiments were being conducted in a controlled temperature room maintained at 25°C. Various concentrations of the chemical substance have been tried. However, the results obtained from preliminary experiments remains so far indecisive for any final conclusion.

D.3.3. Genetics of *Aspergillus niger*

A heterokaryon was developed between strain 13a *me thi* (Avellaneous coloured conidia) requiring both methionine and thiamine and strain 54g *py lys* (Green coloured conidia requiring both pyridoxine and lysine). A heterozygous diploid was formed from the above heterokaryon. The diploid $\frac{13a\ me\ thi}{54g\ py\ lys}$ was studied for the 1st and 2nd order of segregations by the following methods.

- (a) Plating the conidia in complete medium and isolating the individual colony for test of colour and nutritional requirements.
- (b) Isolating and testing the sectors arising out of the colonies developing on complete agar medium.

There were 10 sets of new combinations amongst the first order of segregants, the total number of such isolates being 122. However, there were many missing segregants and there were, 8 first order segregants in the case of diploid 4/12 and 12 such segregants in the

case of the diploid 13/54. Five strains of the second order segregants were developed from the first order segregants of diploid strain—4/12 and 9 such strains of diploid 13/54 were developed in the same way.

D.3.4. Biochemical mutants of *Aspergillus niger*

About 376 biochemical mutants were isolated throughout the whole study, most of which required one or two growth factors. Strains requiring arginine, lysine, methionine, cystine, cysteine, thiamine and adenine were found to occur more frequently.

In a few instances guanine or cytosine inhibited the growth of adenine or hypoxanthine-requiring auxotrophs. Some of the cytosine auxotrophs were inhibited by adenine, hypoxanthine or guanine.

A few biochemical mutants however, behaved very exceptionally. Although they were able to grow in complete medium, their nutritional deficiency remained very indistinct in auxanographic test. They responded well only in minimal medium containing yeast extract as a supplement.

D.4. Studies on the bacteriophage of *Rhizobium* strains

D.4.1. Isolation and study of Root nodule bacteriophage

The following legumes were grown at Falta Experimental Station, W.B. and in pot cultures at the Laboratory (Table 18). Seeds germinated in all cases except *Glycin hispida* (Soya bean) and Berseem. Probably these seeds were supplied from older stocks. The growth was poor to medium in most cases. However, good nodules were formed in the case of *Lathyrus sativum*, *Pisum sativum* (Pea) and *Lens esculentum* (Masoor).

TABLE 18

PLANTS GROWN AT FALTA EXPT. STN. AND IN POTS AT THE LABORATORY

1. Phaseolus aureus (Sonamung)	9. Dolichos lab lab
2. P. mungo var radiatus (Mashkalai)	10. Vicia sp.
3. Lathyrus sativum	10. Vicia sp. (French bean)
4. Pisum sativum	11. Arachis hypogea
5. Vigna catjung	12. Trigonella sp.
6. Phaseolus radiatus	13. Medicago sativa
7. Lens esculentum	14. Glycin hispida
8. Cicer arietinum	15. Berheem

In the Laboratory, the pot cultures of the legumes showed normal growth in most cases and good nodulation occurred. The nodules were isolated from the plants and their number, size and colour were recorded. The Berseem seeds germinated but there was no nodulation in the roots of the plants.

Freshly collected nodules were crushed and the *Rhizobium* strains were isolated by the serial dil. method. This was followed by the isolation of respective bacteriophages from the soil of the root region of the above legumes.

Nodules were formed in the roots of the following Leguminous plants. The numbers, size and morphology of the nodules were recorded in 9 cases and the results are given in Table 19.

TABLE 19
NODULE FORMATION IN LEGUMINOUS PLANTS

Name of Plant	No. of plant	No. of nodule	Mean size of nodule	Morphology of the nodules
1. <i>Pisum sativum</i>	1	19	1.0×1.0 mm —5.0×4.0 mm	Simple, round, some bi- or trifurcated pink, no white strand. Few complex and flat type I
2. <i>Phaseolus mungo</i> var <i>radiatus</i>	1	4	1.0×1.0 mm —2.0×1.0 mm	Pinkish, round and simple. No white strand
3. <i>Lathyrus sativum</i>	1	4	1.0×1.0 mm —4.0×2.0 mm	Elongated, pinkish simple, few compound
4. <i>Lupin</i> sp.	1	1	4.0×3.0 mm	Pinkish, round, simple No white, strand
5. <i>Vigna catjung</i>	1	9	1.5×1.0 mm —2.5×2.0 mm	Simple, pinkish, round, with white strand
6. <i>Trigonella</i> sp.	1	6	1.0×1.0 mm —2.0×1.5 mm	Pinkish, simple, round
7. <i>Lens esculentum</i>	1	3	1.0×0.5 mm —2.5×1.0 mm	Elongated, simple, pinkish type
8. <i>Vicia</i> sp.	1	small swelling	0.5×0.5 mm	All white false nodule
9. <i>Berseem</i>	1	„	0.5×0.5 mm	All white. nodules false

D.4.2. Isolation of bacteriophage from soil

50.0 gms of soil was taken in a 250 ml flask containing 100 ml Laird's medium. 5 ml of 24 hours slant cultures of the susceptible *Rhizobium* strains were used as multiplying agent. After 48-72 hours soil culture became clear, it was first filtered through Whatman filter paper (No. 1) and then passed through sterilized sintered glass filter (No. 5). It was then kept at low temperature in the refrigerator and was used as stock phage.

TABLE 20

BACTERIOPHAGES FROM SOIL IN WHICH LEGUMES WERE GROWN

Index of phage	Name of host from the root region of which the soil collected alongwith adhered soil particle	Area from which the soil collected
P ₁	Sesbania sp. (Nodulating stage)	Falta
P ₂	Aeschynomene sp. (Nodulating stage)	Howrah
P ₃	Pisum sp. (Nodulating stage)	Howrah
P ₄	Pisum sp. (Non nodulated)	Falta
P ₅	Pisum sp. (Early stage of nodule)	Falta
P ₆	Lathyrus sp. (Early stage of nodule)	Falta
P ₇	Lens sp. (Early stage of nodule)	Falta
P ₈	Pisum sp. (Mature stage of nodule)	Falta
P ₉	Cicer sp. (Non nodulated)	Falta
P ₁₀	Vicia sp. (Non nodulated)	Falta
P ₁₁	Phascolus sp. (Non nodulated)	Falta
P ₁₂	Phaselus sp. (Non nodulated)	Falta

After isolation, each phage was tested against the bacterial strains. The experiment was done by screening test. The above table shows the effectiveness of the phage against *Rhizobium* strains.

TABLE 21

PHAGE SCREENING TEST WITH BACTERIOPHAGE AND HOST BACTERIA

Phage	Phase- ollus sp.	Vigna sp.	Cicer sp.	Cajanus sp.	Medi- cago sp.	Crotol- aria sp.	Lupin sp.	Glycin sp.	Pisum sp.	Sesba- nia sp.	Pisum sp.	Aeschy- nomene sp.
P ₁	+++	++	+++	++++	+++	+++	+++	+	+++	+	+	—
P ₂	+++	—	++	+	+	+	+	—	—	+	+	++
P ₃	+	++	++	+	++	—	+	+	+	++	++	+
P ₄	++	—	++	+++	++	—	++	++	++	+	++	+
P ₅	++	+	+++	+	++	++	++	++	+	—	+++	+
P ₆	++	—	+	—	++	++	+	++	+	+	++	++
P ₇	+	—	++	+++	+	+	+	++	++	+	+	—
P ₈	+++	+++	+++	++	+++	++	++	+++	++	+	+++	++
P ₉	+++	—	+++	+++	+++	+++	+++	+++	++	+	+++	++
P ₁₀	++	++	+++	+	+++	++	++	++	++	—	++	+
P ₁₁	++	—	++	+++	+++	+++	+++	+++	++	—	++	+
P ₁₂	+++	—	++	++	++	++	+++	+++	+++	—	+++	+++

D.5. Ecology of Soil Fungi

A study of the Ecology of Soil Fungi has been undertaken in cultivated field conditions. A plot of land was acquired at Falta for the purpose. The problem under investigation may be considered under the following broad heads.

(i) *Record of soil conditions and study of nature of cover crop vegetation*

- (a) Soil temperature
- (b) Soil moisture
- (c) Mechanical analysis of soil
- (d) Chemical analysis of soil
- (e) Soil treatment
- (f) Nature and health of crop
- (g) Free amino acids in soil

(ii) *Soil and Root surface fungal flora*(iii) *Growth requirements of some soil fungi*

- (a) Amino acids requirement
- (b) Influence of amino acids on the colonisation of organic matter.

D.5.1. *Record of soil conditions*

Soil temperature was recorded daily and soil moisture content determined once or twice a week.

D.5.1.1. *Soil treatment.* The following soil treatments were applied in order to see whether agronomical practice do have any bearing on the soil fungal population. Size of the plot was 1/66th of an acre.

F_0 —Control

F —3 seers of mustard cake and one seer of superphosphate of lime was applied per plot and ploughed in.

F_D —These plots were deep ploughed by manual labour upto 2 feet from soil surface. Six inches layer of soil was removed at a time and heaped separately before being replaced exactly at the original layer. 5 seers of mixed paddy fertiliser were divided into 4 equal parts and applied at the bottom of each layer of soil replaced i.e., 24, 18, 12 and 6 inches below the surface level. On the surface again 3seers of mustard cake and one seer of superphosphate of lime were applied per plot. Eight replications for each of F_0 and F treatments and 4 replications of F_D treatments were maintained in randomised design.

D.5.1.2. *Nature and health of crop.*—(A) Aus paddy of *Kele* variety was sown by broadcasting on the 7th and 8th June 1959. Germination was uniform and seedlings were dark green upto the 2nd week of July after which date plants in F_0 and F plots became lighter green while those in F_D plots still maintained the dark green colour. Leaf-tip burn symptoms appeared in July and became widespread especially in F_0 and F plots in August. From July onwards plants in F_D plots were darker green and taller. Due to heavy rains in the months of August and September 1959, water level rose to unusual height (Maximum 75 cm. above soil surface), and hence harvesting had to be done earlier when about 50-60% grains matured. The output was, low though increase in yield was noted in F_D treatments compared to F_0 and F ones.

D.5.1.3. *Free Amino acids in Soil.* Soil samples collected from replicated plots of each treatment were mixed well, kept in 70% alcohol over night with occasional shaking. The supernatant liquid was then decanted off, concentrated on a water bath and the presence of amino acids was detected by spot test on Whatman paper No. 1, and spraying with 0.1% ninhydrin solution. If the result is positive the concentrated solution is desalted by passing through Ion-exchange Resin IR-120, and the individual amino acid is then detected and identified by two dimensional paper chromatography. No amino acid however could to be detected by analysing Falta soil according to the above method.

D.5.2. *Soil and Root surface fungal population*

Fungi from soil and root surface were isolated at monthly intervals by the following methods.

A

- (a) Soil dilution plates on Malt agar media.
- (b) Warcup's soil plates on Malt agar media.

B

Soil washed with sterile water by shaking for two hours and then allowed to settle for about $\frac{1}{2}$ minute after which supernatant liquid was poured off and fresh sterile water was added, shaken and poured off again. Thus the process was repeated several times until turbidity became minimum. Soil organic matter was then picked off aseptically dried on sterile filter paper and placed on malt-agar plates and incubated at 25°C.

C

Roots of growing crops, when available were similarly taken in sterile water in sterile flasks, shaken overnight and the liquid washings were then thrown away. Roots were transferred aseptically to fresh sterile water in flasks and the process continued for 7 days. Thus thoroughly washed roots were dried on sterile filter paper and plated on malt agar. The fungi isolated, are given below.

- | | |
|-----------------------------------|--|
| 1. <i>Aspergillus niger</i> | 15. <i>Rhizopus nigricans</i> |
| 2. <i>Aspergillus terreus</i> | 26. <i>Syncephalastrum</i> sp. |
| 3. <i>Aspergillus versicolour</i> | 17. <i>Verticillium</i> sp. |
| 4. <i>Penicillium</i> —1 | 18. <i>Trichothecium</i> sp. |
| 5. <i>Penicillium</i> —2 | 19. <i>Rhizoctonia solani</i> |
| 6. <i>Mucor</i> sp. | 20. <i>Nigrospora</i> sp. |
| 7. <i>Cephalosporium</i> sp. | 21. Culture No. —3 |
| 8. <i>Sphaeronema</i> sp. | (Sterile coloured hyphae producing black |
| 9. <i>Helminthosporium</i> sp. | sclerotia by repeated cell-divisions) |
| 10. <i>Curvularia</i> sp. | 22. Culture No. 4 |
| 11. <i>Arachniotus</i> sp. | (Sterile brown mycelia nodulated) |
| 12. <i>Trichoderma</i> sp. | 23. Culture—5 |
| 13. <i>Fusarium</i> sp. | (Sterile yellow submerged mycelial |
| 14. <i>Cunninghammella</i> sp. | masses). |

Ecological distribution of the above fungal flora in soil treatments and in time seems to vary widely. Some of them are very rare indeed throughout the whole year e.g. *Sphaeronema*, *Syncephalastrum* and *Nigrospora*. *Aspergilli*, *Helminthosporium* and *Curvularia* are frequent to common fungi in early Summer to Autumn while reverse is the case with *Cephalosporium*, *Trichothecium* and *Trichoderma*. Their occurrence seems also to vary with soil treatments as for example, *Rhizopus*, *Cunninghamella* or *Mucor* appear more frequently in F_D or deep ploughed plots than F_0 and F plots.

D.5.3. Influence of aminoacids on the colonisation of organic matter by soil fungi

Two clean slides were placed on filter paper in a petridish and fragments of stems of gram were placed on it and the whole thing was then autoclaved under 15 lbs pressure for 15 minutes. Filter paper was then moistened with sterile water and stem fragments were inoculated with Malt agar inoculum discs (0.7 cm) of *Trichoderma* and *Cephalosporium*, separately. Stem fragments from half the total number of dishes were dipped in 0.2%, casein hydrolysate just before inoculation. Extent of growth was measured along the stem after 5 days and given in table 21.

TABLE 21
GROWTH IN CM ALONG GRAM STEM IN 5 DAYS

Replications	Trichoderma		Cephalosporium	
	Control	Casein supplemented	Control	Casein supplemented
1	1.0	3.5	5.4	2.0
2	0.5	4.4	4.9	1.0
3	2.4	2.4	2.2	3.3
4	3.3	2.3	2.2	2.9
5	3.0	3.2	2.6	2.2
6	2.5	2.6	2.7	3.5

D.6. Studies on Countercurrent Distribution

D.6.1. An expression regarding partition coefficient in single withdrawal procedure

Distribution pattern of a solute subjected to single withdrawal procedure in counter-current distribution obeys Pascal's distribution which might be represented as :

$$S_{\alpha} = p^n \left[(n'_0 - 1) + (n'_1)q + (n'_2 + 1)q^2 \dots + (n'_r - 1 + r)q^r \dots \right] \quad \dots (1)$$

where S_{α} is sum to infinity, as an infinite number of withdrawal series is possible, n' is the transfer number at which withdrawal begins and p and q have the same significance as used in probability distribution. (Stene, S.-Arkiv Kemi, Mineral. Geol. A 18, No. 18 (1944).

Hence the r th terms of the series, expressed in the conventional term used in counter-current distribution, might be represented as :

$$T_{n,r} = \frac{(n+r)!}{r!n!} \left(\frac{K}{K+1} \right)^{n+1} \left(\frac{1}{K+1} \right)^r \quad \dots (2)$$

Using Stirling's approximation to the appropriate terms in the logarithmic form of equation and then differentiating it with respect to r , we have,

$$\frac{d \ln T_{n,r}}{dr} = \frac{n+r+0.5}{n+r} + \ln \frac{(n+r)}{r} - \frac{r+0.5}{r} - \ln (K+1) \quad \dots (3)$$

Assuming $\frac{d \ln T_{n,r}}{dr} = 0$ at the maximum of distribution pattern and solving the differential equation for K , r being r_{max} .

$$K = \frac{n+r_{max}}{r_{max}} e^{-0.5n/r_{max}} (n+r_{max}) - 1 \quad \dots (4)$$

Equation (4), giving a relationship of K with n and r_{max} , provides a method of obtaining an accurate value of K from the peak of the experimental distribution band which is essential for calculating a theoretical curve of such a distribution. A theoretical curve calculated on the basis of K obtained from equation (4) shows a good agreement with the experimental curve of a withdrawn series of neutral red distributed between n -butanol and 1% HCl.

D.6.2. Characterisation of the antibiotic principle obtained from a *Streptomyces* strain designated as Ac_{14} (475) countercurrent distribution

Amongst a number of Actinomycetes, isolated by us from soil samples collected from various places of India, an antibiotic was obtained from a strain of *Streptomyces* designated as Ac_{14} (475) exhibited a broad-spectrum against a number of bacteria, both Gram-positive, Gram-negative and fungi.

The Culture of the organism was grown in M_{12} medium containing 1% glucose, .05% Peptone, .05% Asparagine and .05% K_2HPO_4 . Stationary cultures were used with seven days of incubation period, the final pH of the broth being almost neutral.

Chemical purification

Isolation of the active principles was performed by passing the fermented broth over a column of an ion-exchange resin, Amberlite IRC-50. The column was then washed thoroughly with N/20 methanolic HCl. The eluate, deep brown in colour, was evaporated to dryness in a vacuum evaporator below 45°C. It was then treated with methanol, filtered and the filtrate was passed through a volume of alumina for purification. The column was subsequently eluted first with acetone and then with methanol. The light yellow coloured eluate was treated with half of its volume of ether when the active principle separated out in the form of white flocculent precipitate.

D.7. Physiology of microorganisms

D.7.1. Microbial synthesis of polysaccharides from a *Pseudomonas* sp

The physiology of the *Pseudomonas* sp. was studied in this laboratory in detail with respect to polysaccharide synthesis. Antimicrobial property was tested against common test bacteria and test fungi.

The strain of bacterium utilised Czapek-Dox broth, a synthetic medium, very easily. The culture filtrate on analysis was found to contain polysaccharides.

Czapek-Dox medium containing 4% sucrose (as the carbon source) at pH 7.2 was inoculated with the bacterium and incubated at 25°C for 15 days. Afterwards, the broth was centrifuged out, 7-8 volumes of alcohol were added and kept at 0°C for 2 days. The supernatant liquid was decanted off and the precipitated mass was centrifuged well and washed with absolute alcohol. The white residue was dissolved in a small volume of water, pptd. out with absolute alcohol and centrifuged again. The centrifugate was dried in vacuo. It was a white powdery mass, fairly soluble in water and gave Molisch's test positive and Benedict's test negative. On hydrolysis Benedict's test for sugar was however positive. Hydrolyzed product on paper chromatography gave two spots one corresponding to that of glucose and another unidentified having R_f 0.15 using Benzene-Butanol-Pyridine-water (1:5:3:3) as the developing solvent.

Further work is now being carried out to study the immunological behaviour of the polysaccharide.

D.7.2. Isolation of a red-coloured bacillus from carrot

Carrot was found infected with a bacterial plant pathogen which produced soft rot. The bacteria isolated in pure culture and was found to produce intense red pigment at temperatures 22°-30°C and none at all 37°C. Growth of the bacteria was poor at 22°C and normal at 25°C and 37°C while it was vigorous at temperatures at 28° and 30°C.

TABLE 23

GROWTH AND PIGMENT PRODUCTION AT DIFFERENT TEMPERATURES				
22°C	25°C	28°C	30°C	37°C
Colour-deep red	Colour-deep red	Colour-reddish pink	Colour-pink	Colour-white
growth	growth	growth vigorous	growth vigorous	normal

The infection of carrot was most favourable at 25°C while at temperatures below and above the severity of infection was comparatively less.

The characters of the bacterial plant pathogen was studied. The strain belongs to the Genus *Chromobacterium* and the associated pigment production at temperatures lower than that at 30°C is considered to be characteristic feature worthy of genetic studies.

D.8. Bacterial Transformation

It has been demonstrated that in a few bacterial species, mutations can be induced under the influence of DNA fractions isolated from genetically dissimilar members of the same species. In transformation reaction the specially prepared extract of a bacterial strain is added to the culture of a related strain. Hereditary alterations occur as a result of acquisition of a genetically active unit by the transformed cell. This was observed in encapsulated *Pneumococcus* and detailed work was done.

D.8.1. Transformation reaction in *Staph. aureus*

In the present investigation an attempt has been made to induce transformation reaction in *Staphylococcus* sp. The *Staph. aureus*, a pathogenic form, produces colonies of golden colour on nutrient agar. The transforming principle, DNA was prepared with utmost care from 25 litres of *Staph. aureus* culture broth. The cells were harvested by centrifugation and after a series of reactions the purified DNA was obtained. The substance was repeatedly precipitated and kept stored at low temperature in the dissolved state in saline.

The transforming principle thus prepared was added to the culture medium of a related species of bacteria—*Staph. albus* which is characterised by its white colony on nutrient agar. The whiteness of *Staph. albus* is a heritable character and it was desired to induce transformation in this bacterial strain with the DNA prepared from *Staph. aureus*. The work is in progress.

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E. ZOOLOGY

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

E.1.1. (a) *Penicillin treatment and intestinal flora of tadpoles.* It was observed earlier that penicillin treatment had a significantly inhibitory effect on the survival of intestinal bacterial flora of tadpoles (*Rana tigrina*); of special interest was the complete suppression and ultimate elimination of the microorganisms producing vitamin B₁₂. This observation was of considerable interest specially in view of the reported action of vitamin B₁₂ in counteracting the retardation of tadpole metamorphosis by penicillin. During the year under review, an attempt was made to examine the question thoroughly with a large number of tadpoles. Unfortunately about 80% of the treated tadpoles died within 15 to 30 days; the actual cause of this high mortality could not be ascertained. Of the remaining ones only a few metamorphosed within three to four months, while the rest of them continued in the larval state for four to six months after which spontaneous transformation took place. The examination of intestinal flora was not taken up in view of this high rate of mortality. It is planned to investigate the question this year during the monsoon when a sufficiently large number of tadpoles would be easily available.

(b) *Thiourea treatment.* Thiourea is known as a highly efficient anti-thyroid drug and in suitable doses inhibit the production of thyroxine by the thyroid gland. It has been reported previously that metamorphosis of tadpoles of different species (*Rana tigrina* and *Bufo melanostictus*) is inhibited by thiourea. This inhibition is probably due to suppression of biosynthesis of thyroid hormone which is indispensable for initiation of metamorphosis. The action of vitamin B₁₂ in inducing metamorphosis of penicillin treated tadpoles may be due to its stimulating effect on the production of thyroid hormone or it may be directly related to the differentiation of the cells. To study the question it was decided to stop the transformation by thiourea treatment (chemical thyroidectomy) and then to observe the effect of vitamin B₁₂ on the retarded tadpoles. A large number of tadpoles were treated with thiourea (50 mg. in 1000 cc. water) on every alternate day, the treatment being continued for 20 days. In thiourea groups the death rate was high after the lapse of 20 days. The surviving tadpoles did not show any sign of metamorphosis upto 5 months even though removed from the treatment. Then they were treated with vitamin B₁₂ (dose-ranging from 20-40 µg. per 1000 ml. water). Some of the penicillin treated tadpoles were also similarly treated with vitamin B₁₂. But satisfactory results with respect to metamorphosis were obtained neither in thiourea group nor in the penicillin group, probably due to low temperature in winter. However, a few tadpoles of penicillin group metamorphosed after 30 days of vitamin B₁₂ treatment.

E.1.2. Studies on the effect of thyroxine and thyroxine-penicillin mixture on the metamorphosis of tadpoles (*Bufo melanostictus*). Ershoff in 1950 observed a prolongation in the survival time of hyperthyroid rats fed with penicillin mycelia in conjunction with a diet containing casein. This observation is an indication of minimising the thyrotoxicosis by

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penicillin, or any other factor present in the penicillin mycelia. Some preliminary studies were undertaken to observe whether penicillin can counteract the effect of thyroxine in tadpoles. With this in view three groups of tadpoles (each group containing 10 tadpoles) were taken and treated with thyroxine only and another three with thyroxine-penicillin mixture. The tadpoles of thyroxine group were kept in 1000 ml. of thyroxine solution containing 0.02 mg. of thyroxine for six hours and then transferred to fresh tap water. The tadpoles in the thyroxine-penicillin group were kept in thyroxine-penicillin mixture in the jars containing 0.02 mg. thyroxine and 40,000 units of penicillin in 1000 ml. for six hours and then transferred to 1000 ml. penicillin solution only containing 40,000 units of penicillin overnight. In such a way these groups of tadpoles were treated on every alternate day. After three treatments they were kept under observation in simple tap water containing hydrilla. It was noticed that about 90% of the tadpoles in thyroxine group metamorphosed within 10 days, but all of them died after metamorphosis. In thyroxine-penicillin group not a single tadpole showed sign of complete metamorphosis. Within 10 days 50% of the larvae of this group showed emergence of three limbs (two hind limbs and one fore limb) and the remaining only two hind limbs. Death rate in penicillin-thyroxine group was somewhat higher during this period. From these observations it may be concluded that penicillin can not only counteract the effect of thyroxine on tadpoles, but it may besides effecting retardation of transformation of tadpoles may also have some effect on other organs or systems. Detailed studies are being continued.

E.1.3. *Studies on the actions of penicillin and thyroxine in albino rats.* In last year's report it was mentioned, that penicillin to a large extent and thyroxine to a smaller extent increased the body weight of young rats in 30 days. The different chemicals were administered either by interperitoneal injection or by direct mouth feeding. It was interesting to note that the results were more pronounced and consistent when the chemicals were injected rather than orally administered.

The mechanism of action of penicillin in stimulating growth of higher animals is not well understood, though several theories have been advanced from time to time to explain it on the basis of its effects on intestinal micro-flora.

E.1.4. The effect of penicillin and thyroxine on the overall growth of healthy young albino rats is being studied. An attempt was made to find out if the observed increase in body weight could be related to true growth. For this purpose moisture, protein and fat content of the skeletal muscles and tissues of different organs are now under examination. A final assessment is possible only when a large number of animals have been subjected to treatment and analysis. However, it can be stated on the basis of the results already obtained, that penicillin treatment generally reduced the moisture content of skeletal muscles but increased the fat content. The results suggest that the addition of solid matter in the body as a cause of increased weight of penicillin treated animals. Increase in fat content also indicates some imbalance in fat metabolism, probably a general retardation of oxidation of fatty acids. An analysis of the fatty acid constituents of these fat and also of phospholipid and cholesterol content is now under investigation and may provide some clue as to the nature of the disturbance in fat metabolism.

E.2. Micromanipulation technique for the establishment of homogeneous clones and enucleation of *Amoeba*

Experiments were continued for the establishment of homogeneous clones for subsequent study of the *Amoeba proteus* using micromanipulation technique. After repeated trials we have been successful in isolating a single amoeba from a culture solution containing numerous heterogeneous organisms. For this purpose both the instruments of Peterfi and of de Fonbrune type are being used.

In order to proceed with the work of nuclear transplantation, primarily we have to raise mass culture of homogeneous clones of amoeba. A single amoeba was transferred into a depression in a slide filled with a nutrient solution. The slide was then removed to a moist chamber. The amoeba in one of the cups was observed to multiply to about three to four minute individuals and then to disappear within a few days. Evidently starvation was the cause of such disappearance. The effect of introducing globular protozoa to the nutrient solution was then studied. The effect was more satisfactory, as more than nine organisms were observed to form, but they all disappeared. However, fresh attempts are being continued for mass culture of homogeneous clones in new culture media, prepared from the mixed broth of germinating wheat, decoction of straw and hydrilla plant.

F. WORKSHOP

During the year under report requisitions for more than 500 were received from the different departments of the Institute. Almost all of them have been completed. Some of the major jobs which were taken up are mentioned here.

For Physics Dept. (Ultrasonic and Microwaves)

1. Microphotometer having attachment for fine movement of exposed film drive.
2. Optical bench for ultrasonic investigations in liquid.

For Cosmic ray

3. Modifications of ionisation chambers.
4. Large number of G. M. counters made of brass and glass tubes.
5. Modifications of the large cloud chamber and its accessories.

For Nuclear Physics

6. Precision spherical absorbers for measurements of gamma ray photoelectric effect.
7. Mechanical gamma-ray intensity averaging arrangement over (i) circle (ii) sphere.
8. Differential Compton scattering measurement apparatus.
9. Neutron non-elastic cross-section measurement apparatus.

For Neutron Generator

10. Eight extra stages of high voltage Cockcroft Walton generator has been added for which (i) corona proof condenser housings and (ii) corona-rings of new design have been constructed.

11. Target cooling arrangement has been completed.

12. Lead shields for counters have been made.

For Cobalt 60 sources

13. Source holder and housing arrangement for the 2 curie cobalt 60 source in the Institute area has been completed. One winch system has been installed for operating the source and the lead lid from a distance.

14. Additional shielding arrangement for 20 curie cobalt 60 source at Nilgunge has been provided by assembling of lead bricks, moulded and machined in the workshop.

For Chemistry

15. Modification of the gas phase chromatography apparatus on a more complicated design has been taken up.

For Plant Physiology

16. One large shaker consisting of 7 trays, made of aluminium frame and perspex bottoms, having reciprocating motions of 30 per minute has been constructed for tissue culture work.

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Refrigeration Dept.

The running of air conditioning plants, maintenance and repair of refrigerators, air conditioning units, refrigerated centrifuge were carried out as usual. One additional room in the microbiology department has been air conditioned.

Water Supply

One 2½" tube well with 4" casing has been sunk to meet the present increasing requirements. The cooling arrangement of the corrugated iron roof of the main building during summer months by spraying of water at intervals by a pump has been completed.

G. LIBRARY

The library consists of 5,015 volumes (2,480 books and 2,535 bound periodicals). During the year under report 209 volumes (80 books and 129 bound periodicals) were added to the stock as against 431 during the previous year. The total number of periodicals received was 215 (inland 62 and foreign 153) out of which 113 were subscribed and the remaining 102 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.

The library is primarily meant for use of its research staff only but it is frequently consulted by many research workers and scientists from other institutions. As the number of outside readers is increasing it has been found necessary to restrict its use to bonafide research workers to whom reader's cards are being issued.

The library is experiencing great difficulty in storing the increasing number of books and periodicals. This year a room on the ground floor of the Library building, of almost the size as the present stack-room has been made available as additional stack-room. It is expected that within the next year the new stack-room will be ready for use.

One of the difficulties in meeting the growing demand for books and journals, is the mounting cost of acquiring books and journals specially of American origin. The annual increase in the grant which the library receives from the limited resources of the Institute is more than eaten up to meet increase in rates of subscriptions and prices of books. Occasionally the library has received valuable presentation of books and journals from the U.S.A. under the "India Wheat Loan Educational Exchange Program". We understand that after this year no further grants will be available from that source.

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PUBLICATIONS

Volume 23 (1959-60) of the "Transactions of the Bose Research Institute" is now in press and is expected to be published shortly.

Many important and popular works of Sir J. C. Bose viz., "Response in the Living and Non-Living", "Plant Response", "Researches on the Irritability of Plants", etc. have been out of print for a long time. Efforts are being made to reprint these volumes in the near future and the reprinting of "Response in the Living and Non-Living" has been taken up and is expected to be published in next year. Other publications during the year under report were (1) Report of the Bose Institute for 1958-59, (2) Jagadis Chandra Birth Centenary Celebration Addresses and Twentieth Memorial Lecture by Dr. S. Radhakrishnan, Vice-President of India, and (3) The Twentyfirst Acharya Jagadis Chandra Bose Memorial Lecture on "Technology and Economic Change in the Third Plan" by Sir Jehangir Ghandy and Director's Report.

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LIST OF PUBLISHED PAPERS 1959-60

Title of papers	Author	Where published	Reference
1. Wachstumsregulatoren und verwandte Stoffe. In Papierchromatographie in der Botanik. Zweite erweiterte Auflage	S. P. Sen	Springer Verlag (Berlin) 1959.	
2. Translocation and utilization of sulphate S ³⁵ and phosphate P ³² in the pea plant.	B. B. Biswas S. P. Sen.	Ind. Jour. Pl. Physiol.	2:1(1959)
3. Relationship between auxins and nucleic acid synthesis in coleoptile tissues.	B. B. Biswas S. P. Sen	Nature	183:1824 (1959)
4. Photosynthesis in Hydrilla.	D. K. Basu S. P. Sen	Proc. Nat. Inst. Sci. (India)	25B:215 (1959)
5. Metabolic changes in photoperiodism: A tracer study.	S. P. Sen	Proc. 9th Intern. Bot. Congress. (Montreal)	p.353 (1959)
6. The relationship between auxins and sulphur metabolism in plants.	A. Sengupta S. P. Sen	Proc. 47th. Ind. Sci. Congress	Part III p.354(1959)
7. Attempted synthesis of psoralidin.	S. K. Chakraborty P. K. Bose	Trans. Bose Res. Inst.	Vol. XXIII (in press)
8. Natural Flavones. Part VI. The yellow pigment of the flowers of <i>Helichrysum annum</i> .	S. K. Chakraborty A. K. Banerjee P. K. Bose	Ibid.	Ibid.
9. The effect of psoralene on tyrosinase activity and melanin pigmentation	D. P. Chakraborty C. Deb. M. Mukherjee	Science and Culture.	25, 386 1959.
10. The constitution of albigenic acid. A new triterpenoid saponine from <i>Albizia Lebbek</i> , Benth.	A. K. Barua S. P. Raman	Tetrahedron	Vol. 7, 19-23, 1959.
11. Thermal neutron absorption in chlorine.	A. M. Ghose.	Proc. Ind. Sc. Cong. II.	p. 76 (1960)
12. Instrumentation for radio-carbon dating	A. M. Ghose A Nath	Ibid.	1.87, 960

Title of papers	Author	Where published	Reference
13. Photon counter with approximately constant spectral sensitivity.	A. M. Ghose	Proc. 4th. Low Energy Nuclear Physics Symposium	(in press)
14. Investigations of the angular distribution of Compton scattered Co-60 photons.	A. M. Ghose.	Ibid.	Ibid
15. Cytotaxonomy of <i>Ludwigia parviflora</i> Rox b.	Mary Korah	Trans. Bose Res. Inst.	Vol. XXIII (in press)
16. Amphivasal vascular bundles in <i>Cyperus</i> .	A. P. Mani	Science and Culture	25, 437-438 1960
17. Development of ovule and embryosac in <i>Croton bonplandianum</i>	A. P. Mani	Ibid.	25, 439-440
18. Preliminary note on comparative study of the monazite sand at Manavala-kurichy in the Madras state.	G. G. Nair K. T. Jacob S. Bose.	Science & Culture	26, 42-43
19. Breeding value of radiation induced morphological mutants of <i>Brassica Juncea</i> . L.	U. K. Rai G. G. Nair	Jour. Ind. Oil-seeds	Vol. III No. 4. Oct 1959.
20. A preliminary study of meiotic nucleolus in <i>Sesamum orientale</i> L.	G. G. Nair.	Proc. of Ind. Sci. Cong. 47th.	1959-60
21. Diurnal and nocturnal changes undergone by chloroplasts in motor cells of <i>Mimosa pudica</i> Linn	Mridula Datta A. Chatterjee	Science and Culture	24:426-428 1959
22. A new interpretation of the structure of the contractile vacuole in sensitive pulvini	Mridula Datta	Trans. Bose Res. Inst.	Vol. XXIII (in press)
23. The cytogenetics of some ornamental jasmynes	Mridula Datta	Caryologia (Italy)	(in press)
24. The effect of visible light on the mitotic spindle.	Mridula Datta	Expt. Cell. Res. (U.S.A.)	(in press)
25. Incorporation of aminoacids into protein of particulate preparation from <i>Azotobacter vinelandii</i> .	D. P. Burma M. Chakraborty	Fed. Prod.	19:348 1960.

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Title of papers	Author	Where published	Reference
26. Free aminoacids in <i>Azotobacter vinelandii</i> and their activation in cell-free preparations.	M. Chakraborty	Trans. Bose Res. Inst.	Vol. XXIII (in press)
27. Study of microbial synthesis of protein in relation to the biogenesis of nucleic acids with <i>Azotobacter vinelandii</i> .	M. Chakraborty	Nature	In press.
28. Purification and properties of of a stereospecific dihydro-lipoic dehydrogenase from <i>Spinacia oleracea</i> .	D. K. Basu D. P. Burma	Jour. Biol. Chem.	235:509 1960
29. Ultraviolet induced bio-chemical mutations in <i>Aspergillus niger</i> .	A. K. Mishra P. Nandi,	Science and Culture	Vol. 25 1959
30. Survey of antibiotic production bacteria from the soils of India and Indonesia.	M Purakayastha P. Nandi	Antibiotics & Chemotherapy	Vol. 10. No.4.1960
31 Inhibition of <i>Rhizobium</i> and <i>Azotobacter</i> by soil fungi.	S. P. Sen P. Nandi.	Trans. Bose Res. Inst.	Vol. XXIII (in press)

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