Printed by Kalipada Mukherjee at Eka Press, 204/1. B. T.

REPORT

OF

THE BOSE INSTITUTE

FOR

1957-58

BOSE INSTITUTE
93/1 UPPER CIRCULAR ROAD, CALCUTTA-9

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REPORT OF THE BOSE INSTITUTE

1957-58

Governing Body. Three meetings of the Governing Body were held during the year.

Council. Five meetings of the Council were held during the year.

The Council at its meeting on 27.3.58, recorded its deep gratification on the election of Prof. S. K. Mitra, one of the members of the Council, to a Fellowship of the Royal Society London, and offered its felicitations to him.

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

Pay scale revision proposals which were accepted with some modifications by the Ministry of Education were given effect to.

Dr. S. K. Roy continued as Acting Registrar till the end of the year 1957-58.

Grants. The following grants were received during the year 1957-58:

(*a*) *Non-recurring grants* (*i*) Rs. 2.00 lakhs from the Government of India which was allocated as follows :

Building	Rs.	96,000.00
Apparatus		75,000.00
Workshop		9,000.00
Furniture & Fittings		20,000.00
	Rs.	2,00,000.00

(*ii*) Rs. 30,000.00, contributed in half the amount each, by the Indian Central Jute Committee and the Indian Central Oilseeds Committee for the construction of a 20-Curie Cobalt 60 field, at the Experimental Farm of the J.A.R.I. at Nilgunge,

(*iii*) Rs. 6,000.00 from the Atomic Energy Department of the Government of India for Cosmic ray measurements for the IGY programme 1957-58, in addition to Rs. 20,000.00 received in 1956-57 for the same purpose.

(*b*) *Recurring Grants*

Govt.of India	Rs.	3,50,000.00
Govt of India—training in scientific research		7,272.48
Govt. of India—Fellowship		5,800.00
Governing Body, Bose Institute		42,856.94
	Rs.	4,05,929.42

Contrary to expectation the Govt. of India recurring grant was reduced from Rs. 4.00 lakhs, received in 1956-57 to Rs. 3.50 lakhs received during the year 1957-58. This has resulted in a deficit of Rs. 32,418.56 in the Income and Expenditure Account.

(*c*) *Grants-in-aid schemes* The following grants-in-aid were received during the year.

Schemes	*Sponsoring Body*	*Amount of grant*
		Rs.
X-ray irradiation on Oilseeds	Indian Central Oilseeds Committee	29,980.00
Weed Control	Indian Council of Agricnltural Research	15,199.48(*)
Antibiotic Production	Council of Scientific & Industrial Research	8,750.29
Radiation mutation in Jute	Indian Central Jute Committee	15,471.50(+)
Cosmic Ray investigations Nuclear Physics investigations Neutron Generator Induced mutagenesis in crop plants using x-rays & radio-active isotopes	Board of Research on Atomic Energy	1,00,000.00
Research scholarships, stipends, and foreign scholarships	Sir J. C. Bose Trust Fund No. 1	14,000.00

+Amount received after 31st March 1958

*Amount not yet received from the Govt. of West Bengal.

Acharya Jagadish Chandra Bose Memorial Lecture.

The Nineteenth Lecture of the series entitled "India and the Atomic Age—Her Natural Resources for Nuclear Power Production" was delivered by Dr. D. N. Wadia, M.A., D.Sc., F.R.S., Geological Adviser to the Govt. of India, Dept. of Atomic Energy, on November 30, 1957. The Lecture has been published along with the Director's Report presented to the 40th Anniversary meeting of the Institute.

Study Abroad.

1. Sri Ananda Mohan Ghose, Junior Research Assistant in the grant-in-aid scheme of the Dept. of Atomic Energy, has been awarded a Smith-Mundt Scholarship and a Full-bright Travel grant for higher studies and research in the Brookhaven National Laboratory, U.S.A. He proceeded abroad on study leave on 26.8.57.

2. Sri Nripendra Kumar Ganguli, Junior Research Assistant in the A.E.C. scheme, has been awarded a Research Scholarship by the North Carolina State College, U.S.A., for undertaking research work on Nuclear Physics. He proceeded on study leave abroad with effect from 28.10.57.

Grants for study abroad.

A grant of Rs. 2000/- each out of J. C. Bose Trust Fund Grant was made available to Sri A. M. Ghosh & Sri N. K. Ganguly to defray their cost of passage to the U.S.A.

Visitors.

The Institute was honoured by the visit of H.E. Dr. Ho-Chi-Minh, President of the Democratic Republic of Viet-Nam on February 13, 1958. The following also visited the Institute during 1957-58 :

(1) Dr. E. H. Wakefield of the Radiation Counter Laboratories Inc., U.S.A. (2) Dr. Schroder of the University of Leipzig. (3) Dr. Kraadz of the University of Berlin. (4) Prof. K. V. Thimann, Professor of Biology, Harvard University, USA., (5) Prof. H. M. Kalckar of the National Institute of Health, Bethesda, Maryland, USA. (6) Prof. E. H. Ennor, Dean of the John Curtin School of Medical Research and Professor of Biochemistry at the Australian National University, Canberra. (7) Dr. Y. Tazima, Head of the Division of Morphological Genetics, National Institute of Genetics, Mishima, Shiznoka, Japan., (8) Prof. Arthur Kornberg, Professor of Biochemistry, Washington University, U.S.A. (9) Prof. J. H. Quastel, F.R.S. of the McGill University, Montreal. (10) Prof. J. Lederberg, Department of Genetics, Wisconsin University. (11) Dr. Shiu-ying Hu, Botanist, Flora of China Project, Arnold Arboretum, Harvard University, (12) Prof. E. G. Richardson of King's College , Newcastle-on-Tyne, U.K. (13) Madam Y. Khouvine, Director of Higher Studies, Institute de Biologie Physico-Chemique, Paris. (14) Prof. K. Mothes, Director, Plant Physiological Laboratory, Gatersleben, East Germany. (15) Prof. Richard Kuhn, N. L., Director, Institute of Chemistry, Max Plank Institute, Heidelberg, West Germany. (16) Prof. Chou-tun-Ching, Professor of Physics, Fu tun University, Shanghai, China., (17) Prof. S. P. Tolstov & Prof. V. P. Peshkov, U.S.S.R., delegates to the Indian Science Congress 1958. (18) Prof. Konard Hruban and Prof. Dr. Otto Jirovec of Czechoslovakia. (19) Dr. Anton Peterlin of Lujubliana, Yugoslavia, (21) Prof. I. I. Emanulloff., Member, Bulgarian Academy of Science.

New Appointments and Awards.

1) Sri Nirmal Kumar Sinha, M.Sc., was appointed Research Scholar in the Dept. of Chemistry with effect from 12-4-57.

2) Sm. Maharani Chakraborty, M.Sc., was appointed Research Scholar in the Dept. of Botany with effect from 1.8.57.

3) Dr. Satyabrata Sarkar was appointed Research Assistant in the Dept. of Botany with effect from 6-7-57.

4) Sm. Anjali Chowdhury, M.Sc., was appointed Research Scholar in the Dept. of Physics with effect from 8-7-57.

5) Sri Bimalendu Mitra, M.Sc., was appointed Junior Research Assistant in A.E.C. scheme with effect from 17-6-57 vice Sri A. N. Banerjee resigned.

6) Sri G. Gopalan Nair, M.Sc., was appointed Assistant Botanist in the I.C.O.C. scheme with effect from 23.8.57 vice Sri R. N. Mukherjee resigned.

7) Sri Deb Kumar Basu, M.Sc., was appointed Chemical Assistant in the I.C.O.C. scheme with effect from 31-8-57 vice Sri N. C. Saha resigned.

8) Sri Balaram Mozumder, M. Sc., was appointed Junior Research Assistant in A.E.C. Scheme with effect from 8-1-58.

9) Sm. Asha Lata Paul, M.Sc., was appointed Research Scholar in the Dept. of Microbiology with effect from 24-2-58.

10) Sri Nirmalendu Chowdhury, M.Sc., was appointed Junior Research Assistant in A.E.C. Scheme with effect from 15-3-58 vice Sri C.R. Mallick resigned.

11) Sri Jotirmoy Datta, M.Sc., was awarded Sir J. C. Bose Fellowship of Calcutta University with effect from 1-9-57.

12) Sri Ajit Kumar Banerjee, M.Sc., was appointed Research Scholar in the Dept. of Chemistry with effect from 1.8.57.

13) Sri D. P. Chakraborty, M. Sc., was awarded Junior Research Fellowship of National Institute of Science of India with effect from 26-8-57.

14) Sm. Mira Purkayastha, M.Sc., was appointed Research Scholar in the Dept. of Microbiology with effect from 1-6-57.

15) Sri V. S. Sarma, M. Sc., was appointed Research Assistant in the Dept. of Botany with effect from 6.1.58.

16) Sri A. K. Mishra, M.Sc., was appointed Research Assistant in the Dept. of Microbiology with effect from 1.1.58.

17) Sri P. P. Mukherjee, M.Sc., was appointed Research Assistant in the Dept. of Microbiology with effect from 1.3.58.

18) Sri K. V. Rao, M.Sc., was appointed Research Scholar in the Dept. of Chemistry with effect from 15-9-57.

Resignations

1) Sri N. C. Saha, M.Sc., Chemical Assistant in the I.C.O.C. scheme, resigned w.e.f. 31-7-57 to join the Fuel Research Institute.

2) Sri Ramendra Nath Mukherjee, Asst. Botanist, in the I.C.O.C. scheme, resigned with effect from 23-7-57

3) Sm. Chitra Ghosh, M.Sc., Mary and Richard Keating Student, resigned with effect from 15-8-57 on ground of ill health.

4) Sri Chittaranjan Mallick, M.Sc., Junior Research Assistant in the A.E.C. scheme resigned with effect from 12.12.57 on ground of ill health.

5) Sri Sachindra Kumar Chakraborti, M.Sc., Research Scholar in the Dept. of Chemistry, was released from 28.2.58 to enable him to take up an appointment under I.C.M.R. in the Institute of Child Health, Calcutta.

6) Sri P. P. Mukherjee, M.Sc., was released from his post of Junior Research Assist. in C.S.I.R. scheme with effect from 1.3.58 to enable him to take up an appointment as Research Asst. in the Dept of Microbiology.

7) Sri Ajit Kumar Medda, M.Sc., Senior Manpower Scholar, resigned with effect from 1.5.57.

Degrees award.

1) Sri B. B. Biswas was awarded D. Phil degree of the Calcutta University.

2) Sri U. K. Rai was awarded D. Phil degree of the Allahabad University.

3) Sri M. G. Srivastava was awarded D. Phil degree of the Calcutta University.

4) Sri T. C. Bhadra was awarded D. Phil degree of the Calcutta University.

Seminar.

Fortnightly seminars were held during the period under review, when the members of the research staff reported on the results of their investigations and reviewed the progress of work done on selected topics. This helps the dissemination of scientific knowledge among the members of the research staff dealing with different subjects.

Date	*Speaker*	*Subjects*
6. 9.57	Sm. Nilima Basu	Absolute differential range of sea-level μ meson at 12° N.
13. 9.57	Dr. P. N. Nandi	Development of Streptomyces mutants for antibiotic production.
20. 9.57	Dr. S. B. Sarkar	Role of Gibberellic acid in flower formation.
27. 9.57	Dr.M. G. Srivastava	Cytotaxonomical studies in Leguminosae.
15.11.57	Sri S. B. Ghosh	Counter-current distribution and its applications in Biochemistry.
22.11.57	Sri D. P. Chakraborty	Chemistry of Indian Guttiferae.
6.12.57	Dr. D. P. Burma	Enzymatic apparatus of Lactobacillus pentosas for the handling of fine carbon-sugars.
14.12.57	Dr. S. B. Sarkar Sri. D. P. Chakraborty	Developments in Mimosa Problem.
14. 2.58	Dr. M. S. Sinha Miss. Nilima Basu	(*i*) New evidence for a particle of mass 525 me (ii) Anomalous scattering of low energy mu-mesons in copper.

Special Lecture & Seminar.

Prof. K. V. Thimann, Professor of Biology, Harvard University, U.S.A. and a visiting Professor in the Department of Botany, Calcutta University, under the India Wheat Loan Education Exchange Programme, gave a talk on "Growth".

2) Prof. H. M. Kalckar of the National Institute of Health, Bethesda, Maryland, U.S.A., gave two talks entitled "Hereditary Galactosemia—a molecular disease" and "Further considerations concerning defects in glactose-metabolism".

3) Prof. Arthur Kornberg, Professor of Biochemistry, Washington University, gave a talk on "Enzymatic Synthesis of Deoxyribonucleic acid".

4) Prof. J. Lederberg of the Department of Genetics, University of Wisconsin, Madison, U.S.A. delivered a lecture entitled "Drug Resistance in Micro-organisms".

5) Prof. E. G. Richardson of King's College, Newcastle-on-Tyne, delivered a lecture on "Ultrasonic Measurements".

6) Madame Y. Khouvine, Director of Higher Studies, Institute de Biologie Physico —Chemique, Paris, gave an illustrated lecture on "In vivo and in vitrio incorporation of P^{32} in the nucleic acids of epitheliomatus tissues."

7) Under the auspices of the Botanical Society of Bengal, Prof. Dr. K. Mothes, Director, Plant Physiological Laboratory, Gatersleben, East Germany, delivered a lecture entitled "Function of root in the life of Plants" in this Institute.

8) Under the auspices of the Indian Chemical Society, a symposium on "Terpenoids" was held in this Institute.

New building and Laboratory extensions.

A two storied block at the western end of the Institute grounds has been completed and equipped with furnitures and fittings. When the workshop is removed to the ground floor of this building, the rooms occupied by the latter on the ground floor of the southern block extension, will be converted into air conditioned laboratories for biomolecular, plant biochemical and physiological investigations.

A three storied block containing 8 rooms in each floor has been erected to house the subordinate staff members of the Institute. Increased water supply both for the new blocks as well as to the older laboratories are being provided by means of a new tube well as well as by the construction of two reservoirs.

The Institute has received a grant of $30,000.00 under the India Wheat Loan Educational Exchange Programme, out of which an Ultracentrifuge and an additional Beckman's Spectrophotometer are being acquired.

A de Fonbrune micromanipulator and a three pen unit pen recorder have been recently purchased. The former is being housed in a special air conditioned room. The latter is being used to study the electric action potentials accompanying mechanical responses in motile plants.

J. C. Bose Birth Centenary.

A Committee has been formed for celebrating the Birth Centenary of the Founder of the Bose Institute, which falls due on November 30,1958. This committee is working in close cooperation with the Bose Institute, whose Director Dr. D. M. Bose is the Treasurer to the Committee.

The special activities assigned to the Bose Institute include

(i) Publication of a Centenary Memorial Volume of the Transaction of the Bose Research Institute. A large number of distinguished foreign and Indian scientists have been invited to contribute papers,

(ii) Arranging an exhibition (a) of apparatus, with charts and experimental demonstrations of J. C. Bose's classical apparatus and of a representative collection of investigations carried on at present in the Bose Institute, (b) momentoes of J. C. Bose, including letters, manuscripts, pictures and other collections,

(iii) Assist in the preparation of a documentary film on the life and work of J. C. Bose, which has been entrusted by the Ministry of Information and Broadcasting to a well known film director.

A long one storied block (120′×40′) in front of the new workshop building is in course of construction, to house this exhibition. This block will later be converted into a special laboratory for pilot plant studies.

RESEARCH ACTIVITIES—NON-TECHNICAL REPORT

Ultrasonic radiation. One of the accepted methods for extracting enzymes and proteins intact from bacterial cells, is to subject bacterial suspensions to ultrasonic radiation in specially cooled vessels. An apparatus made in the laboratory enables this technique being employed for desegregation of conglomerate tissues and disintegration of living cells. The same technique is being employed for loosening some of the bonds in coal molecules, enabling studies on increase in chemical reactivity in coal specimens so treated.

Separate measurements of radiation and streaming pressure components in u.s. radiation propogated through liquids is being continued. It is expected such investigations may throw some light on the nature of bulk viscosity whose existence has recently been postulated, to explain anomalous absorption shown by some liquids.

The intensity distribution in the diffraction spectra produced by passing light through a u.s. radiation field in a liquid medium, is being investigated.

Cosmic rays. A rectangular cloud chamber has been in continuous working in the Mayapuri Research Station, Darjeeling. Special arrangements have been made to photograph unstable particles of life time $\sim 10^{-8}$ sec. inside the chamber. These include studies on heavy mesons and hyperons. Amongst the interesting results obtained are (i) evidence of cascade decays of a superheavy meson (1480 m_e), (ii) the associated production of a Σ_0 and a $K^+\mu_2$ particles. The most interesting feature of this photograph is that besides showing the usual decays of $\wedge^\circ$ ($\rightarrow\pi^-+P$) and K_μ ($\rightarrow\mu^+ + \nu$) it shows that the materialisation of the photon resulting from the transition $\Sigma^\circ \rightarrow \wedge^\circ + \gamma$; an overall energy balance is obtained for the whole event.

New evidence for the existence of a particle of mass$\sim$525 m_e has been obtained among the pictures of stopped particles photographed in a Wilson Chamber in Calcutta. They go to cofirm the findings of the Russian group of observers working under Alikhanyan.

A large cloud chamber (36″×30″×16″) which can house 17 half inch plates is under construction; it will be ready in a few months' time. This apparatus will be very suitable for the study of long range secondaries of unstable particles.

IGY programme. Two cubical telescopes installed at Mayapuri, Darjeeling, are operating successfully since August 1957. A neutron monitor unit employing three $B^{10}F_3$ proportional counter tubes has been recently installed in Mayapuri and is working in a satisfactory manner.

The data collected after preliminary analysis are being sent to the Indian IGY Coordination Committee.

Continuous recording of cosmic ray intensity with a pressure ionisation chamber (vol. 45 litres) coupled with a Lindemann electrometer has been going on in Calcutta. The work has been temporarily interrupted due to a defect in the recording electrometer. A new Lindemann electrometer has been ordered from England.

Preliminary investigations have been commenced on the existence of different maxima in the Rossi curve, using a plastic phosphor in place of the usual G. M. counters or small

pressure ionisation chamber . A multi channel pen recorder has been used to note the different kinds of coincidences between the primary and secondary (charged and uncharged) components.

Nuclear Physics. Systematic data on thermal neutron cross section for different light elements using the spherical symmetry method, devised in the laboratory, have been collected and analyzed. Similarly the 1.3 MeV gamma rays from Co-60 has been used to measure the absorption coefficient of several elements; these have been compared with the corresponding theoretical values.

Two workers in this section are at present in the USA for special research training.

Neutron Generators. A new large neutron generator has been set up. With it a larger ionic deuteron beam current upto 50 μ c, accelerated upto a beam potential of about 100 kv can be used, to strike a tritium saturated target. At the present housing it is not possible to utilise the maximum deuteron current with a neutron production equivalent to 50 Curie Ra Be source. The whole apparatus is being set up on the first floor hall in the new workshop building. Arrangements for air conditioning and for sufficient radiation shielding of the neutron generator room are being provided.

Physical Chemistry. Work on the isolation, fractionation, and physico chemical investigations of seed proteins is being continued. This year detailed studies on the proteins obtained from *Brassica campistria* (Mustard) has been carried out. The total precipitates obtained on electrophoretic analysis at 20° C by Tisellius method showes two major and one minor fast moving components. The isoelectric point of one was in the region of *p*H 6.0–6.2 and of the other around *p*H 10. Work with other proteins is in progress.

Animal Metabolism. The investigation on the effect of ionising radiation on albino rats is being continued. As a preliminary to it some detailed investigations have been carried for desalting, prior to chromatographic analysis of biological fluids, particularly of urine. A new method of desalting of micro quantities of urine by the technique of paper electrophoresis has been devised.

A gas phase chromatographic apparatus has been constructed with assistance from the workshop. It can be worked at temperatures upto 100°C. Complete separation of mixtures of a number of volatile organic fluid mixtures has been achieved. For automatic recording a pen recorder is being assembled for use with this apparatus.

Plant Chemistry. Survey of saponin containing plants occurring in India, is being continued. Seven new plants have been found to contain saponin. Modified methods have been introduced for isolation, which gave higher yields of the sapogenins. The method of separation of the individual constituents from the sapogenin mixture has been perfected. Further studies on the acutogenol B and C have been made.

Another survey is being continued on the occurrence of alkaloids in the so-called nonmedicinal group of plants. Out of 25 plants examined, alkaloids have been detected in three. The relationship between chemical constitution and the antibiotic activities of the coumarins as well as of mesuol are being investigated.

Detailed study of the compound clerodin, the bitter principle of *Clerodendron informatum* has led to the formulation of its chemical structure, which is believed to be the correct one.

Studies on other plant products are being continued.

Radio Chemistry. Translocation of sulphates and phosphates in plants has been investigated with inorganic salts containing the radioactive isotopes of sulphur and phosphorus.

Investigations using C^{14} as tracer have been continued, for interpretation of J. C. Bose's observation that during summer months the aquatic plant *Hydrilla* utilises malic acid as substrate. For this purpose a method has been devised for the synthesis of isotopically labelled compound dl-malic acid–2, 3–C^{14}, using fumaric acid–2,3-C^{14} as starting material.

The C^{14} isotope has also been employed to study carbon dioxide fixation in relation to auxin induced growth and inhibition in plant tissues. The possibility of isolating the so-called 'flowering hormone', which may be expected to be formed in plant leaf exposed to light of suitable wavelength in an atmospheric containing CO_2, has been investigated by using $C^{14}O_2$ as tracer. No qualitative difference in the synthesized carbon compounds was detected in the growing point of Wintex barley plants, supplied with $C^{14}O_2$ during a prescribed light period at the flowering stage.

Enzyme chemistry. On his return after three years' study in Canada and in the USA, Dr. D. P. Burma is organizing a special section dealing with Enzymes. At present the suitability and the relative efficiency of different methods of disrupting bacterial cells without denaturing the enzyme contents is being investigated. Purification and study of the properties of "Zwischen ferment." from *Phascolus mungo,* as well as of ribulose diplosphotase from spinach has been taken up.

Plant Physiology. One of the striking parallelisms between plant and animal responses studied by J. C. Bose, was on the similarities in the correlation between mechanical and electrical responses found in animal and plant contractile tissues eg.

(i) nerve and muscle units in animal, with the petiole pulvinus units in *M. pudica*, (ii) myogenic pulsations in animal hearts with similar pulsations in *Desmodium gyrans* side-leaflets.

Previously the recording of electric pulsations by sensitive long period galvanomenter suffered from the disadvantage of its long period of oscillation; this prevented the recording of the finer details of the electric pulsations.

Recently a pen recording unit, which permitted the recording of electric oscillations of periods upto ten thousand cycle per second, has been set up. With this pen recorder it has been possible to record the finer details of the electric action potential of *D. gyrans* side leaflets. Preliminary studies have revealed some similarities with the P Q R S T characteristics of animal electrocardiograms.

It has been possible to record simultaneously the mechanical and electric responses both in *Mimosa* pudica and *D. gyrans* pulvinus petiole units. It has been found for example

that in *M. pudica* the electric action potential preceded by 1/20″ to 1/10″ the mechanical fall of the pulvinus. Many other interesting observations; *eg* on the course of the reflex arc in a *M. pudica* leaflet are being studied in detail.

Weed Control. Detailed experiments were conducted to find out the most appropriate stage of application of the herbicides, such as amines, esters, sodium salts of 2, 4-D and M.C.P.B. in paddy, for effective weed control, and the cost yield relationship has also been worked out. It was concluded that Na-2, 4-D ethyl sulphate was the most economic of all these chemicals.

Radiation Genetics. The mutagenic action of radiation on seeds of economic plants has been undertaken in the Institute for several years. The sources of radiation employed are x-rays, B-radiation from the isotopes P-32, S-35. Arrangements are nearing completion for installing a 20 curie Co-60 gamma radiation source in a specially fenced 3 acre field at the Jute Agricultural Experiment Station at Nilgunge. Mutagenic action of some herbicides have been studied.

The plants investigated are Jute, Sesamum, Mustard, Rice and Tomato. Preliminary studies with groundnuts have been started. It is expected that the Institute will receive an additional grant-in-aid from the Central Oilseeds Committee for the study of effects of mutagenic radiation on groundnut.

Some of the interesting results obtained are summarized below:

Sesamum

a. The *white flowered* and *small seeded* mutants isolated from the X-radiated progenies of *Sesamum orientale type 16* from West Bengal, had again higher oil contents of 4% and 17% respectively over their controls, with better keeping qualities, both in the fresh as well as one month old oils, as indicated by their lower peroxide values.

b. Some of the other higher yielding and/or early flowering selections from the X-radiated progenies in *S. orientale*, continued to maintain these characters in the subsequent generations.

Mustard

Some of the higher yielding or early maturing selections from the irradiated progenies in Mustard, also continued to exhibit these characters in the subsequent generations.

Jute

The yield of fibre in *Corchorus olitorius* vars., *Tall Mutant*, *R.* 26, *Chinsurah Green* and *C. capsularies*, vars. *D*154 and *D*386 have been found to be 36, 27, 34, 27 and 31 maunds per acre, respectively.

b. As in the previous years, a number of plants with branches emanating from the bottom have been isolated in the first generation of the treatments with P^{32}; the total fibre yield per plant is much more than that in the control.

c. In the unusually thick plants isolated from the P^{32} treated population of *Corchorus*

Paddy

a. Some of the P^{32} treated plants in their first generation gave significantly higher grain yields than their controls, but there were no significant differences in the X-rayed and S^{35} treated populations.

b. Two of the higher grain yielders of the previous P_1 generation gave significantly higher yield in the P_2 generation also.

c. Some non-lodging selections have been made and their behaviour studied.

Tomato

a. The behaviour of the high yielding mutant, which gave 103 fruits, weighing 5253 gms, as against the best plant in the control with 62 fruits weighing 3362 gms in the X_2 generation of the previous year, has been investigated, in addition to the breeding behaviour of the dwarf and other mutants, isolated from the X-radiated progenies. The cytological abnormalities in these have also been studied.

b. The relative potentialities of the mutagenes used in inducing variability in tomato appear to be in the following order : X-rays-P^{32}-S^{35}-Ultraviolet.

Modifying factors in induced mutagenesis.

In paddy it was found that when the seeds were X-radiated in a chamber containing O_2, the variability of the progenies, and the abnormalities were the maximum, followed by X-radiation in air and in nitrogen.

Microbiology. After return in August 1957 from study leave abroad, Dr. P. Nandi has started some new lines of investigations which include more systematic study biochemical mutants of *A. niger*. It is now possible to measure more accurately the UV dosage given to the microorganisms. Auxanographic technique is being employed to detect the biochemical mutants.

The microorganism *Aspergillus niger* propagate asexually. They are not suited for the study of the Mendelian characteristics of the mutants produced. A new line of study on chemical and physical mutagenesis in a micro organism like *Neurospora*, which is propagated by sexual reproduction method, is being started.

The comparative study of mutagenesis in two types of micro-organisms with asexual and sexual methods of reproduction will lead, it is expected, to some interesting results.

Zoology. Delayed metamorphorsis in tadpoles of *Rana tigrina* and *Bufo melanastictus* due to penicillin treatment, and how in such retarded tadpoles, metamorphosis could be induced by application of vitamin B_{12} and thyroxin, have been reported last year. Recent investigations have indicated that many sulpha drugs produce hyperplasia and hypertrophy of the thyroid gland in rats, mice, and dogs. Since it has been observed that metamorphosis in tadpole is controlled by the activity of the thyroid, it was thought worthwhile to investigate the effect of some sulpha drugs, insulin, and methyl thiourate on the retardation resp. acceleration of metamorphosis in tadpoles of *Rana t.* and *Bufo m.*. No clear cut conclusion on the effect of these drugs on tadpoles metamorphosis could be established.

It was reported last year that retarded tadpoles of *Bufo m.* when treated with thyroxine produced a novel intermediate type of froglets living under water with four feet, tail, and gill breathing mechanism. Such froglets could be kept alive last year upto a period of 22 days by special feeding. By combination of several nutrient materials, the period of longevity could be extended upto 38 days.

Studies on Amoeba proteus. This amoeba has been the subject of many important investigations. We have been interested in the study of its mechanism of movement, which is supposed to be caused by transformation from gel to sol condition of its plasmagel. The same mechanism is supposed to be responsible for the imbibition of solution by its contractile vacuole, also for solute excretion. Recent studies on the contractility of the pulvinus in M. pudica and show that expulsion and subsequent reimbibition of solutes in the many contractile vacuoles present in the pulvinus are responsible for it, dorsiventral movement.

It has been thought worthwhile to make a comparative study of the mechanism of contractility of the vacuoles in *A. proteus* and *M. pudica.*

Specimens of *A. proteus* are being cultured in suitable nutrient media in petri dishes.

A. PHYSICS

A. 1. Apparatus

A.1.1. *Renovation of Dr. J. C. Bose's physical apparatus and instruments*

Dr. J. C. Bose's Birth Centenary falls due on November 30, 1958. It will be a befitting occasion to exhibit and demonstrate before the younger generation, some of his original and more important apparatus and instruments, to infuse in them the lofty spirit expressed in those researches. The chief characteristic features of his apparatus and instruments the (i) the simplicity and delicacy in construction and (ii) the many-sided implications of are results.

With this end in view the following physical apparatus and instruments have been renovated :

1. *Microwave apparatus.* (a) The original one has been reconditioned for demonstrating all optical analog experiments performed by him.

(b) A new transmitter based on the original model has been made for generating microwaves at higher intensity level for distant transmission.

2. *Detector of Microwaves.* Two types of detectors, (i) point contact type and (ii) galena (Pbs) type, used by him, have been restored.

3. "The Strain Cell" and 'the Magnetic Crescograph and Radiometer' have been reconstructed after his model.

Ultrasonics

A.1.2. *Application of high intensity ultrasonic waves.*

It was reported in the annual report 1951-52 that the high intensity ultrasonic generator which was built in this Institute yielded an output of about 42.2 watts/cm^2 at 1000/kc/s. This generator was so long used to investigate physical problems only. Now seeing the immense possibilities of utilizing high intensity ultrasonic energy in various fields of science and industry as an important tool for investigations, this untrasonic unit has been equipped with some additional facilities, particularly suitable for biological and chemical investigations. Devices have been incorporated in the equipment (1) to control the inside temperature of a double jacketted glass cell which contains the specimen under test and (ii) to vary the magnitude of ultrasonic intensity. Provision for varying the frequency of ultrasonic waves is being incorporated. Dosages for each specimen irradiated with ultrasonic waves were standardised by trial experiments.

Following specimens were irradiated with high intensity ultrasonic energy under varied conditions of temperature and intensity of ultrasonic energy.

(a) Desegregation of cells of crown gall tumor tissue—Sri V. N. Gadgil, Plant Physiology Department,

(b) Desegregation of filamentous blue green algae and further rupturing of the cell wall to extract the cell contents for bio-chemical study—Dr. B. B. Biswas, Plant Physiology Department,

(c) Disruption of bacterial cells to extract enzyme and protein—Dr. D. P. Burma, Chemistry Dept.,

(d) Rupturing cholera vibro to extract its protein content in intact condition—Prof. S. N. De, Medical College, Calcutta,

(e) Irradiating bacterial cells for study of the lethal action in u.s. radiation—Dr. J. C. Roy, Indian Institute of Biochemical and Experimental Medicine, Cal.

(f) Irradiating natural coal with different moisture contents and temperatures for applied studies—Dr. A. K. Lahiri, Central Fuel Research Institute, Jealgora, Dhanbad,

A. 1.3. *Experimental investigations on the separation of streaming and radiation pressures produced in a liquid medium due to the passage of ultrasonic waves at different intensities.*

The present investigation is an extension of the work reported in § A.3.3 of the annual report, 1954-55.

The measuring apparatus is the same as was designed and used before. Modifications are made in the generator and power supply. A Hartley oscillator with the provision of generating various frequencies and tank circuit of high Q value have been built up. The power input to the oscillator valve can be varied by inserting a variac in the primary side of the H.T. transformer. This investigation has been started with the idea of determining the ratio of μ'/μ, bulk to shear viscosities, for different liquids, by measuring the radiation and the streaming pressures at different perpendicular distances along the axis of the acoustic beam, so as to throw some light on the existing anomalous absorption coefficient of ultrasonic radiation in some liquids.

Experiment : Measurement of radiation and streaming pressures were made at different input energies supplied to the oscillator i.e. at the different intensities of ultrasonic waves. The effect observed is that the streaming pressure increases with distance at first, reaches a maximum value at a particular region which is about 6-7 cm from the source, after that it decreases with distance.

It has been further observed that the region at which the streaming pressure attains its maximum value is more or less the same at different intensities.

In the case of radiation pressure it has been found that it decreases with distance from the source and the curve, radiation pressure vs. distance is exponential in nature.

While measuring the total pressure some difficulty was experienced. The total pressure as measured by cavity radiometer method was found to be lower than the sum of the radiation and the streaming pressures determined separately. According to the postulate of Borgnis, the measured total pressure should be equal to the sum of the separate measures of streaming and radiation pressure but can never be less than the sum. The reason for such an effect has not yet been clarified. Exploration of the possible causes revealed that the crystal transducer tuned to oscillate at any particular harmonic, was excited at the same time by lower harmonics also.

Determination of the frequency of lower harmonics which is very essential for this type of investigation, could not be ascertained as no requisite wave meter was available. The

frequency of the desired harmonic was determined roughly by calibrating the oscillator with the transducer load on by Debye & Sears optical method.

A.1.4. *Acoustic Interferometer*

It was reported last year that an acoustic interferometer has been installed in the laboratory for investigating the intensity distribution in the successive order of the diffraction patterns in a beam of monochromatic light passing through the liquid medium traversed by ultrasonic radiation. By using this interferometer provided with a photographic device, attempt is being made to measure the intensity distribution in the different orders in order ro evaluate the absorption co-efficient of ultrasonic radiation in liquids.

(a) Attempts were made to measure the intensity of light passing through the slit and also of the light diffracted in different orders, directly by means of (i) vacuum photocell and an associated D. C. amplifier built here by using electrometer value & (ii) a special type of slit arrangement to allow the light of the desired order of the diffraction pattern to be incident on the photocell. No satisfactory results could be obtained; any displacement of the photocell carrier to receive the light of a particular line, disturbed the whole system. Further the intensity of light in the higher order of diffracted lines is very low, almost comparable to that of the background. This is also another drawback of the direct measurement method.

(b) Attempts were also made using a barrier layer photocell and a galvanometer to achieve the objective mentioned in the previous paragraph. This device proved to be even less efficient than the previous one. Hence this method was discarded.

(c) In the third method, the diffraction spectra of different orders is photographed on a film & the intensity of blackening of different lines produced on the photographic film is compared by a micro photographic device. A series of photographs of the diffraction patterns produced in benzene only, were taken. The next step is the installation of a microphotometer for recording intensity of blackening along the diffraction pattern. For this purpose an apparatus incorporating a sensitive photomultiplier (RCA 6810 A) and a pen recorder has been designed. For the time being no progress along this line is possible as the photomultiplier and the recorder are not available. Attempt is being made to find out some alternative method to solve the problem.

The following papers have been published during the year 1957-58 :

(i) Part I. On the measurement of ultrasonic power radiated from a quartz crystal transducer into liquid medium' (Trans. Bose Inst. XXI)

(ii) Part II. On the measurement of acoustic energy density and streaming velocity to determine the absorption co-efficient by the streaming method" (Trans. Bose Inst. XXI)

(iii) On the making of electret and measurement of the changes of dielectric constant of a polarised electret forming material with time' (Ind. Journal of Phys. 41, 281, 1958).

A. 2. Cosmic Ray Investigations

A.2.1. *High altitude studies*

The cloud chamber operating at Darjeeling has now been set up with a special triggering device to facilitate detection of unstable particles inside the cloud chamber. The producing layer of Pb has been shifted to an average distance of 80 cm above the chamber so as to get the maximum number of decays of unstable particles of mean life $\sim 10^{-8}$ sec. The chamber has been operating smoothly except for brief periods when the power supply at Darjeeling fell below 150 Volts. This is the first time that we have attempted to run the cloud chamber continuously even during the winter months; and we have been successful. We made special heating arrangements to keep the laboratory at a temperature 60°—65° F when the temperature outside fell to freezing point or even lower. About 12,000 pictures have so far been taken with the new arrangement of triggering system, yielding about 60 pictures of decay of the new unstable particles. Analysis of the whole set of pictures is now in progress for the following informations:

(i) Number of stopped particles inside the chamber with their ranges and interactions,

(ii) Number of nuclear interactions produced by slow secondaries of penetrating showers and their mean free path,

(iii) Relative yields of slow pions to K-mesons as identified from their residual ranges.

During a detailed study of the pictures taken at Darjeeling in the summer of 1956 a very interesting picture of associated production of a $K^+\mu^2$ and Σ° has been noticed. The most interesting feature of this photograph is that besides showing the decays of a $\wedge^0$ and $K_{\mu 2}$ it shows the materialisation of the photon resulting from $\Sigma^\circ \rightarrow \wedge^\circ + \gamma$ and an overall energy balance is obtained for the whole event. A detailed analysis of this picture was submitted in the form of a paper to the last symposium on Cosmic rays at Bombay (Feb. 26, 27, 1958) and has been subsequently published in the *Nuovo Cemento*.

We have also undertaken the construction of a big size cloud chamber ($36'' \times 30'' \times 18''$) which can house seventeen half inch plates and will be very suitable for the study of long range secondaries of unstable particles . After a long and painstaking effort we have been able to make this chamber leak tight. The other accessories for making it completely mechanised are being constructed. Special flash lamps of illuminating length 85 cm have been purchased for this chamber. We hope to run this chamber within a few months.

A.2.2. *Sea-level investigations*

The apparatus set up for the study of range spectrum of muons at the low energy end have yielded further important results. Besides confirming the previous value obtained for the muon intensity we have been able to determine the fine structure of the mu-meson spectrum at the extreme low energy end : (6-32)g/cm² of air equivalent. It has been found that the spectrum gradually falls off as predicted by Sands from his computations on the production spectrum of mu mesons at high altitudes.

A final analysis has now been made of the scattering of the stopped muons in the $\frac{1}{2}$inch copper plates inside the chamber. From a total number of about 800 stopped particles 250 were selected in which the ionisation at different gaps and residual ranges of these particles definitely identified them as mu mesons. These muons had momenta varying from 100–170 MeV/c when they entered the chamber. The projected angle of scattering at each Cu-plate was measured together with the momentum of the muons as obtained from their residual ranges and ionisation after the scattering. Altogether 446 scattering angles of muons with their momenta were analysed and a differential distribution of $p\beta\theta$ have been obtained. It has been found that the distribution agrees well with the theory of Olbert and Moliere or Snyder and Scott upto $p\beta\theta = 2.5$ and is too large by a factor of 2 to 3 for values of $p\beta\theta = 3$ to 5. The disagreement has been observed to be much more prominent in the low energy region than that reported by other workers for the high energy muous. The results on the scattering of slow mu mesons in copper were reported in the Bombay symposium and they are now in the course of publication.

New evidence was obtained for the existence of a particle of mass $\sim 525\ m_0$ among the pictures taken of the stopped particles at Calcutta. Nine pictures could be singled out in which the rate of change of ionisation of the particles and their residual ranges were not compitable with that shown either by mu-mesons or by protons. Their mass values were obtained by measuring their change in ionisation in the successive gaps and their decrease in residual ranges. Ionisation was measured by drop counting. It was found that five of these particles gave an average mass value $528 \pm 34\ m_e$ and the remaining four an average of $280 \pm 21\ m_e$. The close proximity of the latter value with the pion mass is a strong indication for the correctness of the method employed. A preliminary report of this result appeared in *Science and Culture* (March 1958) and the detailed paper is in the press (Ind. Journal of Physics). The following papers have been published during the year under review.

1. Cascade decay of a heavy K-meson-Phil, Magz. Vol. 2, No. 19, 936, 1957,
2. Differential Range spectrum of Sea level muons at 12°N–Trans. Bose Res. Inst. Vol. XXI, 67, 1957,
3. On the existence of a particle of mass $\sim 525\ m_e$—Science and Culture vol.23, 496, 1958,
4. New evidence for a particle of mass $\sim 525\ m_e$—Ind. Journ. Phys. (in the Press),
5. Associated production of Σ° and $K^+\mu^2$-Nuovo Cimento (in the Press),

A.2.3. *IGY Programme*

Two cubical meson telescopes installed at Mayapuri, Darjeeling, are operating successfully since August 1957. The data collected so far are being analysed and these will be sent to the IGY Committee very soon. One neutron monitor has also been installed recently. Another neutron monitor unit employing $B^{10}F_3$ counters which being fabricated in the workshop and filled up in our filling system, will be installed soon.

A.2.4. *Ion Chamber Investigations :*

Two ionisation chambers have been assembled. They will operate as electron collector ion chambers. One ion chamber along with its electronic accessories has already

been tested at Calcutta and is being installed at Darjeeling. Operation of ion chambers in coincidence will be utilized for the study of spatial distribution of atmospheric showers with special attention to the distribution of neutral particles in the core of such showers. These chambers will also operate continuously during the 'ALERT' periods, to substantiate independently the data on intensity variations during or after any solar flare and magnetic storms obtained by the counter telescope assembly.

A.2.4.1. Plastic phosphor plates have been used in place of ionisation chamber to investigate the existence of successive maxima in Rossi's curve, due to cosmic ray secondaries produced in different thicknesses of lead absorbers. Elaborate anticoincidence circuits have been used to eliminate the effect of side showers. Multiple pen recorded circuits have been used to correlate the secondaries with primary photons as well as with charged and uncharged primary particles. The energy distribution in the secondaries is also being determined.

A.2.4.2. Recording of cosmic ray intensities by means of a large pressure ionisation chamber (5 liter capacity) and a Lindemann electrometer is being continued. Due to a defect in the recording electrometer there has been a temporary interruption. A new Lindemann electrosater has been ordered.

A. 3. Nuclear Physics

A.3.1. *Measurements on thermal neutron absorption cross section*

The thermal neutron absorption cross section in Cl has been measured by the spherical symmetry method developed in the laboratory. For precision measurement of σ_a a mechanical arrangement has been devised which smooths out small deviations from spherical symmetry in the apparatus. The absorption spheres of aluminium are rotated about a vertical axis with small angular velocity. A foil holder carrying a detector foil (Indium) is rotated about a horizontal axis, the two axes meeting at the centre of the spheres. The speed of rotation of the foil was large compared to that of the spheres, while the period of rotation of the spheres was small compared to the half life of indium. Thus during each rotation of the foil, there was, in effect, an averaging of neutron intensity over a great circle of the spheres, while during each rotation of the sphere there was practically an averaging out over the entire solid angle. The activity of indium was measured by a thin walled halogen filled beta counter, to eliminate the effect of 4.5 hour isomeric transition gamma rays. The absorber used in this experiment was pure CCl_4. The use of liquid absorber ensured homogenity of the arrangement.

From the measured value of transmissions through spheres of different radii, the value of σ_a for chlorine comes out as 31.9 ± 1.6 barns, after correcting for detector efficiency, multiple scattering, shape of neutron spectrum used in the experiment and other minor effects. This value is in complete agreement with the standard accepted value of 32.1 ± 1.0 barns.

A.3.2. *Experiments on slow neutron scattering by bound protons.*

Using the experimental arrangement described in previous reports we have measured the scattering cross-section of bound protons for slow neutrons. The compounds used in this experiment were water and glacial ortho-phosphoric acid. The experimental results have been analysed in the light of mass tensor approximation of Sachs and Teller as interpreted by Koles and Janik. The bound proton cross-section of proton comes out as 37.86 $\pm$ 0.81 barns, which agrees with the non-linear structure of water molecule. The absorption coefficient of orthophosphoric acid (1.72 cm^{-1}), however, shows complete disagreement with the theoretical value (2.48 cm^{-1}). This indicates that —CH groups are probably hindered rotationally.

A.3.3. *Experiments on gamma rays*

Using a scintillation gamma ray spectrometer we have measured the absorption coefficient of 1.3 Mev cobalt 60 gamma radiation and compared them with the corresponding theoretical values. The measured values are shown in the table :

	Theoretical value of atomic scross-section	Experimental value	Deviation
Zinc	5.554 barns	5.578 barns	+ 0.4%
Bromine	6.517	6.519	—
Silver	8.952	8.922	— 0.4%
Cadmium	9.168	9.142	— 0.3%
Tin	9.609	9.567	— 0.4%
Mercury	18.30	18.26	— 0.2%
Lead	19.04	19.08	+ 0.2%

Since the theoretical estimates of elastic scattering processes are not known very accurately and the calculation of photoelectric cross sections are also not known very precisely, we conclude that the measurements agree with theory within $\pm$ the range of uncertainty of the theoretical and experimental values.

A. 4 Neutron Generator

14 Mev neutrons have been produced by T^3 (d, n)H_e^4 reaction in a powerful neutron generator built up in this laboratory. A photomultiplier scintillation detector has been used for detection of neutrons. Neutron irradiation of seeds for induced mutagenetic studies has been undertaken, along with studies of physical properties of fast neutrons.

Earlier, a prototype neutron generator was built up and yield measured. That machine was capable of focussing 2 μ amp of total ionic beam current in a spot less than 1 cm in diameter, at a distance of nearly 34" under total acceleration potential of 52 kilovolts. The yield of reaction measured by a secondary method, was about 800 mc/μ amp. of deuteron beam current. A previous magnetic analysis of ions produced under the experimental condition showed that nearly 60% of ions produced were atomic ions.

The present machine is an improvement upon the earlier one, since in it (1) larger beam current with a max of 50 μ amps. can be used for acceleration giving nearly 25 times

more yield, (2) greater acceleration potential can be applied resulting in further increases in yield, (3) the instrument is more compact, overall length of the accelerator being 28″ excluding the ion source.

In construction it is similar to the earlier apparatus but with a number of improvements. The vacuum system has been made more efficient by using more robust pumps with higher pumping speed, nearly 100/120 litres per sec at 10^{-4}mm of Hg reaching an ultimate vacuum of 10^{-6}mm. The vacuum seals have been made more dependable by dispensing with wax seals and utilizing greased neoprine O'rings and clamps in between stages. The accelerator consists of two cylindrical aluminium electrodes 4″ & 18″ in length of internal diameter 2.8″. Only a part of the focussing electrode 18″ in length is of different diameter, equal to 1.8″. The ion source is made similar to that described by Moak Reese and Good but with modifications. It is of much larger volume to minimise space charge effect.

The target is supplied by AERE, Harwell, and consists of tritium gas absorbed in Zirconium, evaporated on copper disk and certified to contain .17 cc of 90%T, distributed over a circular area of nearly 1″. D_2 gas generated by electrolysis of heavy water is introduced in the ion source through a brass tube beaten flat, so that by altering the curvature of it, the flow can be regulated.

R.F. discharge is produced in the ion source by an oscillator of frequency 22 mega cycles/sec and delivering 200 watts of power. At first the pressure in the ion chamber is maintained such that the discharge is just kept going then gradually it is increased to get more ionic beam current.

D.C. high voltage for the electrostatic accelerator has been taken from a rectifier voltage doubler circuit and 50 kv transformer, thus 100 kv potential can be given from the generator. A Cockroft Walton generator has also been constructed which can generate voltage upto 240 kv at 400/μ amps. This utilizes 24 condensors arranged in 4-stacks and 24 selenium rectifiers. Initial voltage of 10 kv at 100 kc/s can be multiplied 24 times.

A focussing potential of 5 kv and acceleration potential from 30–75 kv has been applied. More accelerating, potential can be used and arrangements for minimizing corona loss are being made.

Neutrons have been detected by a photomultiplier scintillation detector, which detects recoil protons produced in the activated phosphor itself. The tube type EMI 6260 has been employed. The counts in photomultiplier circuit are directly proportional to neutron yield. A curve showing increase in c.p.m. against increasing acceleration potential is obtained, which shows almost linar increase in yield with increasing deuteron energy. Thus curve fits well with a part of the curve of Bretscher and French for yield of reaction in (T, d) source. Yield of reaction is now being measured by comparison with a standard source by a secondary method i.e. by activation of silver foil, in the same way as it was earlier done by Banerjee.

A collimator of neutron beam and shielding with 'Boroffin' (Paraffin+20% B_2O_3) is being made.

The following electronic circuits have been constructed :

1. A type 100 amplifier
2. A decade scaler
3. An oscillator of frequency 22 mc/s, 200 watts output
4. Photomultiplier circuit of EMI tube type 1562
5. Power supplier for G. M. counter

Repairs to all the existing electronic apparatuses were mace. At present the following works are proposed to be done :

1. Irradiation of seeds with known dosages of fast neutrons for induced mutagenetic studies. A sample of sesamum seeds has already been given irradiation.
2. (n, 2n) reaction studies for heavy elements
3. Determination of transmission coeffs of shielding materials
4. Neutron spectroscopy by means of neutron telescope and pulse height analyser.

The following electronic circuits have been proposed to be built :

1. Power supply, amplifier, discriminator and Cathode follower, circuit of the photo-multiplier tube type RCA 5819 for use in neutron telescope
2. Proportional counter circuit for recoil proton counting
3. Coincidence circuit
4. Scaler
5. PH analyser for neutron spectroscopy
6. BF_3 proportional counter.

B. CHEMISTRY

B. 1. Physical & Biochemistry

B.1.1. *Physico-chemical studies on proteins*

Work on the isolation, fractionation and physico-chemical investigation of proteins from seeds, as reported last year, has been continued. Investigations, in the main, have been carried out on seeds of *Brassica campestris* (mustard) obtained through the courtesy of the Botany Department of the Institute. Several extracting solvents were tried and finally the following precedure was found most suitable for extracting maximum nitrogen from the meal. The decorticated seeds were coarsely ground and after defatting with petroleum ether and further grinding to 50-mesh size, the meal was extracted with 10% sodium chloride solution for 6 hours at about 25° C. After centrifuging and filtering through paper pulp, the clear filtrate was saturated to 80% with ammonium sulphate. The resulting precipitate which was strongly coloured and contained carbohydrates, was redissolved in 5% sodium chloride solution and reprecipitated in the same manner. At this stage it was free from carbohydrates but considerable colouring matter still remained. The precipitate was stored over saturated ammonium sulphate solution in the cold room.

The total precipitate on electrophoretic analysis at 20° C by Tiselius' method showed two major and one minor fastmoving components over the *p*H range 4.0-11.0, in buffer solutions of ionic strength 0.1. The isoelectric point of one component was in the region of *p*H 6.0-6.2, while that of the other around *p*H 10.0. Fractional precipitation with ammonium sulphate at about 22% and 80% saturation gave two fractions, the former being smaller in quantity. This fraction was homogeneous as shown by a single peak in electrophoretic pattern at different *p*H values, but the other revealed a minor impurity. Attempts are being made to remove it. The 22% saturation fraction has a very low solubility below 5° C, especially near its isoelectric point, in the usual buffers employed in electrophoretic work of ionic strength 0.1. The solubility increased when the temperature was raised to 20° C; at this temperature electrophoretic analysis was carried out over wider range of *p*H and at *p*H values closer to isoelectric point.

Work on proteins from other seeds is in progress.

B.1.2. *Effect of ionising radiation on albino rats*

In continuation of previous studies (vide Annual Report 1956-57 p. 16) it was proposed to measure the changes in the amino-acids, urea, uric acid, free ammonia, creatinine and sulphate contents of the urine of albino rats following wholebody ionising radiation. The amounts of the above substances excreted in the 24-hour urine of a batch on non-irradiated rats have been collected. These will be taken as normal readings. The amino-acids were detected and estimated by means of paper chromatographic methods, the sulphate by gravimetric method, urea and free nitrogen by semimicro Kjeldhal method and the rest by colorimetric methods.

An interesting finding with the normal rats is that an unidentified blue (ninhydrin) spot is observed in the aminoacid chromatogram of urine of some of the rats. The Rf value

(phenol) of this spot is very close to phenylalanine but the butanol-Rf is much lower. Work on the identification of this spot is in progress.

B.1.3. *Desalting of urine*

Desalting of amino-acids in biological fluids, particularly urine, prior to paper chromatographic analysis is important. A new method of desalting of micro-quantities of urine by the technique of paper electrophoresis has been developed. Using N/10 hydrochloric acid as electrolyte and applying 200 volts for three hours, a complete separation of salts and amino-acids has been achieved. The papers used were Whatman no. 1 and no. 3. Very good separation is also obtained within 2 hours using 2 :1 (v/v) mixture of pyridine and N/20 hydrochloric acid and by applying a potential of 50 volts.

B.1.4. *Gas-phase chromatography*

A gas-phase chromatographic apparatus with special features, such as (1) interchangeable columns and (2) interconnectable column containing different liquid phases has been designed and built in the Institute. The different columns are : (1) a ten ft. carbon column, (2) a 5 ft. high-vacuum pump oil column and (3) a decyl phthalate column.

The supporting solid for the last two columns is Celite -545. The apparatus can be worked at temperatures up to 100°C.

Complete separation of mixtures of (a) ethyl, methyl and propyl alcohols, (b) diethyl ether, acetone, methyl alcohol and (c) n-pentane and n-haxane, has been achieved.

B.1.5. *On the antibiotic properties of mesuol and mesuone*

Further studied on the antibiotic activity of mesuol and mesuone have revealed that they inhibit the growth of *Mycobacterium* phelei. Mesuol is 1% and mesuone 0.1% as active as streptomycine. The antibiotic activity of both the compounds is inhibited in presence of serum.

B.1.6. *On the relationship between chemical constitution and antimicrobial activity of the coumarins*

Dalbergin, nor-dalbergin, wedelolactone, tri-O-methyl wedelolactone, 5 : 7-dihydroxy -4-phenylcoumarin. 2 : 2-dimethyl-5-hydroxy -7 : 8 (4¹-phenyl-α-pyrano) -chroman are being examined for their antimicrobial activity.

B.1.7. *Studies on the ultraviolet spectra of natural coumarins*

To study the ultraviolet absorption spectra in relation to the structure, the following natural coumarins and related compounds are being examined by spectrophotometric method : Seselin, luvangetin, xanthotoxin, byakangelicin, angelicin, murrayin (scopolin), marmesin, wedelolactone, Tri-O-methyl wedelolactone, dalbergin, nor-dalbergin.

B. 2. Plant Chemistry

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

Survey of the saponin-bearing plants of India undertaken with a view to discover new sapogenins which might be utilized for the commercial production of cortisone and sex hormones in India has been extended. In the year under review some of the investigations

mentioned in the previous report have been continued and also preliminary investigations of a number of new plants have been carried out. The following have been found to contain saponin.

1. *Pongamia pinnata* 2. *Zephyranthes* spp. 3. *Argyreia tiliaefolia.* 4. *Cestrum noctarnum.* 5. *Clitoria ternatea.* 6. *Solanum indicum.* 7. *Aphanamixis polystachya.*

B.2.1.1. *Barringtonia acutangula*, Gaertn.

In the previous report, the isolation of two acid sapogenins and three neutral sapogenins was mentioned. One of the acid sapogenins was characterised as barringtogenic acid ,a triterpene reported to be present in *B. recemosa.* In the year under review a modified method of isolation, giving higher yields of the sapogenins, has been worked out. The method of separation of the individual constituents from the sapogenin mixture has also been perfected. Further studies on the constitution of acutagenal B and C have been made.

Infra-red spectrum of acutagenol B showed characteristic bands for tri-substituted double bond, hydroxyl and *gem*-dimethyl groups. Acutagenol B appears to be a triterpene alcohol of the β-amyrin group.

To acutagenol C, m.p. 305-310°, has been tentatively assigned the molecular formula, $C_{30}H_{50}O_4$. It formed a triacetate $C_{36}H_{56}O_7$, m.p. 230-32°. I.R. spectrum of acutagenol C showed striking similarity with that of acutagenol B, indicating the presence of tri-substituted double bond, hydroxyl and *gem*-dimethyl groups. It did not react with sodium borohydride or lithium aluminium hydride under standard conditions. Further work on the constitution of the *Barringtonia* sapogenins is in progress.

B.2.1.2. *Luffa cylindrica* Roem :

The sugar constituents of the crystalline saponin, m.p. 271° (decomp.), isolated form the seeds of *L. cylindrica* (vide previous report) have been characterised as galactose, arabinose, xylose and rhamnose following the technique of paper chromatography.

B.2.1.3. *Luffa acutangula*

In the previous report, the isolation of a neutral compound, m.p. 275°, and a crude acid sapogenin from the seeds of *L. acutangula* was mentioned. It has now been possible to isolate a pure crystalline bitter principle, $C_{32}H_{48}O_8$, m.p. 182-85° :$[\alpha]_D$+87°.4.(96% ethanol). The physical and chemical properties of the above compound agree closely with those of cucurbitacin B reported to be present in the various species of *Cucurbitaceae* family. Their identity was established by mixed melting point determination, and also by paper chromatography.

The acid sapogenin fraction mentioned above could be obtained in a pure state, $C_{30}H_{48}O_3$, m.p. 304-8°, $[\alpha]_D$+78°.3 ($CHCl_3$). The monomethyl ester of the acid, $C_{31}H_{50}O_3$, m.p. 198-200°, furnished a monoacetate, $C_{33}H_{52}O_4$, m.p. 222°, $[\alpha]_D$+70°.4 ($CHCl_3$). This acid sapogenin was identified as oleanolic acid by direct comparison of the mixed m.p. of the methyl ester with an authentic sample of methyl oleanolate.

B.2.1.4 *Albizzia lebbeck* Benth.

From the alcoholic extract of the beans of *A. lebbeck* a good amount of crude saponin (vide previous report) was obtained. This on hydrolysis gave a mixture of acid and neutral

sapogenins. The yield of the neutral sapogenins was poor. This fraction on benzoylation gave two benzoates, m.p. 192-4° and 285-8° respectively showing that two compounds are present. The acid sapogenin fraction which was obtained in fairly good yield was directly esterified with diazomethane and on chromatographic resolution over Brockmann's alumina gave two methyl esters A and B.

The ester A, $C_{31}H_{50}O_4$, m.p. 211-13°, $[\alpha]_D$+33°.5 (ethanol 95%) gave positive Liebermann-Burchardt test and a pale yellow colouration with tetranitromethane. It formed a monoacetate, $C_{33}H_{52}O_5$, m.p. 205-206°, with glacial acetic acid and H_2SO_4 in cold and a diacetate, $C_{35}H_{54}O_6$, m.p. 198-200°, with pyridine and acetic anhydride at 100°C. Ester A could be hydrolysed to an acid, m.p. 305-8° and on oxidation by Sarett's method gave a diketone, $C_{31}H_{46}O_4$, m.p. 167-68°. The physical and chemical properties of this dihydroxy triterpene acid and its derivatives agreed closely with that of echinocystic acid and its derivatives. The identity was finally established by a comparison of the mixed melting point of ester A with an authentic sample of methyl echinocystate.

The ester B, $C_{31}H_{50}O_4$, m.p. 225-226°, $[\alpha]_D$-9°.5 $\pm$ 1 (ethanol) showed positive Liebermann-Buchardt test and a pale yellow colouration with tetranitromethane indicating unsaturation. On hydrolysis it gave an acid, $C_{30}H_{48}O_4$, m.p. 248-50°, $[\alpha]_D$-12°.3 $\pm$ 0.5 (ethanol). The ester B gave a diacetate at 100° C with pyridine and acetic anhydride, $C_{35}H_{54}O_6$, m.p. 189-190°, $[\alpha]_D$-53° $\pm$ 0.3 ($CHCl_3$).

This second acid appears to be a new triterpene acid as the physical and chemical properties of the acid and its derivatives do not agree with those of any known triterpene acid reported in the literature. This acid has been named as '*albigenic acid*'. Methyl albigenate showed the presence of one double bond (perbenzoic acid titration). It could not, however, be hydrogenated over Adam's catalyst showing the hindered nature of the double bond. It also did not react with osmium tetroxide. Methyl albigenate, on oxidation with CrO_3 in pyridine, gave a diketone, $C_{31}H_{46}O_4$, m.p. 196-98°. The diketone gave Zimmermann colour reaction indicating th presence of a 3-keto group. It did not give any colouration with alcoholic ferric chloride. The diketone on hydrolysis with alcoholic caustic potash furnished a decarboxylated nor-ketone which indicated the presence of a keto-group in β-position to the carboxyl group. Albigenic acid on treatment with 48% hydrobromic acid, gave a lactone, m.p. 281-82°, indicating the presence of the double bond either in $\beta\gamma$ -or $\gamma\delta$ -position with respect to the carboxyl group. Albigenic acid appears to be a pentacyclic triterpene acid of the oleanen type closely resembling echinocystic acid. As albigenic acid is laevoratory, it was thought that it might be a 3-epimer of echinocystic acid but it was found not to be the case as the diketo derivative of methyl albigenate depressed the melting point of diketomethyl echinocystate. It is well-known that the triterpene compounds of the oleanen series, which have the double bond at the usual 12 : 13 position, consume perbenzoic acid very slowly and the rapid consumption of perbenzoic acid in the case of albigenic acid is indicative of an unusual disposition of the double bond. Work on further elucidation of the structure of albigenic acid is in progress.

B.2.1.5 *Ervatamia coronaria* Stapf.

The petroleum ether (b.p. 60-80°) extract of the bark of *E. coronaria* on chromatography over Brockmann's alumina furnished four triterpene compounds, α-amyrin, α-amyrin acetate, lupeol and lupeol acetate besides a sterol mixture from which β-sitosterol could be obtained in a pure state.

B.2.1.6 *Daemia extensa* R. Br.

The petroleum ether extract of the plant on chromatographic resolution over Brockmann's alumina furnished α-amyrin acetate, lupeol and lupeol acetate and β-sitosterol. It has not yet been possible for us to crystallise the bitter fraction obtained from the benzene, chloroform and alcoholic extracts of the plant.

B.2.2. *Alkaloid survey*

The survey of alkaloidal potentialities of the local flora including non-medicinal has been continued. Twenty five plants of different families were subjected to preliminary screening and alkaloid could be detected only in the following—(1) *Cestrum noctarnum*, (2) *Bignonia sp.*, (3) *Clitoria ternatea.*

B.2.3. *Chemical Examination of Mesua ferrea* Linn.

(a) *On the constitution of mesuol*

Further studies on the constitution of mesuol have revealed that it can be methylated with diazomethane to its dimethyl derivative ($C_{25}H_{26}O_5$, m.p. 132°), identical with the product obtained by methylation of mesuol with methyl iodide (J.I.C.S., 1940, **17**, 277). This conclusively proves that mesuol has two phenolic hydroxyl groups.

That mesuol is a monolactone has been proved from lactone titration values of dimethyl mesuol. Its ultraviolet absorption spectrum also suggests the presence of a lactone function.

The infrared spectrum of dimethyl mesuol exhibits bands characteristic for hydroxyl, lactone, C-methyl and unsubstituted phenynlucleus. The presence of the hydroxyl band in dimethyl mesuol proves that in addition to two phenolic hydroxyl groups, it has another hydroxyl group which cannot be methylated. Dimethyl mesuol however could not be acetylated; hence the hydroxyl group is believed to be tertiary in nature.

The ultraviolet absorption spectrum of tetrahydromesuol ($C_{23}H_{26}O_5$, m.p. 181°) showed a strong peak at 287 mμ (logε 4.06) and a broad weak band at 342 mμ (logε 3.59). The curve is typical of coumarins. The infrared spectrum of tetrahydromesuol also showed characteristic bands for hydroxyl, lactone, C-methyl and unsubstituted phenyl nucleus.

Mesuol on hydrolysis with 40% aqueous caustic potash gave acetone, (presumably drived from a gem-dimethyl group) acetophenone, and two other crystalline constituents of m.p. 268° ($C_{20}H_{18}O_4$) and 189° ($C_{15}H_{14}O_4$) respectively.

These degradation products are alkali-soluble and do not give ferric reaction. The U-V curves of these compounds are very similar to that of 5 : 7--dihydroxy 4-phenylcoumarin (Bull. Soc. Chim. 541, 1955). Acetophenone has recently been obtained by alkaline degradation of 7-methoxy-5-hydroxy-4-phenylcoumarin. It is therefore assumed that mesuol is a 4-phenyl-coumarin derivative.

Mesuol also undergoes degradation in presence of 5%, 10%, and 20% aqueous caustic potash. In all the cases acetophenone has been obtained as one of the decomposition products. Dimethyl mesuol on the other hand is indifferent to 20% aqueous caustic potash.

Dimethyl mesuol on oxidation with neutral permanganate yields benzoic acid and oxalic acid. This observation confirms the presence of an unsubstituted phenyl nucleus in mesuol. Oxalic acid might have been derived from carbon atoms in position 2 and 3 of the coumarin ring.

Chromic acid oxidation of dimethyl mesuol gives acetone and by ozonolysis a steam volatile acid and a phenolic product, besides acetone, have been obtained. The acid reduces ammonical silver nitrate solution and decolourises potassium permanganate solution. By paper chromatographic technique the compound has been identified as formic acid. The phenolic product could not be crystallised.

Phloroglucinol has been obtained by fusion of mesuol with caustic potash at 200-210°C.

Further work is in progress.

(b) *On the constitution of mesuone*

The isolation of a 136°-melting colourless crystalline compound from the seeds of *Mesua ferrea* Linn. was reported previously (Annual report 1956-57). It has been named mesuone.

Analytical and molecular weight data point to the molecular formula $C_{29}H_{42}O_4$ which has been tentatively assigned to the compound. It is insoluble in 5% sodium bicarbonate but soluble in 4 % sodium hydroxide solution. With alcoholic ferric chloride it gave a pale red colouration. It does not give Liebermann-Burchardt reaction for steriods. It decolourises bromine water and alkaline permanganate solution. With tetranitromethane the compound gives a pale yellow colouration indicating the presence of unsaturation in the compound.

The ultraviolet absorption spectra of the compound show a sharp absorption maxima at 224mμ (logε 3.27) and at 353mμ (logε 3.45) with a minima at 275 mμ .

The infrared absorption spectrum of the compound gives bands for chelated hydroxyl, carbonyl function in conjugation with a double bond, phenyl group, butadiene type with a CH_3 group at the end, *gem*-dimethyl and monosubstituted phenyl nucleus. Further studies are in progress.

B.2.4. *Synthetic experiments in the coumarin series*

(a) The ultraviolet absorption spectra of the isomeric psoralidin methyl ether (*vide* previous report), natural psoralidin methyl ether, and angelicin, an angular furanocoumarin, have been determined (Table I).

TABLE 1

Compound	λ max in mμ*
Our synthetic methyl ether	222 (4.42), 251 (4.30), 266 (4.25), 310 (4.12).
Natural psoralidin methyl ether	213 (?), 243 (4.24) 305 (3.78), 345 (4.32).
Angelicin	247 (4.34), 300 (3.93)

*Figures in the parenthesis denote the log ε values.

Since our product has obviously the linear furano-coumarin structure it is probable that psoralidin has a structure different from what has been assigned to it by Siddiqui *etal.* (J. Sci. Ind. Res., 1948, **7B**, 24).

(b) In an attempt to synthesise suberosin (Aust. J. Sci. Res., **3A**, 342, 1950) the following compounds have been prepared : (i) $\gamma\gamma$-Dimethyl allyl ether of umbelliferone, colourless crystals, m.p. 75-77° with a characteristic sweet smell. It distils in vacuum at 130°. In order to study Claisen rearrangement of this ether, it was heated at 190°-200° for $\frac{1}{2}$ hr. at 8-12 mm. when umbelliferone was obtained. Similar decomposition occurred when heated above 160° in an evacuated sealed tube.

(ii) *$\gamma\gamma$-Dimethyl allyl ether of 8-formyl umbelliferone*, colourless needles, m.p. 163-4°

In order to prepare the 6-alkyl derivative of 8-formyl umbelliferone, the above ether (ii) was subjected to the condition of Claisen rearrangement (thermal treatment) when the degraded product, 8-formyl umbelliferone, m.p. 185°, was obtained.

(c) Attempts at nuclear alkylation of umbelliferone with $\gamma\gamma$-dimethyl allyl bromide in benzene in presence of sodium ethoxide did not prove successful. Similar was the case with umbelliferone 8-aldehyde.

B.2.5. *Further investigation on Eupatorium odoratum* Linn.

The crystalline flavone, ($C_{17}H_{14}O_7$, m.p. 232-3° M.W. 323; methoxyl 17.65%), isolated from the leaves of *Eupatorium odoratum* (*vide* previous report) gave an acetyl derivative, m.p. 207-9°.

Owing to the very poor yield of the flavone and seasonal variation, and also due to great difficulties encountered in its isolation, satisfactory progress could not be made in the study of its constitution.

B.2.6. *Constitution of Clerodin, the bitter principle of Clerodendrum infortunatum*

Studies on the constitution of clerodin have been continued (see Annual Report 1956-57, p. 26). The molecular formula of clerodin has been revised to $C_{21}H_{34}O_7$ with the help of X-ray data. We have also studied the infrared spectrum of clerodin and our inferences and one of Chaudhury and Dutta (J. Ind. Chem. Soc. 1954, **31**, 8) are tabulated below :—

Chaudhury and Dutta		Present authors	
Observation	Inference	Observation	Inference
3660 cm^{-1}	Hydroxyl	3435 cm^{-1}	Hydroxyl
1725 cm^{-1} 1250 cm^{-1}	Acetoxy	1725 cm^{-1} 1250 cm^{-1}	Acetoxy
1617 cm^{-1}	Ethylenic double bond	1613 cm^{-1}	Conjugated Ethylenic double
		1365 cm^{-1} 1385 cm^{-1}	*gem*-Dimethyl
		1450 cm^{-1} 864 cm^{-1} 750 cm^{-1}	Furan

The observed acetyl value (19.4%) is compatible with the assumption that two acetoxy groups, instead of one, are present in clerodin. Clerodin could best be deacetylated by treating with 0.5% alcoholic caustic potash at room temperature for three hours. The resulting major product namely, desacetyl clerodin, $C_{20}H_{30}O_5$, crystallised from dioxan, m.p. 256-60°, which is higher than that reported by previous workers. The presence of acetic acid in the hydrolysate was proved by paper chromatography and its conversion into *p*-phenylphenacyl acetate 112°. Desacetyl clerodin when acetylated with acetic anhydride and pyridine regenerated clerodin. Desacetyl clerodin showed, as expected, the absence of characteristic bands for acetoxy but a band at hydroxyl appeared.

We could not confirm the presence of four hydroxyl groups in clerodin as suggested by Chaudhury and Dutta and failed to obtain tetraacetyl clerodin by following their methods. Clerodin gave nearly 0.5 mole of active hydrogen. But the hydroxyl group could not be acetylated by the usual procedure nor could it be oxidised by Sarett's method. These facts eliminated the possibility of the above hydroxyl group being primary or secondary and led us to believe it to be of tertiary character.

The infrared spectrum of clerodin showed many bands common with marrubiin at frequencies regarded as characteristic for the furan ring. Moreover, clerodin gave a positive Ehrlich's test and a pink colour with antimony trichloride and acetic anhydride-reactions which are reported to be characteristic for the furan ring. Clerodin was also found to be unstable towards acid and the furan ring could account for its acid sensitiveness. By ozonolysis of clerodin a mixture of volatile and non-volatile acids could be obtained. The volatile acid was identified as formic acid. The non-volatile acid on crystallisation from methanol formed a low-melting solid which is believed to be an α-keto acid from its colour reactions. The low yield of the acid could not permit further studies. The results of ozonolysis could be explained by assuming the presence of a β-substituted furan ring in clerodin.

Desacetyl clerodin was found to undergo further hydrolysis on treatment with hot 5% alcoholic caustic potash giving a neutral compound, $C_{18}H_{28}O_4$, H_2O, m.p. 132-33° and acetic acid. The same compound could also be directly obtained from clerodin. This neutral compounds showed the bands for the following groups in the infrared spectrum :

Hydroxyl, conjugated ethylenic double bond, gem-dimethyl and furan. From what has been stated above it will be seen that, prima facie, clerodin contains three acetoxy groups but the third one does not show in the infrared spectrum of clerodin. It is therefore inferred that the third acetoxy group is masked. Some indirect evidence of the presence of masked acetoxy groups in clerodin and desacetyl clerodin has been obtained but more definite evidence is being sought. The seven oxygen functions in clerodin could thus be attributed to acetoxy (4) furan (1) and masked acetoxy (2).

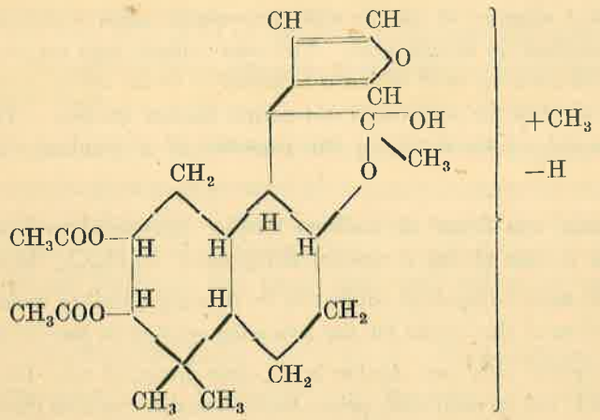
The occurrence of a masked acetoxy group in a natural product is rare. Barton *et al.* reported the presence of such group in tenulin. The masked acetoxy group could account for the tertiary OH. An attempt was also made to fix the relative position of the two normal acetoxy groups in clerodin. For this purpose desacetyl clerodin was made to undergo

quantitative reaction with periodic acid. From the amount of periodic acid consumed it was evident that desacetyl clerodin contains an α-glycol system. In other words, the acetoxy groups in clerodin are vicinial. Further, desacetyl clerodin could be oxidised to a light yellow crystalline compound, m.p. 218-20°C by Sarett's method. It gave a violet colour with alcoholic ferric chloride which indicates the presence of a diosphenol system in the compound. The U-V spectrum supported this conclusion.

To determine the nature of the basic carbon skeleton of clerodin, it was subjected to selenium dehydrogenation, when, besides other products, a liquid hydrocarbon was obtained, which formed an orange coloured symmetrical trinitrobenzolate, m.p. 153-55°. The hydrocarbon was regenerated over Brockmann's alumina and its ultraviolet spectrum was found to be superimposable on that of 1 : 2 : 5-trimethylnaphthalene. An authentic sample of 1 : 2 : 5-trimethyl naphthalene formed a trinitrobenzolate which had the same properties as our product and the mixed m.p. of the two was undepressed.

1 : 2 : 5-Trimethyl naphthalene has also been obtained from two bitter principles, marrubiin and columbin under similar conditions by other workers. Incidentally both these contain a β-substituted furan ring.

It is therefore, concluded that clerodin belongs to the diterpenoid type of bitter principles. Its reactions and other properties so far studied by us would seem to suggest formula (I) as a possible one for clerodin. Further work is in progress.



B.2.7. *Triterpenoid sapogenins from Aegiceras majus* Gaertn.

Kincl and Gedeon (Arch. Pharm., 1956, **289**, 221) reported a new triterpene sapogenin, Kanjalagin, $C_{30}H_{52}O_3$, m.p. 230-2° from the bark of *A. majus* (syn. *A. corniculatum* Blanco) and stated that it gave no colour with tetranitromethane. It therefore appeared to us that it might prove to be a member of the friedelane group, probably occurring for the first time in nature as a saponin.

In the present investigation three neutral triterpenoid sapogenins were isolated from the above source and purified through their acetates and/or benzoates. All of them gave a pink Liebermann-Burchardt test and a yellow colour with tetranitromethane. Sapogenin I had m.p. 184-6° (acetate, m.p. 189-91°, benzoate, m.p. 232-5°). Sapogenin II melted at 238-40°, $[\alpha]_D$ -17° ($CHCl_3$); acetate m.p. 194-6°. Sapogenin III, $C_{30}H_{50}O_3$, m.p. 240-42°, $[\alpha]_D$ +29.4° (absolute ethanol) gave a triacetate, m.p. 158-59°, $[\alpha]_D$-9.2° ($CHCl_3$). Only sapogenin III was obtained in good yield and was identified as genin-A by comparison of physical properties and determination of mixed m.p. with an authentic sample, which was undepressed. It is possible that kanjalagin is genin-A. The acetate of sapogenin III gave an intense colour with tetranitromethane, though the parent substance developed only a faint colour with this reagent on account of its sparing solubility in chloroform.

B.2.7. *On a flavonol constituent of A. majus* Gaertn.

Hydrolysis of the crude saponin also yielded a small quantity of a flavonol, $C_{15}H_9O_6 \cdot OCH_3$, m.p. 300° (decomp.), R_f 0.80 (Butanol-acetic acid-water 4 : 1 : 1). It formed a tetraacetate of m.p. 207-8°, and a fully methylated product identical with quercetin pentamethyl ether. It appears from the physical properties and colour reactions that the flavonol may be isorhamnetin. It is possible that the flavonol is present as a glycoside as no free compound could be isolated from the ether or ethyl acetate extracts of the alcoholic residue, the latter, however, yielding a polyphenolic acid, m.p. 207-8°.

B.2.8. *Constitution of a lactone from Pachyrrhizus angulatus* Rich.

Norton and Hansberry (J. Amer. Chem. Soc., 1945, **67**, 1609 and earlier papers) found the resin from the seeds of *P. angulatus* Rich (syn. *P. erosus* Urban) to be insecticidally active and isolated six crystalline substances of which one was rotenone and another pachyrrhizone.

'Compound III' of Norton and Hansberry, (*loc. cit.*) a yellow crystalline substance of m.p. 204° has now been taken up for detailed study. It has a molecular formula $C_{19}H_{12}O_6$ ($\pm CH_2$). The oxygen functions are accounted for in a lactone, furan, methoxyl and methylene dioxy groups. With dimethyl sulphate and alkali it formed an 0-methyl acid, m.p. 220 -3° which on hydrogenation over palladium charcoal melted at 178°. Hydrogenation of the lactone under the above conditions gave a derivative of m.p. 214° which still gave colour with tetranitromethane. Further work is in progress.

B.2.9. *Chemistry of gum 'Guggul'.*

The sterol reported in the earlier report has been identified as β-sitosterol. A neutral compound of m.p. 226-8° and a methyl ester of the acidic fraction, m.p. 68-70° have also been isolated from the gum resin.

B. 3. Radiochemistry

B.3.1. *Synthesis of isotopically labelled compounds*

DL-Malic acid -2,3-C^{14} has been prepared. 500 μ C of fumaric acid -2, 3-C^{14} weighing 9.5 mg. and 5 ml. water were heated in a sealed tube at 170°-180°C for 96-100 hours. The reaction mixture was cooled and an aliquot was analysed by paper chromatography in ethanol ammonia-water (85 : 5 : 10). Viewed under ultraviolet light of 2537Å two spots

were visible on the chromatograms—one corresponding to malate and the other to fumarate (quenching). When, however, the paper was scanned with a Nuclear Chicago automatic chromatogram scanner or autoradiograms of the same paper were prepared; the presence of three C^{14}-labelled compounds was revealed; the third compound has not been identified yet.

For isolation and purification of malic acid the reaction mixture was fractionated on a Dowex 1 anion exchange resin in the formate form. 1.0 N acetic acid was used as the eluting agent. 1.5 ml. fractions were collected on a Shandon automatic fraction collector, aliquots of each fraction plated out on stainless steel planchets and counted with an end-window β-counter. Aliquots of each fraction were also subjected to paper chromatographic analysis for identification of the compound. Malic acid was eluted first with a peak in the 25th fraction. The contents of tubes containing malic acid were then evaporated to dryness, extracted with acetone and dried. Further purification was obtained by band chromatography of the residue on Whatman no. 1 filter paper with butanol-acetic acid-water as the developing solvent. The malic acid band was eluted with warm water and evaporated to dryness. Yield 48%, specific activity 6 mC/mM.

B.3.2. *Translocation of sulphate and phosphate in plants*

In earlier experiments (This Report 1956-57, p. 28) it was observed that in the pea plant the sulphate -S^{35} ion, within 15 minutes after entering the roots, is translocated to the topmost leaves and rapidly converted to a large number of organic compounds. That the sulphate ion moves towards the topmost leaves first and then comes down has been indicated by the following experiments. When the topmost leaves were cut and inorganic sulphate ion was fed through the cut end, a similar distribution pattern was observed. This type of distribution was also observed when the sulphate ion was supplied through the roots in complete darkness—a condition under which the stomata are closed; thus the relative rates of transpiration of different regions of the plants are not responsible. When the top-leaves were cut and sulphate was fed through the roots the sulphate ion spread over the entire plant. The phosphate ion behaved in the same way in many respects. When, however, the top leaves were cut and the phosphate-P^{32} was fed through the cut ends, the distribution was found to be onesided . For a 15 min. period the phosphate ion translocated to the top leaves was present almost exclusively as inorganic phosphate and apparently took longer time for conversion to organic compounds.

B.3.3. *Photosynthesis in Hydrilla*

Experiments have been continued to find an explanation using isotopic tracers for the observation of Sir J. C. Bose that during the summer months the aquatic plant *Hydrilla* utilises malic acid as a substrate for photosynthesis. Experiments were designed to show whether the CO_2 arising from decarboxylation of malic acid was used in photosynthesis or whether the entire or a part of the malic acid molecule is incorporated into sugars. Earlier (This Report 1955-56 p. 23) it was observed that the CO_2, fixed photosynthetically, follows the normal pathway.

When *Hydrilla* leaves were incubated with acetate-1-C^{14} in darkness, the acetate was rapidly metabolised and a number of organic acids, amino-acids and sugar phosphates were formed. On exposure to light for 15 mins. rapid decarboxylation took place; the C^{14} -activity in the alcohol-soluble fraction dropped to less than half the value at the end of the dark treatment. This was accompanied by an increase in the specific activity of starch. When the *Hydrilla* leaves were incubated with acetate -2-C^{14} in Warburg vessels in darkness and caustic soda was introduced into the central well at the time the vessel was exposed to light for 15 mins., one sixth of the C^{14} supplied could be recovered as CO_2 trapped in the alkali. Thus there are rapid decarboxylation reactions going on in the *Hydrilla* plant immediately on exposure to light. A part of the CO_2 coming out was fixed back into the plant with a resultant increase of radioactivity in sucrose. C^{14}-activity in malic acid decreased during exposure to light, but malic acid is not a major acid of *Hydrilla* leaves in light or in darkness and it is quite possible that other organic acids contribute the CO_2 required for photosynthetic fixation. The major organic acid of *Hydrilla* is an unidentified compound which moves with the solvent front when chromatographed in ethanol-ammonia-water (80 : 5 : 15). Experiments with malic acid —2, 3-C^{14} are in progress.

B.3.4. *Carbon dioxide fixation in relation to auxin-induced growth and inhibition*

The earlier observation that indoleacetic acid (IAA) promotes CO_2 fixation in *Avena* coleoptiles has been confirmed in numerous experiments performed during the year under review. The distribution of C^{14} in the alcohol-soluble and insoluble fractions is a function of time. For short periods (4 hrs. or less) IAA had a stimulating effect on the incorporation of C^{14} in the alcohol-insoluble fraction but with relatively long periods of incubation (14 hr.) no such stimulation was observed.

In the alcohol-soluble fraction of all these experiments a consistent stimulation of C^{14} -incorporation into malate was observed. If the DPNH-linked malic enzyme is involved in malate synthesis it might be expected that addition of DPNH to the system in absence of IAA would yield similar results and this is exactly what was found. TPNH, AMP or ATP stimulated the rate of CO_2-fixation but the stimulation of malate synthesis in none of the cases was comparable to that obtained with IAA or DPNH. It may be suggested tentatively that application of IAA leads to reactions generating DPNH and the electron transfer mechanism is coupled to the phosphorylative synthesis of energy-rich nucleotides utilised for a number of metabolic reactions including CO_2 fixation. IAA however appears to act on other loci as well, since the incorporation of C^{14} into other cell-wall components like pectates, uronides, hemicelluloses etc. as affected by DPNH, TPNH, AMP or ATP does not always appear to be similar to that observed with IAA.

IAA has also been found to stimulate the metabolisation of acetate -2-C^{14}, pyruvate -3-C^{14} or fumaric acid -2, 3-C^{14}, but in different ways.

B.3.5. *Carbon dioxide fixation and photoperiodism.*

Based on the observation that the presence of CO_2 in the experimental atmosphere is essential for photoperiodic induction the CO_2 fixation products in relation to the partial processes of photoperiodism have been studied.

The previous observation that in the low-intensity light process exposure to red light results in considerable decarboxylation, has been confirmed. The decarboxylation effect is observable even at 20°C, but at 10°C a sharp increase of C^{14}-activity in the alcohol soluble fraction has been observed. Among the compounds malic acid experiences the most marked change. In the long-day Wintex barley there is a sharp depletion of C^{14}-activity in malic acid; in the winter months, when the induction is less effective, larger number of inductive cycles are required for this effect to be discernible. The rate of photosynthesis in red light during the low intensity light process decreases sharply in the short-day Biloxi soybean but is practically unaffected in the long-day Wintex barley. The products of $C^{14}O_2$ fixation in blue light have also been studied. In blue light CO_2 is fixed at a relatively high rate, but sucrose synthesis appears to be inhibited.

If the contention that during photoperiodic induction treatment some compound(s) is formed in the leaves which on translocation to the growing points initiates floral primordia is correct, it may be expected that if $C^{14}O_2$ is supplied during the induction treatment to intact plants for a relatively long period and the induction treatment is continued in natural air permitting sufficient equlibriation of the C^{14} fixed, analysis of the growing points should reveal the presence of the so-called "flowering hormone" which should be tagged with C^{14}. Wintex barley plants which require CO_2 during the light period for flowering were supplied $C^{14}O_2$ during the light period for $4\frac{1}{2}$ hrs. on the third day of a 6-day induction period and the growing points were analysed on the tenth day. No qualitative difference in the compounds was observed. There were however marked quantitative changes. The compounds detected were the usual cell metabolites, namely sugars, organic acids, amino-acids, etc. Earlier experiments with the short-day *Xanthium* also failed to reveal any qualitative difference in the tagged products of *Xanthium* buds.

B. 4. Enzyme Chemistry

B. 4.1. *Disruption of bacterial cells*

Different methods of cell-breakage were tried for the nitrogen-fixing bacteria, *Azotobacter vinelandii* (strain 0), brought from the University of Wisconsin, Madison, U.S.A. The cells were grown on Burk's nitrogen-free medium, harvested in a refrigerated centrifuge and washed with phosphate buffer. To measure the extent of breakage the amount of soluble protein in the extract was estimated by Folin-Ciocalteu method. To judge the suitability of the method for enzyme isolation, the extract was assayed for 'Zwischenferment' (glucose-6-phosphate dehydrogenase) activity.

(a) *Alumina grinding.* Cells were ground with Merck's alumina in a chilled mortar in a 0° room with frequent cooling in between the grindings. The pasty mass was kept "just wet" by adding drops of phosphate buffer.The contents were transferred in a centrifuge tube by repeated washings with phosphate buffer and the supernatant collected by centrifugation in the refrigerated centrifuge.

(b) *Sonication.* Raytheon Magnetostriction Oscillator was used for the purpose. The cup in which the cells were exposed to sonication was cooled to 5°C by running ice-cold water. Usually the cells were suspended in phosphate buffer and exposed to 10 KC for a total time of 15 minutes, each treatment lasting for 5 minutes.

(c) *Ultrasonic treatment*.* Cells suspended in phosphate buffer were exposed to ultrasonic (1 Mega cycle) in a specially constructed glass cell*. This cell was kept at 4°C by passing ice-cold water through its jacket. The position of the cell was so adjusted that the peak of the wave generated in the suspension was at its maximum height. Preliminary trials were made for different time intervals and it was found that maximum amounts of enzyme and protein were extracted in 15 minutes. Specific activity of the enzyme was highest at this stage.

(d) *Shaking in a Nossal shaker.* The construction of a slightly modified Nossal shaker in the Institute's workshop has almost been completed.

The results are presented below.

Method	Units/ml.	mg. protein /ml.	Specific activity
Alumina treatment	1.0	12.0	0.08
Sonication	1.0	8.6	0.11
Ultrasonication	0.8	6.7	0.12

B.4.2. *Autolysis of yeast :*

Yeast from different sources was autolysed by suspending in 0.1M $NaHCO_3$ and incubating at 40°C for 5-6 hours. The extent of autolysis was determined by measuring the protein content and "Zwischenferment" activity of the extracts. Following table shows that a sample of dried yeast yielded the maximum amount of enzyme of high specific activity

	Units/ml.	mg. protein /ml.	Specific activity
Dried yeast (stored for long time, obtained from Dr. Nandi)	0.1	1.4	0.07
Dried brewer's yeast powder (obtained from Science College)	0.2	6.3	0.03
Dried yeast granules (obtained from Russa Distillary)	0.6	3.2	0.20

B.4.3. *Metabolism of sucrose in plants*

Sucrose is the first free carbohydrate formed in plants during photosynthesis and is the storage product in some plants. It is also actively metabolised which is apparent from sucrose-starch interconversion during day and night. It is speculated that an active sucrose-kinase may mediate this interconversion. Preliminary attempt at detection of this speculated enzyme in the acetone powder prepared from *Spinacia oleracia* (palang sak) by measuring

* The kind permission and help of Dr. T. C. Bhadra of the Department of Physics in using his ultrasonic equipment and glass cell are gratefully acknowledged.

the disappearance of sucrose failed. More sensitive and specific methods as formation of ADP would, however, be tried.

B.4.4. *Purification and properties of 'Zwischenferment' from Phaseolus radiatus seedling*

The presence of glucose -6-phosphate dehydrogenase (Zwischenferment) and 6-phosphogluconate dehydrogenase activity in the seedling of *Phaseolus radiatus* has already been reported. Attempt was made to purify the former enzyme by the usual methods. The optimum time for harvest was determined by preparing extracts from the seedling after different periods of germination and measuring the "Zwischenferment" activity and protein content of the extracts. The following table shows that the seedling after 24-48 hours' germination is most suitable for the preparation as after this period the activity falls off rapidly.

Age in hr. of the seedling after soaking	Protein (mg./ml.)	G-6P-dehydrogenase activity		6-Phosphogluconate dehydrogenase activity	
		Units/ml.	Specific activity	Units/ml.	Specific activity
24	9.9	0.50	0.05	0.50	0.05
48	7.7	0.50	0.06	0.40	0.05
72	4.8	0.27	0.05	0.25	0.05
96	3.2	0.08	0.02	0.11	0.03

The following operations were done at 2°-4°C. The crude extract was prepared by homogenising the seedling with sucrose-phosphate buffer in a Waring blender. The crude extract was subjected to ammonium sulphate fractionation. The protein precipitating at 50-70% saturation was collected by centrifugation and dissolved in water. The enzyme was then adsorbed on calcium phosphate gel and eluted with 0.1 M phosphate buffer, *p*H 7.5. The eluate was subjected to ammonium sulphate fractionation. The enzyme precipitated between 40% and 60% saturation and was dissolved in water. About 12-fold purification was achieved in this way as shown in the following table.

	Units/ml.	Total units	mg. protein /ml.	Specific activity
Crude extract	0.7	273	12.9	0.06
Amm. sulf. I	8.2	160	26.9	0.30
Gel elution and Amm. sulf. II	12.0	24	19.1	0.70

The partially purified enzyme had still some 6-phosphogluconate dehydrogenase activity although the latter was found not to interfere with the assay of G-6-P. The enzyme is active only with TPN and shows no activity with DPN. The enzyme has a rather sharp *p*H optimum at 7.5 and the activity drops rapidly below *p*H 6.0 and above *p*H 9.0. The enzyme showed no Mg^{++} requirement in barbitone buffer, which was routinely used in the assay

system. There is no inhibition of the enzyme activity up to a phosphate concentration of 0.1 M. Phosphate of molarity 0.2 and 0.9 brought about 25% and 87% inhibitions respectively.

B.4.5. *Purification and properties of ribulose diphosphatase*

The presence of ribulose diphosphatase in plants was speculated on the basis of photosynthetic cycle. If there is some deposition of ribulose diphosphate (RuDP) due to its incomplete conversion to phospho-glyceric acid there should be some device to lower the level of RuDP by its conversion to ribulose monophosphate. Indication of the presence of this enzyme in spinach was obtained while working in Dr. Horecker's laboratory in U.S.A. Now the enzyme has been partially purified and some of its properties have been studied.

All operations were done at 2°-4°C unless otherwise stated. Acetone powder of the leaves (*Spinacia oleracia*) was prepared by blending the leaves with acetone (-15° C). The powder collected by filtration was dried and stored in a vacuum desiccator at 0°C. It was extracted with 0.1 M Na-acetate and the extract was subjected to acetone fractionation at -5° C. The precipitate obtained at 0–50% acetone concentration was collected and dissolved in TRIS buffer, *p*H 7. This acetone fraction was then subjected to ammonium sulphate fractionation. Most of the enzyme precipitated at 50-70% saturation. This was dissolved in TRIS buffer again and then adsorbed on calcium phosphate gel. The elution was done with M/15 phosphate, *p*H 7. The enzyme was precipitated from the eluate by making it 90% saturated with ammonium sulphate and redissolved in TRIS buffer. The enzyme was thus 17-fold purified.

	Units/ml.	Total units	mg. P. ml.	Specific activity
Crude extract	8.0	1280	6.0	1.3
Acetone (0–50%)	40.0	1480	7.3	5.5
Ammonium sulfate (50–70%)	210.0	630	18.7	11.0
Ammonium sulfate precipitate of gel eluate	130.0	299	5.8	22.4

The enzyme is not entirely specific for ribulose diphosphate as it reacted with fructose diphosphate though at half the rate of the former. In fact fructose diphosphate was used as the substrate for routine assay due to the limited supply of ribulose diphosphate. The partially purified enzyme has still some monophosphatase activity. The enzyme has rather a sharp optimum at *p*H 5.5 with both FDP and RuDP as substrates. The activity drops rapidly after *p*H 5.5. Using FDP as substrate the liberation of phosphate was measured. The rate dropped when a little more than one mole equivalent of inorganic phosphate had been liberated.

C. BOTANY

C. 1. Plant Biochemistry & Physiology

C.1.1. *Studies on Crown Gall Problem*

C.1.1.1. *Causal Organism :* A strain of *Agrobacterium tumefaciens* has been isolated from naturally occurring crown gall tumor from *Tropaeolum majus* plant host. This strain numbered BI-16 differs from the original strain B-23 in its host range (no gall production in Marigold, Snapdragon & Bryophyllum), polysaccharide and indole production.

C.1.1.2. *Bacteria-free tumor tissue :* Employing the antibiotic treatment reported previously bacteria-free tumor tissue has been obtained from *Tropaeolum majus, Bryophyllum calycinum & Balsam impatiens* and the tissues are being maintained satisfactorily both in solid and liquid media. The Tropaeolum tissue which failed to grow for a long time in Robbin's medium recorded continued growth in Tobacco medium (modified).

C.1.1.3. *Polysaccharide from Tumor Hosts :* Attempts to separate Polysaccharide mixture by using electrophoresis (continuous) mentioned previously were not successful even after trying different *p*H values, voltage & solvent strength. As such attempt was made with success, using precipitation with organic solvents viz., Absolute alcohol, Acetone, Methyl alcohol & Dioxan in increasing concentrations by steps of 5-10%. Fractionation was done in MSE refrigerated centrifuge at 0°C at 3500 r.p.m. for 20-30 minutes with a precipitation period of 30-60 minutes after increasing the concentration from lower percentage value to next higher one. Qualitative and quantitative estimation of sugar groups and individual sugars by colorimetric methods in different fractions has been completed. For a comparative evaluation of polysaccharides from gall tissue and normal tissue, crown gall tumors in sufficiently large number will be produced during the rainy season at Falta Experimental Farm of the Institute using Balsam & Marigold as the host plants. Bacteria-free gall tissue is also being grown on large scale in 5″×1″ test tubes (agar slant). For this purpose special wooden stands fitted with aluminium angles for keeping the tubes in slanting position enabling an easy access to the tubes and easy observation of the tubes at a glance have been prepared. Each stand accommodates approximately 400 tubes.

C.1.1.4. *Medium for continued in-vitro culture of bacteria-free crown gall tissue.* From comparison of growth of bacteria-free gall tissue (Hollyhock (H-23)) in modified 'Sunflower' and 'tobacco' medium it has been concluded that the Tobacco medium has better combination of the nutrients. (In Tobacco medium the growth of the tissue is about 2-3 times more than that in Sunflower medium during the 4 weeks growth period). Further work is in progress to finalise the medium for H-23 gall tissue using triangular method of permutations with 25 flasks for each set of nutrient combination and 10 such combination sets at a time. For this purpose the tissue culture shaker has been modified by attaching one more tier of three trays to provide a total capacity of 250 flasks at a time. During the experiment on comparative growth in two different media, both in shake culture and steady culture,

in which the increase in fresh weight of the tissues in culture at defined intervals was followed, it was observed that growth rate in Sunflower medium gradually declined with age of the tissue. Further the tissues in shake culture turned brownish. Whether this was due to the continued exposure of the tissue to light or other factors like the total volume of the media, *p*H, period of subculture and shaking speed, remains to be elucidated.

C.1.1.5. *Antibiotic from Gall tissue.*

During the period under review, sufficiently strong antifungal property of crown gall tissue (H-23) has been observed when there was a chance fungus contamination in the culture tubes. Work is at hand to separate and identify the contaminants and to study the nature of the antifungal principle in crown gall tissue.

C.1.2. *Nucleic acid metabolism in Bluegreen algae*

C.1.2.1. *Nucleic acid control of Polysaccharide synthesis in Nostoc muscorum.* In view of the fact that a polysaccharide was associated with the nucleic acid fraction DNA_2 of *Nostoc muscorum* it was considered worthwhile to investigate the dependence of polysaccharide synthesis on the presence of nucleic acids. The polysaccharide is composed of glucose, galactose, ribose, arabinose, xylose, rhamnose and two unidentified components. It has been observed that when DNAase treated cells were allowed to metabolise $NaHC^{14}O_3$ in presence of light, the C^{14} fixed in polysaccharide fraction was much less than that of the control; that was also the case with DNA_1 and DNA_2 fractions, indicating a probable relationship between the syntheses of polysaccharide and DNA in this species. With RNAase, the effect was less marked. As the DNAase treatment hampers the synthesis of DNA it is expected that the incorporation of C^{14} in DNA would be less than that of the control, but the RNA should not be affected. It has been found in the present experiments that there was an increase in activity in RNA when the cells were treated with DNAase. In later experiments the DNAase treated cells were washed twice with phosphate buffer *p*H 7.0 (after incubation with DNAase) and it was observed that the increase in activity in RNA was reduced. It seems therefore, probable that the degradation products of DNA might have enhanced the incorporation of C^{14} in RNA. It was thought worthwhile to study whether the decrease in activity in DNA and polysaccharide fractions after DNAase treatment could be restored by the addition of DNA_1 and DNA_2, isolated from the same species. It was found that the activity in DNA and polysacchride were increased after the addition of DNA_1 and DNA_2 but complete restoration has not yet been achieved. With the addition of glucose the C^{14} activities in polysaccharide, DNA and RNA fractions were more than with arabinose alone. Glucose and arabinose together were as effective as the mixture of the five sugars, glucose, galactose, arabinose, ribose and xylose. With ATP the C^{14} activity in polysaccharide, DNA and RNA fractions increased showing thereby, that the synthesis of polysaccharide and nucleic acids are coupled with energy transference systems.

C.1.2.2. *Nucleic acid control of the primary reactions in photosynthesis.* As has been pointed out the nucleic acids may play a role in the synthesis of polysaccharides in *Nostoc muscorum* it was of considerable interest to investigate whether there is any relationship between

photosynthesis and nucleic acids. Nostoc cells were incubated with $NaHC^{14}O_3$ in presence of light for 2 hrs. after treatment with DNAase or RNAase for 2 hrs. It was observed that the total amount of CO_2 fixed was lowered by these treatments with nucleases, the inhibitory effect of DNAase being more marked than that of RNAase. The C^{14} activity in nucleotide & sucrose fractions deviated much from that of the control in which 53% of the total activity was incorporated in the nucleotide area on the paper chromatograms. After DNAase treatment this increased to 65%, probably indicating that the nucleotide synthesis remained unhampered and its accummulation was due to its nonutilisation for DNA synthesis. The same was the case with RNAase. In sucrose only 20-21% of the total activity was incorporated after DNAase treatment whereas in control at least 31-32% of the activity was recorded. This showed that after DNAase treatment the synthesis of sucrose was hampered. With RNAase the percentage of activity in sucrose was much less than that of the control, suggesting that RNA may be more closely connected with sucrose synthesis. Beside these, the percentage of activity in the aspartate and glutamate of treated cells was much higher than that in the control cells. This might suggest an effect of the nucleases on the relative distribution of the fixed carbon into the metabolic products; the primary CO_2 fixation reactions of the TCA cycle being preferred to sucrose synthesis. It was observed here that free nucleotides accumulate high activity after DNAase and RNAase treatments. With this in view a short term experiment was designed with $Na_2HP^{32}O_4$. The cells were exposed to $Na_2HP^{32}O_4$ in presence of light only for 1.25 secs. TCA soluble part was fractionated with barium acetate. Barium soluble and insoluble fractions were analysed for phospho compounds. The tentative identification of the two compounds where perceptible change in the radioactivity has been observed are (1) ATP and (2) ADP. This might be interpreted to indicate that the photosynthetic phosphorylation step in photosynthesis remained unaffected by treatment with nucleases even though the CO_2 fixation reactions were affected.

C.1.2.3. *Auxin control of nucleic acid synthesis in Rice Coleoptiles.* Auxin and nucleic acids are both connected with the growth of cells. With this end in view an experiment was performed with rice coleoptiles. Six mm. sections of coleoptiles were floated on water to free them from endogenous auxins. The sections were then treated with 10^{-6} M IAA, a growth promoting concentration and a parallel control also being run. The sections were incubated in the dark with $Na_2HP^{32}O_4$ for 2 hours. It was observed that both in DNA and RNA fractions the P^{32} activity increased remarkably as compared to the control. IAA was thus found to stimulate the synthesis of nucleic acids during growth.

C.1.2.4. *Phycocyanin and nucleic acid metabolism.* In the previous report it was mentioned that phycocyanin was isolated from *Nostoc muscorum*. Chemical composition of phycocyanin was determined qualitatively. The following amino acids have been detected. Cystein, lysine, aspartic acid, glutamic acid, threonine, α-alanine, tyrosine, γ-aminobutyric acid, methionine or valine, leucine, phenylalanine, proline and methionine sulphoxide. With RNAase treatment C^{14} incorporation in the phycocyanin fraction was much less than that of the control. It seems probable that RNA is related with the synthesis of protein fraction of phycocyanin. Detailed studies are in progress.

C.1.3. *Gibberellic acid and plant growth*

C.1.3.1. *Response of tropical plants to Gibberellic acid.* Gibberellic acid ($C_{19}H_{22}O_6$) and other Gibberellins resemble the naturally occurring plant growth substance, β-indole acetic acid, in some of their actions on growth and development of plants. Both are effective in very low hormonal concentrations. Preliminary experiments were undertaken to study the overall effect of Gibberellic acid on typical Indian plants as most of the previous observations relate to those of temperate climates.

Three-week old seedlings of *Tagetes patula* showed that they respond to an external supply of Gibberellic acid (10^{-4} and 10^{-5} Molar) by rapid elongation of the young internodes. After 15 days from the beginning of the treatment the plants were about twice as tall as the controls. The absence of a significant increase in length due to the higher cocentration of Gibberellic acid (10^{-4}M) over that produced by the relatively lower concentration (10^{-5}M) may be due to the fact that other limiting factors are operative. Among such factors, one may be the restricted amount of auxin available, since it is known that isolated coleoptile sections react to Gibberellic acid only when they have endogenous or applied auxin. Further studies on the response of other tropical plants and on the role of Gibberellic acid on flowering are in progress.

C.1.3.2. *Growth and metabolism of some aquatic plants as affected by growth-regulators.* The aquatic plants, *Lemna* and *Wolffia* were collected and maintained in tap-water enriched with nutrient salts. For investigations of the effects of Gibberellic acid and other growth regulators on their growth and development, they were grown in liquid inorganic culture media in Erlenmeyer flasks and petri dishes under controlled condition of temperature and artificial illumination. After several trials a modified Knop's solution with certain alternations was found to be capable of maintaining rapid growth of *Wolffia*. Further improvement in the composition of the medium particularly for the growth of *Lemna* is in progress. The plants are found to grow best in neutral or slightly acid media, but in alkaline media they soon become pale and gradually die out. After a series of transfers the plants were fed with Gibberellic Acid (GA) with or without the addition of β-indole acetic acid (IAA) in different concentrations. Under the combined action of GA and IAA there was a greater multiplication of the fronds, although each of the two growth-substances alone could also produce a slight favourable effect over the controls. There was, however, hardly any increase in dry weight and no detectable change in the moisture-content. The chlorophyll-content of the fronds of *Lemna* was reduced by the addition of GA (10^{-4} Molar) both in light and in the dark. There is some indication that, so far as the lowering of the chlorophyll-content is concerned, the effects of GA and darkness are additive.

C.1.4. *Tracer studies on phosphorus metabolism in higher plants using radioactive isotope P^{32}*

C.1.4.1. *Influence of some glycolytic inhibitors on the incorporation of P^{32} in organo-phospho compounds.* In last year's report the effect of Fluoride on the incorporation of inorganic P^{32} into the phospho-intermediates in different tissues was described, and considering the relative distribution of esterified P^{32} into various sugar-esters and nucleotides, particularly in mung and oat excised roots, it was suggested that in a condition where the metabolism

of triose phosphate via the EMP pathway to pyruvic acid is inhibited, the pentose-phosphate pathway provided a bypass for sugar metabolism. During the year under review the effect of iodoacetate, another inhibitor of triose-phosphate dehydrogenase, on the relative distribution of P^{32} in the esters of the EMP and pentose-phosphate pathways was studied.

Iodoacetate inhibited the total uptake of P^{32} in all the excised tissues studied and also the rate of esterification . As with fluoride the effect was most evident in roots rather than in any of the aerial tissues. In this case also the root-tissue revealed a switch over to the pentose phosphate pathway. There was indication of a proper block of the triosephosphate dehydrogenation as revealed by a greater accumulation of activity in hexose diphosphate.

The above results with fluoride and iodoacetate, however, are for the present, to be treated as qualitative since the experiments were more or less of exploratory nature. In so far as the incorporation of P^{32} from inorganic phosphate pool to different esters is an indication of the turnover of the individual esters they are quite interesting. But for confirmation it is necessary to isolate the individual esters in relatively pure form and to consider their concentration and specific activity under different treatment of the tissue.

A comparative study of the effects of fluoride and iodoacetate on the distribution of P^{32} in the compounds involved in triosephosphate dehydrogenation led to an interesting observation. Fluoride treatment did not inhibit the formation of 3PGA, but definitely inhibited the turnover of P^{32} in ATP. At the same time the chromatogram revealed a significant labelling in a new compound suspected to be inosine triphosphate, which is normally poorly labelled. Iodoacetate treatment did not permit the formation of labelled 3PGA to any reasonable extent as might be expected and also the labelling in ATP was abolished. However, in this case the new compound (suspected to be ITP) which appeared in fluoride treated tissue, along with the oxidation of triosephosphate to 3PGA did not appear. Comparing the results of the two experiments it may be suggested that phosphorylation of ADP in this sequence is not directly linked with the oxidative step and could be separated under suitable condition. The thiol ester formed between the oxidation product of the aldehyde and the –SH group of the enzyme may undergo a cleavage under the influence of a "phosphorylating enzyme" to produce the acid, and an intermediate nucleoside triphosphate (probably ITP). This reaction would naturally be inhibited by iodoacetate which inactivates the –SH group of the enzyme as observed. The nucleoside triphosphate so formed may phosphorylate ADP under the influence of nucleoside diphosphokinase to form ATP and this later kinase reaction may be susceptible to fluoride inhibition. The above postulation necessarily demands that 1,3-diphosphoglyceric acid is not formed as an intermediate in triose-phosphate dehydrogenation. In the present method of analysis this could not be conclusively ascertained. For a better elucidation of the mechanism proposed above, it is necessary to study the problem carefully with isolated enzyme systems and experiments in this respect are now in hand.

C.1.4.2. *The influence of some plant growth regulators on incorporation of P^{32} in organo-phospho-compounds.* Considering the suggested roles of auxins and blastocholines in plant respiration, an attempt was made to find out if these factors had any role in the metabolic

turnover of phosphate in phospho-esters. The results obtained did not establish any significant effect of IAA in the distribution of P^{32} in the phospho-esters, although the overall process in roots was to some extent retarded. The blastocholine, hexenolactone had but an effect on the total activity incorporated which gradually diminished with the age of normally germinated seedlings. However the phosphorus esterfication in germination inhibited seeds appeared to have been affected to a greater extent. The incorporation was much lowered and probably the kinase systems were affected. The high energy adenine nucleotides retained a good fraction of the small amount of P^{32} found inside the tissue.

C.1.4.3. *Effect of pH of the nutrient solution and some metabolic inhibitors on the absorption and translocation of P^{32} in whole seedlings.* The kinetics and mechanism of phosphate absorption have been studied in detail in excised root tissues. However, the data on root absorption of phosphate and its translocation to aerial tissues, in whole seedlings are not many. This problem was studied to some extent during the period under review. Whole seedlings of mung and oat, both etiolated (grown in dark in the laboratory at 25°C) and field grown, were used in the experiments as test materials, and effect of different *p*H of the nutrient medium, glycolytic inhibitors like fluoride and iodoacetate, respiratory inhibitor fluoroacetate, and auxins on the absorption in the root and translocation in the aerial portion, of phosphate was studied with P^{32} as the tracer.

The *p*H of the bathing solution appeared to affect both absorption and translocation of phosphate. Within a range of 4.5 to 8.0, the lower *p*H values seemed to accelerate the translocation of phosphate in the aerial part of the seedling whereas the absorption in root seemed to be retarded. Increasing *p*H gradually brought about a reversal of these effects, and at *p*H 8.0, though maximum absorption of phosphate in the roots took place, the translocation was retarded. It was confirmed that the phosphate absorbed and retained in the roots was mostly absorbed in the active process as only a small fraction of it would diffuse out when the roots were placed in a phosphate free solution. It thus could be observed that active absorption of phosphate in roots of whole mung or avena seedling was *p*H dependent and further, translocation of phosphate was not governed by or related to the quantity of actively absorbed ions in the roots. The present observation would tend to support the "enzyme kinetic" mechanism of active absorption of ions at least in cases of phosphate absorption.

The three metabolic inhibitors used had different types of effects on absorption and translocation. Fluoride retarded the absorption of phosphate in roots and practically completely inhibited translocation of the salt. Iodoacetate very largely inhibited the absorption but apparently had little effect on translocation. Fluoroacetate did not affect absorption but inhibited translocation. These effects of the inhibitor on absorption and translocation of phosphate once again demonstrated that translocation was not directly dependent upon the actively absorbed phosphate. However the inhibitory effect of Fluoride and iodoacetate on absorption suggested that the "Carrier" in active absorption of phosphate is probably an intermediate of the glycolytic pathway. Again the observed results also indicated some relation of translocation with metabolic processes. Elucidation of the nature of the "Carrier" is under investigation.

C. 2. Plant Physiology

C.2.1. *Study of electrical variations in plants*

For detailed study of the electrical variations in plants a modern apparatus with electronic amplifiers and pen recorders has been installed. Previously such electrical variations have been studied in the Institute with the help of highly sensitive galvanometer. The main disadvantage of using galvanometer is that it has a considerably long period of oscillation which stands in the way of correct detection of intermediary electrical changes occurring within that period. Further, continuous recording of galvanometric responses entails laborious photographic process and the changes cannot be followed visually. These short-comings of the galvanometric study have been removed in the modern pen recording system, where even ten thousand cycles per second can be correctly recorded and the recording can be followed visually.

The pen recorder that has been indented has three amplifiers, out of which two have been set to working order and the other due to some defect in one of its valves, is yet out of commission. Some preliminary observations on the electrical changes in Mimosa and Desmodium have already been performed using two amplifiers. The results of the observations are summarised below :

C.2.1.1. *Electrical changes in Mimosa*

Magnitude of action potential . By the application of thermal stimulus to the leaf action potential varying from 30 to 80 mv. has been found to be generated during the propagation of excitation through the tissue. Magnitude of the action potential was found to be greater in the petiole than in the subpetiole. Again, in the petiole the magnitude is greater towards the basal than in the apical region. Such variation in magnitude occurs both in acropetal and in basipetal conduction.

Velocity of conduction. Velocity of conduction of action potential in the petiole varies from 16 to 110 mm per second in different specimens.

High speed electrical impulse. During the fall of the pulvinus a sharp diphasic response is found to occur almost simultaneously at different parts of the petiole and the subpetiole. This indicates that this transmission is probably of the nature of an electric conduction than by means of an action current. This transmission is manifested also in the stem though in a lesser magnitude.

The action potential as such does not induce excitation. The action potential produced in the excited petiole was led to another petiole by connecting them electrically, with a piece of metallic wire, to find out whether the action potential produced in a tissue can itself induce fresh excitation to another tissue. It was found that the action potential of the excited leaf was manifested simultaneously in the other leaf without initiating any excitation there. This shows that the transmission of action potential as such through the tissue is not the cause of excitation of the responding organ. The action potential may be said to be only the electrical version of the transmission of excitation,

C.2.1.2. *Reflex arc phenomenon.* When the tip of one of the subpetioles of Mimosa leaf is stimulated, the excitation after passing through the subpetiole and the petiole, brings about the excitatory contraction of the pulvinus. After the fall of the leaf due to the contraction of the pulvinus, the other subpetioles close one after another, in a definite sequence in relation to the proximity of the originally stimulated subpetiole. J. C. Bose concluded that the subsequent closure of the subpetioles is due to the excitation reflected back from the pulvinus, through the petiole, and he compared this with the reflex arc phenomenon of the animal nerve system.

Attempt was made to pursue this reflex arc porcess by the study of electrical transmission of excitation. No conclusive result has yet been obtained. A measured source of stimulation instead of the hot point, as is being used at present, might be helpful in the study. Whether a feeble electrical stimulus can be applied to achieve the purpose is being considered.

C.2.1.3. *Electrical potential change and the fall of the pulvinus.* It has been mentioned before that when the action potential, due to the transmitted excitation, reaches the pulvinus a sharp diphasic electrical response occurs in the pulvinus during its fall. In order to find out whether the electrical response and the mecahnical fall of the leaf are simultaneous or there exists a time difference between the two, a device has been made by which the mechanical fall of the leaf can be automatically recorded on the same paper in the pen recorder.

The main principle of this device is that when the petiole just begins to fall an electrical circuit is completed through pen recorder, thus making a sharp mark in the line record. A thin piece of copper wire fixed with a glass fibre is vertically suspended from the petiole. An adjustable mercury cup is placed at a distance of about 0.5 mm beneath the suspended copper wire, so that the electrical contact is made just at the initiation of the fall.

Records taken in this method show that the electrical response in the pulvinus preceeds the mechanical fall of the leaf by a time difference varying from 1/20th to 1/10th of a second in different specimens.

C.2.2. *Simultaneous recording of the mechanical and electrical pulsation of Desmodium*

The normal frequency of Desmodium pulsation is between two to four minutes. The minimum speed of the recording paper being 1 mm. per second, it was found necessary to reduce it further for suitably recording the electrical pulsation. In doing so the electric motor of the recorder for moving the paper had to be put off and a separate arrangement required to be made for moving the paper at a slow speed. Moreover, for convenience of comparison of the electrical and the mechanical pulsations, the synchronization of the movements of the paper of the pen recorder, and the smoked plate of the mechanical recorder was considered to be most essential.

To achieve this, the same pulley which is worked to move the smoked plate of the mechanical recorder was simultaneously used to pull the paper of the pen recorder, suitably attached with a piece of string. In this process the plate and the paper being moved at the same speed, the relation between the mechanical pulsation and its electrical component could be correctly studied.

For taking records a detached specimen was mounted in a U- tube filled with water. The leaf was attached to the lever of the mechanical recorder by a silk fibre to record the movement of the leaf. To pick up the electrical activity of the pulvinus, which initiates the movement, two connections were made through fine tinsil wire and kaoline, one at a point on the lower pulvinus and the other at an indifferent point, either on a stipule or on a peeled out strip of skin from the stem. The two leads were then connected to the pen recorder through the amplifier.

Resemblance of electric pulsation of Desmodium with that of animal heart beat. It can be seen from comparison of the electrical and the mechanical records of the leaf movement that each mechanical pulsation has its electrical component. But the periods of mechanical and electrical pulsations are not the same, the electrical pulsation being completed within a much shorter time. The characteristic of the electrical pulsation is a quick negative impulse followed by recovery, with the down movement of the leaf. The negative impusle may occur just before or after the down movement has started and its recovery is completed much ahead of the completion of the down movement. During the rise of the leaf there are some minor electrical variations. The overall picture of the electrical activity of the Desmodium leaf is comparable with that of the animal heart beat. In some of the records the electrical pattern of the Desmodium pulsation seems to bear some similarity with the P.Q. R.S.T. phenomenon of the electro-cardiogram record of the animal. The magnitude of the negative impulse indicates generation of 10 to 50 m.v. in different specimens.

Effect of forced stoppage of movement. When the movement of the leaf is stopped by fixing the blade its electrical activity is found to persist unabated.

Effect of chloroform. It is known that the administration of a small dose of chloroform brings about stoppage of the pulsatory activity of Desmodium. It was proposed to find how the electrical activity is affected as a result of the application of chloroform.

Chloroform effects complete stoppage of both the mechanical and electrical components of pulsation. But the stoppage of the mechanical movement is a gradual process, while the stoppage of the electrical activity is spasmodic in nature; immediately after the application of chloroform there is a sharp negative inpulse followed by a slight shift towards positivity and then stopping completely. The mechanical movement does not stop in such abrupt way and it persists, though for a short time, after the stoppage of electrical activity.

In order to understand whether the electical activity preceeds the mechanical movement of the leaf some experimental device is being contemplated by which the pulsatory activity of the leaf can be kept temporarily stopped. If that be possible the heenomenon can be studied when the activity restarts.

C.2.3. *Loss of turgor in the pulvinus of Mimosa pudica due to excitatory contraction*

A note on the above work has been published in 'Science & Culture' and the detailed paper is going to be published in Trans. Bose Res. Inst.

In this work a device has been described by which the expulsion of sap from the puivinus of Mimosa, due to its excitatory contraction can be studied quantitatively.

C.2.4. *Autogenic responsive movements of the leaf of Mimosa pudica*

A short note on the above work has also been published in (Science & Culture) and the detailed paper has been made ready for publication.

The young leaf of Mimosa undergoes contractile response randomly at different hours of day and night without any apparent cause of stimulation. This autogenic behaviour of the leaf disappears when the top bud is removed and is partially revived by the application of auxin at the cut stump. It has been suggested that in the developmental phase of the leaf some internal reaction associated with hormone is the cause of such antogenic responses.

C.3. Cytology.

C.3. *The free floating mitotic figures in milk of coconut and other fruits of Palmae.*

As reported previously, floating nuclei in the milk of young coconut fruits may be observed to form spindle figures and undergo regular mitosis, although not bounded by cytoplasm or cell wall.

During the period under review the technique was developed of so mounting drops of meristematic endosperm gel under aseptic conditions that chromatic nuclei would complete the course of division on the slide while being watched through the microscope.

For material, *Areca* (betel nut) was available during the rainy season and *Cocos* (coconut) almost the whole year round, except for a few weeks of drought in summer. Young green fruits at the inception of endosperm gel formation were considered the most favourable material.

Dividing nuclei occur not only in the syncitium of the endosperm, but as well in the milk which fills the embryo sac cavity of the fruit.

They can with care be observed in the living fresh untreated condition under high power of dry lens. Permanent preparations were made by fixing and staining material, with certain modifications due to it being a gel. Brilliant Feulgen stained preparations resulted. Plates tended to be well spread out if coumarin was added to the fixing fluid.

The tissue nuclei are generally unorientated. Nevertheless perfectly placed polar views of metaphase may often be observed.

The course of Mitosis has been followed from prometaphase to telophase, the process being continuous. Nuclei greatly differing in size but always round in shape, elongate prior to division. The spindle figures might be perfect in shape, or rounded, or elliptic, or elongate with rounded ends, or somewhat attenuated, the shape varying with external conditions.

Chromosomes first become clearly visible at prometaphase, when approaching the equator. At metaphase they are assembled in what appears at X420 magnification, a rigidly straight row. They generally pass from metaphase to anaphase in 15 minutes and from anaphase to telophase in 15 minutes. The ranks might be broken and there might be some precocity during the beginning of anaphase but they finish their journey together, ending it a little way behind the spindle apex.

The changes that take place at telophase i.e. despiralization, joining up of chromosome ends etc., are not directly observable but within the hour the cleavage plate is laid down. Minute incretions appear at the equator, grow in numbers and merge to form a rigid plate, cutting the spindle across transversely. There is no elongation of the spindle to form the stem body. At this point the remaining spindle substance appears very gradually to draw away from the daughter nuclei and at a later stage this plasm and the cleavage plate are sloughed off together possibly with the remnants of the nuclear membrane, and the two new nuclei are set free. The stages after cleavage are not directly observable but inferred from the various stages encountered in preparations.

Mitochondria move freely about in the endosperm water and are not particularly attracted to the resting nuclei, but when the achromatic figure is beginning to form, mitochondria not only gather thickly about its periphery serving to outline it, but are greater in numbers at the two poles. So far as can be gathered from direct observation, mitochondria do not penetrate the dividing spindle figure.

Chromosomes were individually observable in only one rare instance when a giant polyploid nucleus of *Areca* which was fortunately detected at polar view metaphase, could with care be brought under the oil immersion lens and not only observed, but photomicrographed as well. Chromosomes which were spread out in the plate were bent at the centromere, but secondary constrictions were not observable. They appeared as in fixed preparations though less sharply defined. When the spindle turned over on its side due to pressure from the lens, it was revaled as a flattened figure with a large (polyploid) plate, which explained why polar view in this instance was observable. As in all previous cases the elongate spindle had been on its side, side view only of mitosis was seen, and individual chromosomes could not be distinguished. In side view under oil immersion (X950) in the above case, a few chromosomes had progressed to anaphase. They were more rounded than elongate and in the case of the longer chromosomes the ends of the two arms were not distinct from each other. Shorter chromosomes lay vertically along the spindle rather than horizontally across it. In all cases of anaphase, short chromosomes appear to be placed vertically upwards. This appearance is also noticed, though not commented upon, in Bejar's cinemicrographs of mitosis in living cells in which some individual chromosomes appear distinctly.

Chromosome ends are always confined to within the spindle figures and do not project beyond it.

The Spindle figure appears homogeneous, outlined by an adhering film of cell inclusions but in certain cases, and photographed in the case of the giant *Areca* nucleus mentioned above, striations stand out very clearly in the living untreated material, though they cannot be said to correspond to fibres. A difference in appearance due to consistency may be observed at anaphase between the spindle caps bounded by the moving line of chromosomes and the central portion over which the chromosomes have passed. This may with caution be interpreted as a difference in appearance between the chromosomal fibres and the continuous fibres.

Often the spindle figure may not occupy the entire nuclear area and there may be a clear zone between it and the nuclear membrane. This clear zone carries no inclusions and

doubtless corresponds to that portion of the nucleoplasm which is not utilized in forming the achromatic figure in the case of complete cell division.

The nuclear membrane : The membrane of the free floating nucleus is persistent throughout the course of division. It does not dissolve away during synaptic prophase, and is probably persistent even after telophase till ultimately it is sloughed off. It has never been observed to disappear. Normally the spindle outline is indistinguishable from its membrane but when such a spindle contracts from Coumarin effect, it draws away from the membrane leaving a clear gap, showing that the membrane is there. Literature on observations on cell division naturally refer to the complete cell, but even in such cases the membrane may persist. Wada mintains there is always a phase boundary between spindle and cytoplasm and Bajer mentions instances when the dissolved membrane is replaced by a secondary one. The membrane of coconut nucleus may deliquesce at a probe from the micromanipulator needle.

The function of the *Cleavage Plate* is primarily to cleave the cytoplasm of the dividing cell in two. As in this context there would appear to be no necessity for the cleavage plate to appear in a case of division involving only the nucleus, the persistence of the cleavage plate would appear to be a relict of complete cell division. The cleavage plate has therefore its origin in the spindle and later extends beyond it through cytoplasm to cell wall. In the present case, nuclear components would appear to complete their role in the cycle of division and later on those portions of the older nucleus that are redundant after formation of the two new nuclei are discarded.

The Effect of Coumarin is known to be C-mimetic. Attempts were made to study the course of the effect of this reagent on the living spindle under the microscope. The method of placing a small crystal of Coumarin on the floor of a grooved slide, and inverting a hanging drop mount over it so that the two surfaces did not touch, then sealing the whole mount with neutral vaseline, was found to be most successful. Nuclei directly above the crystal were kept under observation and as gradually the Coumarin permeated the sealed chamber, the spindle structures were visibly affected. Metaphase and anaphase figures at once began to shorten and at length became fully rounded out while chromosomes remained stationary at their original positions. The nucleus then reverted to resting stage. This nucleus would now contain the doubled complement, which is the known effect of C-mitosis.

When 0.1 M Coumarin solution was placed on the floor in place of a crystal, the reaction took a less severe course. There was a slight contraction of the spindle figure due to which it drew away from the membrane before slightly expanding out again but not to its former dimensions. Metaphase and earlier stages reverted back to resting stage, while anaphase continued on to telophase.

Resting nuclei appeared in every instance to be totally unaffected.

Types of Nuclei. Nuclei have been measured to range in size from 160 to 642.6μ. In addition to confirming the previously reported chromosome counts of 32, 48, 64, 80 and 160 the following counts were made : 34, 43, 45, 52, 57. These aneuploid numbers may

have arisen by partial endoploidy, and by the fusion of 2 nuclei with irregular numbers. Readings were taken of the incidence of nuclei in water of fruit in which the endosperm has barely been deposited. 5 nuclei only were encountered in 10 readings. Nucleoli appear to be alveolated.

Inclusions in endospermal fluid—In the earlier stages of coconut formation only nuclei of an even size are encountered. With increase in age of the fruit more inclusions come to be incorporated into the water and the sizes of nuclei begin to differ widely. These inclusions are variously fibre cells, plates of tissue, fat drops, crystals, what may possibly be bacteria, and lastly in fully mature fruit, yeast cells, which grow and increase in numbers and turn the milk cloudy in appearance and acrid in taste.

In the case of *Areca*, fibres and plates of tissue may be seen in the very early stages, and rarely giant nuclei. Later on with growth and deposition of endospermal tissue, the water becomes clearer and relatively free of inclusions, nuclei are rarely discovered and then never of a giant size.

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

C. 4. Radiation mutations

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

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

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

The season was favourable for the growth of *sesamum* plants, resulting in comparatively better yields than in the previous year.

The following varieties of *Sesamum orientale* were studied, as in the previous year, such as *types* 10,12,16 and 20 from West Bengal and *T.M.V.I.* and *II* from Madras.

C.4.1.a.1. *Breeding behaviour of the recessive mutants.* During the year under report, the further behaviour of the *white flowered* mutant, isolated in the X_3 generation from the 20,000 r treatment and the *small seeded* mutant isolated in the X_4 generation from the 14,400 r treatment in *S. orientale type* 16, were studied with regard to their oil content, flowering etc. and the data included in Table 1.

TABLE 1

Mutants and Control	X_8—1957			
	Mean Percent of oil, over Control ± S.E.	Peroxide value of oil		Difference in time of flowering ± S.E.
		Fresh oil	Oil kept for a month	
Control	100.00±0.89	6.0	21.0	—
White flowered mutant	103.90±0.89	6.0	13.6	+3.4±0.36
Small seeded Mutant	116.70±0.89	3.4	14.2	−1.2±0.33

It will be seen from the above table that data on oil content of X_8 progenies of the white flowered and the small seeded mutants isolated from *Sesamum* type 16, when analysed statistically revealed an increase of about 4.0% and 17.0% respectively over the control, as against X_5, 1954-1955 data, where the increase was 6.0% and 26.0% respectively.

There was no appreciable change in the characteristics of oil and composition of mixed fatty acids between the mutants and their control. But the keeping quality of the oil of the mutants continued to be better than the control, as indicated by their relative peroxide values of 6.0, 6.0 and 3.4 in the freshly extracted oils of the control, white flowered and small

seeded mutant respectively. Oils kept for a month gave peroxide value 21.0, 13.6 and 14.2 respectively.

While the white flowered mutant flowered about 3 days later than the control, the small seeded mutant flowered 1 day earlier than the control.

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

TABLE 2

Type and Locality	Control and Treatment	Plant Population	X_8—1957 Best Selection		Age of the plant at the time of flowering, in days	Peroxide value	
			No. of fruits	Wt. of seeds in gms.		Fresh oil	Oil kept for a month
Type 12 (W. Bengal)	Control	229	645	20.15	44	2.7	16.8
	14,500 r	141	678	34.20	45	4.8	11.3
	20,000 r	170	490	42.20	42	5.4	12.2
Type 16 (W. Bengal)	Control	122	604	32.50	44	3.8	27.80
	14,500 r	183	1070	63.70	42	4.8	24.85
	20,000 r	132	805	58.77	46	6.1	29.28

In the above table, the total number of plants raised and studied both in the control and the particular treatments have been included under "Plant population". In the next two columns, under "No. of fruits" and "Weight of seeds in gms", the number of fruits harvested from the highest yielders and the total quantity of seeds obtained from them, both in the control and the particular treatments, have been included.

It will be seen from the table 2 that the best plants selected in X_8 generation in 14,500 and 20,000 r treatments in type 12, gave 34.20 and 42.20 gms of seeds as against 20.15 gms of control.

Similarly, in *type* 16, the best plants in these two treatments gave 1070 and 805 fruits respectively, as against 604 of control with 63.70 and 58.77 gms of seeds, as against 32.50 of control.

The breeding behaviour of the selections which are in the X_7, X_5 and X_4 generations during the year under report have been summarised as follows :

In the X_7 generation, the best selection in 14,500 r treatment in *type 12*, gave 904 fruits and 57.80 gms of seeds as against 67.9 fruits and 43.40 gms of seeds of control. In *type 16*, although the fruits were greater in number, the quantity of seeds was less than the control.

In the X_4 generation of *Sesamum types* 10, 12, 16 and 20 where 40,000 and 56,000r treatments were given, the best selections in *types* 10 and 12 only gave satisfactory yield of seeds. In *type* 10, while the highest yielder in the control gave 694 fruits and 60.50 gms of seeds, the best individual selections in 40,000 and 56,000r gave 598 fruits and 61.80 gms of

seeds and 739 fruits and 62.20 gms of seeds, respectively. In *type* 12, the control gave 560 fruits and 27.30 gms of seeds. The respective figures in 40,000r selection are, 495 and 60.90 and in 56,000r selection, 470 and 37.30.

In the X_4 generation of *Sesamum type* 10, where 10,000, 12,500, 56,000 and 80,000r treatments were given, the best selections in all the treatments gave greater number of fruits and more quantity of seeds than their control.

The behaviour of two of the types in the X_3 generation during the year under report, with special reference to the yield of fruits and seeds, date of flowering and characteristics of the oil are included in Table 3.

It was noticed that in the X_3 generation of *Sesamum types* 10, 12, 16 and 20, where seeds were treated with 8,000, 16,000, 24,000, 40,000 and 80,000r, the best selections in *types* 10, 16 and 20 gave good results. In many of the selections in these three types it was noticed that with the increase in the number of fruits, there was an increased seed weight also. The best selections in 16,000, 24,000 and 80,000r of *type* 20 gave double the quantity of seeds than their respective controls (Table 3).

TABLE 3

Type and Locality	Control and Treatment	Plant Population	X_3—1957 Best Selection		Age of the plant at the time of flowering, in days	Peroxide value	
			No. of fruits	Wt. of seeds in gms.		Fresh Oil	Oil kept for a month
Type 10 (W. Bengal)	Control	147	507	28.80	44	20.0	32.06
	8,000 r	107	709	38.45	43	—	—
	16,000 r	73	821	38.40	44	—	—
	24,000 r	130	469	33.55	44	16.5	29.06
	40,000 r	111	760	45.40	43	16.9	37.40
	80,000 r	96	458	26.20	43	15.8	28.60
Type 20 (W. Bengal)	Control	122	445	23.50	43	23.2	36.40
	8,000 r	154	456	38.90	43	—	—
	16,000 r	8	519	44.10	—	—	—
	24,000 r	84	690	48.80	44	21.55	32.70
	40,000 r	79	645	59.80	42	28.60	42.95
	80,000 r	61	863	64.85	44	30.50	36.40

In the X_1 generation of *Sesamum types* 10, 12, 16, 20 and *T.M.V. I*, where the seeds were treated with 10,00,000 r in the 1956 season and sown in 1957, the best selections in *types* 10 and 20 and *T.M.V. I* gave higher seed weight than their controls. The number of fruits in the selection of *type* 20 and *T.M.V. I* were also greater than their controls.

In the fresh irradiation of *Sesamum types* 10,12, 16 and 20 and *T.M.V. I* with 75,000, 1,00,000 and 1,25,000 r, the best selections in 75000 r of *type* 12 and 1,00,000 r of *type* 20 gave greater number of fruits and much higher seed weight than their respective controls.

C.4.1.a.3. *Flowering behaviour of selections.* The selections of *types* 12 and 16 which were in the X_8 generation during the season, did not show any significant difference in the data

of flowering (Table 2). The date of flowering in the X_7 parent population of these selections in *type* 12 were same as that of control, *while in type* 16, they were 3 to 8 days earlier than the control.

The date of flowering in the selection of *T. M. V.* 1, which were in the X_6 generation during the season, were 2 to 5 days later than the control.

The date of flowering in the selections of treatments 8,000, 16,000, 24,000, 40,000 and 80,000 r of *types* 10, 12, 16 and 20 which were in the X_3 generation during the season, were recorded. Some of the selections in *type* 10 flowered 1 day earlier and in *type* 20, 1 day later than their respective controls (Table 3).

C.4.1.a.4. *Pollen grain sterility and diameter.* The percentage of sterile pollen grains in 75,000, 1,00,000 and 1,25,000 r treatments of *type* 10 were, 18.41, 44.88 and 50.88 respectively, as against 12.09 in their control. The pollen grains in 75,000 r treatment alone were bigger than those in the control. In *type* 12, although the percentage of sterile pollen grains in the 75,000 and 1,00,000 r treatments are 5.89 and 23.95, as against their control with 6.94, the dimater of pollen grains in these two treatments are bigger than in the control. In *type* 16, the treatment 75,000 r gave lower and 1,00,000 r higher percentage of sterile pollen grains than their control; but in both the treatments, the pollen grains were bigger than the control. In type 20, the treatments had smaller pollen grains with greater percentage of pollen sterility than their control. In *T.M.V. I*, in the treatment 75,000 r, the percentage of sterile grains was 3.89 as against 8.44 of the control. It has smaller pollen grains also. In this connection it may be mentioned that when X_1 progenies were raised from the seeds kept for a year after the irradiation, it has been found that the greater the percentage of sterile pollen grains, the smaller is the size of pollen grains and vice versa.

C.4.1.a.5. *Characteristics of oils.* All other characteristics of oil such as colour, refractive index, saponification value, iodine value, peroxide value, non-sap and mixed fatty acids in the X_8 progenies of the 14,500 and 20,000 r treatments of *types* 12 and 16 and the X_3 progenies of the 24,000, 40,000 and 80,000 r tretaments of *types* 10 and 20 analysed during the year, remain practically unchanged from the previous year's analysis. But some change has been noticed in the peroxide value. In the X_1 generation of *type* 12 and 16, the oils of the selections show greater peroxide value than the control when they are fresh, but when kept for a month, the increase in the value is less than the control (Table 2). On the contrary, in the X_3 generation of *type* 10 and 20, the peroxide value in the oils of the selections remained low in fresh as well as one month old oil (Table 3).

C.4.1.a.6. *Multicoloured seed mutant.*

The ash, grey and white coloured seeds isolated from the 56,000 r treatment in 1954 from *S. orientale type* 12 continued to give much higher percentage of oil i.e. 52.0, 51.0 and 53.0 respectively, as against 43.0 percent of oil in their control. The mixed population of grey and ash seeds gave still higher percentage of oil i.e. 54.50 percent. Various other shades of seed colour gave percentages of oil ranging between the control and the mixed (grey and ash) type.

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

C.4.1.b.1. *The yield of previous selections.* The selections from S. *orientale type* 12, which were in the S_2 generation, on comparison with the control revealed that both the activities of 89 and 123μ c of 72 hours duration, gave higher seed yield i.e. 32.70 and 39.22 gms as against the control with 32.35 gms (Table 4).

Most of the selections also gave more fruit and seed yields than their wet control. Their parents were also the best yielders of the S_1–1956 generation.

TABLE 4

Type and Locality	Duration of Treatment in hrs.	Control and initial activity in μc	S_1–1956 Best selection: No. of fruits	S_1–1956 Best selection: Wt. of 1000 in gms	S_2–1957 Best selection: No. of fruits	S_2–1957 Best selection: Wt. of seeds in gms.	Age of the plant at time of flowering, in days	Percentage of sterile pollen grains	Plant Population
Type 12 (W. Bengal)	(Dry) 48	Control	—	—	281	32.35	41	10.94	192
		Control	80	1.42	190	27.20	41	11.57	287
		89	353	2.78	185	31.40	41	4.90	210
		123	155	2.97	141	22.30	45	10.30	267
	72	Control	88	2.50	153	22.00	41	4.44	321
		89	265	3.28	358	32.70	46	6.87	272
		123	174	3.25	254	39.22	41	7.14	232
	96	Control	88	2.20	138	29.22	41	8.31	297
		89	194	2.36	64	30.05	43	7.68	199
		123	267	2.66	274	23.00	43	9.95	262

C.4.1.b.2. *Flowering behaviour of the selections.* The selections of *type* 12, which are in the S_2 generation during the year, started flowering 2 to 5 days later than the dry control (Table 4). The dates on which 50 percent of the plant flowered in the various treatments, were also the same as that of control.

C.4.1.b.3. *Percentage of sterile pollen grains.* The pollen grain sterilities of the wet controls (except the wet control for 48 hours, where the sterility is 11.57%), were lower than the dry control with 10.94. The treatments also gave lower percentages of sterile pollen grains ranging from 4.90 to 10.30 (Table 4). Contrary results were obtained in the S_1 generation of 1956, where the sterility in the treatments ranged from 4.94 to 12.51 percent, as against 5.65 percent in the control.

C.4.1.c. *Effects of radiophosphorus (P^{32}) in Sesamum*

C.4.1.c.1. *Germination percentage.* The dry seeds of *types* 12 and 16 were treated with various activities ranging from 116 to 1000 μc for 24, 48 and 96 hours.

It was found that both in *types* 12 and 16, in 24 and 48 hours duration of treatments, the germination percentages ranged from 6.5 to 22.5 as against control with 24.5 and 27.0 respectively. In the 96 hours duration, the treatment gave better germination i.e. ranging

from 5.5 to 23.5 in *type* 12, as against 5.5 in dry control and 3.5 to 10.5 in *type* 16 as against 2.5 in dry control. The germination recorded on three dates at weekly intervals showed both delayed germination and mortality. This low germination and survival were due to the late sowing.

TABLE 5

Type and Locality	Duration of treatment in hours	Control and initial activity in μc	Plant population	Best Plant: No. of fruits	Best Plant: Wt. of seeds in gms.	Age of the plant at the time of flowering	Percentage of sterile grains	Diameter of pollen grains in μ
Type 12 (W. Bengal)	(Dry) 24	Control	32	232	21.30	41	6.93	57.47
		Control	28	226	14.02	42	4.46	61.63
		1000	23	170	16.35	41	6.13	65.49
		800	21	317	21.86	41	5.00	60.66
		600	33	127	5.90	41	6.30	60.02
		490	22	124	0.24	44	9.10	59.44
	48	Control	10	198	15.35	41	4.53	57.83
		572	9	308	29.30	44	5.21	60.47
		476	4	258	18.40	43	5.61	61.37
		352	12	108	5.50	41	4.70	60.14
		280	7	321	16.00	41	5.49	61.12
	96	Control	14	162	12.80	44	11.56	58.67
		259	15	387	15.70	48	3.71	59.12
		216	8	167	10.50	46	8.04	64.21
		159	14	135	11.20	46	7.02	60.02
		127	8	78	10.45	45	5.75	61.63
Type 16 (W. Bengal)	(Dry) 24	Control	32	255	36.52	41	7.00	57.51
		Control	21	163	20.30	39	3.19	57.77
		1000	27	288	36.20	40	2.65	61.63
		800	20	515	38.40	39	4.39	61.69
		600	21	233	34.50	40	4.37	62.01
		490	19	346	32.30	41	5.86	62.21
	48	Control	9	242	32.50	42	5.99	61.05
		548	9	252	31.30	43	5.16	63.43
		534	6	98	18.95	47	4.22	64.99
		400	6	342	30.40	43	4.03	63.09
		257	5	267	31.60	44	4.16	61.44
	96	Control	7	174	15.02	49	12.71	63.76
		249	15	421	6.40	46	10.23	63.88
		242	7	146	13.70	49	5.32	62.27
		182	8	131	14.75	44	7.47	64.34
		116	11	132	14.85	45	5.79	64.34

C.4.1.c.2. *The yield of the previous selections.* In the P_2 generation, only the selection from 300 μc of the 72 hours duration in *type* 12 gave higher number of fruits i.e. 306 and greater seed weight i.e. 29.50 gms, as against control with 185 fruits with 15.0 gms of seeds. In *type* 16, a selection in control 48 hours, gave greater seed weight i.e. 40.16 gms as against 38.70 gms in the control.

Table 5 gives the plant population, yield of best plant, age at the time of flowering, percent sterile pollen grains and and diameter of pollen grains in the selections which are in the P_1 generation during the year and their control *types* 12 and 16.

In the P_1 generation in *type* 12, the selection in 800 μc/24 hours with 317 fruits and 21.86 gms of seeds and in 572 μc/48 hours with 308 fruits and 29.30 gms of seeds, are better than the dry control with 232 fruits and 21.30 gms of seeds. Two other selections of the 280 μc/48 hours, and 259 μc/96 hours treatments gave, 321 and 387 fruits respectively, which are higher than the control. In *type* 16, only one selection in 800 μc/24 hours, gave 515 fruits and 38.40 gms of seeds, which was higher than the control with 255 fruits and 36.52 gms of seeds. The selections in 1000 μc/24 hours, 490 μc/24 hours, 257 μc/48 hours and 249 μc/96 hours, however, gave only higher fruit but not seed yield than the dry control.

C.4.1.c.3. *Flowering behaviour of selections.* One of the selections *in type* 12 was as late as 7 days than its control in flowering, while in *type* 16, the majority of the selections in the various treatment of 24 hours duration were early by 1 to 2 days than the dry controls. The selections in 48 and 96 hours durations are however late by 1 to 8 days than their dry control. However, some of these selections flowered earlier than their wet controls.

C.4.1.c.4. *Pollen grains sterility and diameter.* The pollen grain sterility and diameter were recorded in the P_1 population. The dry control has 6.93 percent sterile pollen grains with a diameter of 57.47 μ. Although all the activities in all the three durations gave pollen grains with greater diameter ranging from 57.83 to 65.49 , the percentage of sterility in the control/96 hours was 11.56, in the 216 μc/96 hours was 8.04 and in the 160μc/96 hours was 7.02 , which were higher than the control. In *type* 16, the control (dry) has 7.00 percent sterile pollen grains with a pollen grains diameter of 57.51 μ. Here also, as in *type* 12, all the activities in all the durations gave bigger pollen grains than the control. The diameter ranged between 57.77 to 64.99 μs. The wet control/96 hours, 249 μc/96 hours and 182 μc/96 hours treatments only, gave greater sterility i.e. 12. 71, 10 23 and 7.47 percent respectively, than the dry control with 7%.

C.4.1.c.5. *Cytological observations in Sesamum.* Delayed cell division, inversion bridges, fragments, laggards and extrusions were commonly observed at meiosis of the X-and Beta -rays treated materials.

C.4.2.a. *Effects of X-rays in Mustard.*

Irradiation experiments were continued during the year with the same types of *Brassica*, as in the previous year, such as, *B. Campestris types Tori* 7 (W. Bengal), *Brown Sarson* (E. Punjab), *T* 71 and *T*88 (New Delhi), *B. juncea types Rai* 5 (W. Bengal), *R.L.* 9(E. Punjab), *T* 185 and *T*164 (New Delhi).

C.4.2.a.1. *The yield of the previous selections.*

It will be seen from the Table 6 that X_6 selections of *Rai* 5 in all the treatments, except 40,000 r, gave higher yield of seeds only than the control. The behaviour of the X_5, X_4, X_3 and X_2 selections grown during the current year have been summarised as follows :

TABLE 6

Type and Locality	Control and Treatment	Behavour of selection		Age of the plant at the time of flowering, in days	Peroxide Value	
		No. of fruits	Wt. of seeds in gms.		Fresh oil	Oils kept for a month
Rai 5 (W. Bengal)	Control	615	8.10	41	10.60	29.04
	20,000 r	947	22.00	40	7.20	15.90
	26,000 r	339	8.72	41	8.90	20.40
	40,000 r	415	4.60	39	14.90	22.90
	56,000 r	718	10.20	40	16.40	22.60

Regarding *Tori* 7, it will be seen from the Table 7, that X_5 selections in all the treatments except 26,000 r, gave higher yield of seeds than the control. But in the case of *R.L.*9, only the selection in 1,00,000 r treatment gave higher fruit and seed yields than its control.

TABLE 7

Type and Locality	Control and Treatment	Behavour of Selections		Percentage of oil	Peroxide Value	
		No. of fruits	Wt. of seeds in gms.		Fresh oil	oils kept for a month
Tori 7 (W. Bengal)	Control	346	7.07	32.15	20.37	28.63
	26,000 r	184	4.55	31.65	6.70	13.20
	40,000 r	232	7.46	—	—	—
	56,000 r	471	7.32	35.47	6.40	9.80
	80,000 r	378	10.11	33.48	12.20	19.10
R. L. 9 (E. Punjab)	Control	1066	30.35	35.95	15.60	33.20
	40,000 r	700	22.00	35.49	7.00	14.30
	56,000 r	452	11.60	41.27	6.80	10.60
	70,000 r	520	12.40	42.96	5.20	9.80
	Control	705	17.10	—	—	—
	80,000 r	700	9.50	38.61	4.80	8.40
	1,00,000 r	880	19.30	38.53	4.60	10.40

The X_5 selections of 26,000 r treatment in *Rai* 5; 26,000, 40,000 and 80,000 r treatments in *Brown Sarson* and 40,000 r treatment each in *T* 71 and *T* 88, gave higher seed yields than their respective controls.

The X_4 selections from the 40,000 r treatment in *Tori* 7; 40,000 r treatment in *Rai* 5 and 40,000 and 1,20,000 r treatments in *Brown Sarson*, gave higher fruit and seed yields than their respective controls.

The X_3 selections from the 16,00,000 r treatment in *T* 185 gave higher fruit and seed yields than the control.

The X_2 selections from the 40,000, 50,000 and 80,000 r treatments in *Tori* 7, *Rai* 5 and *T* 185 and 40,000 r treatment in *Brown Saron* gave higher fruit and seed yields than their respective controls.

C.4.2.a.2. *Flowering behaviour of selections.* The X_6 selections of *Rai* 5 flowered 1 to 2 days earlier than their control (Table 7).

In the X_5 generation in *Brown Sarson*, the selections in 76,000 and 80,000 r treatments flowered 1 and 7 days earlier than the control. In *R.L.* 9, the selections in 40,000 and 70,000 r treatments flowered 11 and 9 days earlier and in *T* 71, the selections in 40,000 r treatment flowered 6 days earlier than their controls.

In the X_4 generation, the selections of *Rai* 5 in 40,000 and 1,20,000 r treatments flowered 4 and 6 days earlier and in *T* 185 in both 40,000 and 1,20,000 r treatments, the selections flowered 3 days earlier than their controls.

In the X_3 generation in *R.L.*9 and *T* 185, the selections in 5,00,000 r treatment flowered 4 and 13 days earlier respectively than their controls.

In the X_2 generation, the selections of *Rai* 5 flowered 3 to 4 days earlier and in *T* 185, they flowered 4 to 8 days earlier than their controls.

C.4.2.3. *Percentage and peroxide value of oils.* It will be seen from the Tables 7 and 8 that the peroxide values of the oils in most of the selections of *Rai* 5, *Tori* 7 and *R.L* 9 both in fresh and after one month are much lower than their controls, indicating thereby their better keeping quality. The selections of *Tori* 7 and *R.L.* 9 (Table 8) gave increased percentage of oil, which in the case of treatments 56,000 and 70,000 r of *R.L.* 9, are much more than their control.

C.4.2.a.4. *Pollen grain diameter and sterility percentage.* The controls of *Tori* 7, *Brown Sarson*, *R.L.* 9 and *T* 185 gave greater percentages of sterile pollen grains than the X_1 selections from the treatments 35,000, 45,000 and 55,000 r. In *Rai* 5 and *T* 164, however, the selections gave greater sterility than their respective controls.

Though the selections in *Tori* 7, *Rai* 5, *Brown Sarson*, *R.L.* 9, *T*185 and *T*164 gave pollen grains with bigger diameter than their respective controls, the differences are not significant.

C.4.2.a.5. *Behaviour of early selections* The progenies of early selections in *Tori* 7, *Rai* 5 and *R.L.* 9 continue to be early with increased fruit/or seed yields.

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

C.4.2.b.1. *Yield behaviour of selections.* Table 8 gives the behaviour of the selections in *Rai* 5 and *Brown Sarson* which are in the S_2 generation during the year under report. The yield of the previous generation (S_1) is also given for purposes of comparison.

It will be seen from the above table that *Rai* 5 dry control gave 672 fruits and 14.48 gms. of seeds. One selection each in 679 μc/72 hours and 453 μc/96 hours treatments, gave 726 fruits with 24.38 gms of seeds and 963 fruits with 19.10 gms of seeds respectively, which are higher than the dry control. Wet control/96 hours also gave higher seed weight than the dry control.

Brown Sarson dry control gave 632 fruits and 19.20 gms of seed. A selection each in 453 μc/24 hours, 905 μc/24 hours and 905 μc/48 hours, gave 671 fruits with 23.25 gms of seeds, 1153 fruits with 33.20 gms of seeds and 412 fruits with 22.60 gms of seeds respectively.

TABLE 8

Type and Locality	Dry and Duration in hours	S_{35} Control* and initial activity in μc	S_1—1956 Selection No.	Best Plant: No. of Fruits	Best Plant: Seed weight in gms.	S_2—1957 Selection No.	Best Plant: No. of Fruits	Best Plant: Seed weight in gms.
Rai 5 (W. Bengal)	Dry	Control	1/71	627	13.98	IIIb/5/4/4	672	14.48
	24	Control	2/61	784	9.06	IIIa/4/5/7	397	6.35
	,,	453	3/66	320	16.02	IIIa/3/5/2	520	10.84
	,,	679	4/31	639	10.28	IIIa/2/5/3	215	4.25
	,,	679	4/49	448	6.10	IIIa/2/3/8	309	6.77
	,,	905	5/69	490	7.89	Ib/7/4/5	351	5.94
	,,	905	5/44	360	4.86	IIa/6/3/2	432	9.35
	48	Control	6/55	403	5.35	Ia/4/4/6	240	2.93
	,,	453	7/11	333	5.95	IIb/1/1/6	529	8.99
	,,	679	8/34	569	4.92	IIIa/9/1/7	369	5.95
	,,	679	8/41	495	7.85	Ia/2/5/2	448	11.97
	,,	905	9/57	398	8.70	Ia/3/3/3	247	6.00
	,,	905	9/40	365	7.57	IIb/2/4/4	370	7.55
	72	Control	10/38	519	6.81	Ia/6/1/7	650	9.12
	,,	453	11/59	694	10.62	IIIb/1/4/6	372	6.57
	,,	453	11/37	408	2.39	IIIb/1/5/7	463	8.12
	,,	679	12/49	539	12.02	IIIb/4/1/3	330	8.84
	,,	679	12/37	316	5.20	IIIb/4/5/3	726	24.38
	,,	905	13/75	470	5.57	IIIb/2/5/1	425	8.31
	,,	905	13/21	320	6.13	IIIb/6/4/7	411	9.68
	96	Control	14/10	407	8.87	IIIb/6/5/9	670	20.10
	,,	453	15/43	270	2.52	IIa/1/1/6	963	19.10
	,,	679	16/22	363	6.50	IIa/2/1/6	523	8.34
	,,	679	16/40	244	3.11	IIa/2/2/6	438	10.38
	,,	905	17/42	425	8.75	IIa/4/4/3	607	9.40
	,,	905	17/23	160	2.92	IIa/4/5/6	455	11.12
Brown Sarson (E. Punjab)	Dry	Control	18/4	320	4.95	a/7/2/6	632	19.20
	24	Control	19/15	399	7.30	IIb/3/5/5	448	13.50
	,,	453	20/29	212	3.25	Ia/3/2/5	671	23.25
	,,	679	21/50	386	2.38	Ia/4/5/9	291	8.90
	,,	679	21/21	135	2.20	Ia/4/1/8	310	11.40
	,,	905	22/2	560	4.57	Ia/1/2/1	158	7.50
	,,	905	22/1	116	2.39	Ia/1/3/7	1153	33.20
	48	Control	23/29	115	1.59	Ia/9/4/6	896	15.30
	,,	453	24/95	467	4.83	Ia/8/4/5	492	13.50
	,,	453	24/3	90	1.50	Ia/8/2/4	270	16.80
	,,	679	25/32	496	2.82	IIa/3/3/10	320	7.00
	,,	679	25/25	99	1.10	IIa/3/1/5	659	15.80
	,,	905	26/81	438	8.48	IIa/1/2/1	316	9.80
	,,	905	26/13	239	3.44	Ia/6/2/2	412	22.60
	72	Control	27/20	353	6.20	Ib/5/4/8	302	13.50
	,,	453	28/28	540	12.20	b/1/5/8	265	9.60
	,,	453	28/47	253	3.94	IIIa/2/4/2	663	17.70
	,,	679	29/49	338	3.86	Ib/2/5/2	170	6.50
	,,	679	29/48	106	2.94	IIIa/5/5/7	302	9.90
	,,	905	30/48	298	4.61	Ib/3/5/8	214	10.70
	,,	905	30/30	250	3.98	IIIa/3/1/7	340	13.90
	96	Control	31/43	624	8.00	IIa/6/5/8	304	12.15
	,,	453	32/42	300	4.22	IIIb/3/4/1	285	7.00
	,,	453	32/3	100	2.95	Ib/7/1/7	267	11.40
	,,	679	33/4	305	5.25	IIIb/1/4/7	237	7.20
	,,	679	33/49	197	2.42	IIa/8/2/7	372	14.60
	,,	905	35/43	945	9.00	Ib/9/2/1	205	7.40
	,,	905	35/28	692	17.10	IIa/10/1/9	335	8.80

* Soaked in distilled water.

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

In *Rai* 5 in the current treatment (S_1-1957), some of the selections of the wet controls in 24, 48 and 72 hours durations and the selections in 24 and 48 hours durations of all the three activities 973, 1459, and 2431 μcs flowered 1 to 3 days earlier than the dry control.

In *Brown Sarson*, some of the selections in all the three activities of 973, 1559 and 2431 μcs of the three durations flowered 1 to 5 days earlier than the dry control.

C.4.2.b.3. *Pollen grain sterility and pollen diameter.* In Rai 5, in the S_1 selections of all the activities, the pollen grain diameter is lesser than the dry control. The selections in all the activities gave greater percentage of sterile pollen grains than the dry control.

In *Brown Sarson*, the pollen grain diameter in some of the selections in all the activities was greater than the dry control. The pollen grain sterility in all the selections was lesser than the dry control.

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

C.4.2.c.1. *Yield behaviour of selections.* Table 9 gives the behaviour of the selections in *Rai* 5 and *Brown Sarson*, which are in the P_2 generation during the year under report. For purposes of comparison, the yield of the previous P_1 generation is also given.

TABLE 9

Type and Locality	Dry and duration of soaking in hours	P^{32} Control* and initial activity in μc	Selections No.	P_1—1956 Best Plant: No. of fruits	P_1—1956 Best Plant: Weight of seeds in gms.	P_2—1957 Selection No.	P_2—1957 Best Plant: No. of fruits	P_2—1957 Best Plant: Weight of seeds in gms.
Rai 5 (W. Bengal)	Dry	Control	1/6	526	12.50	Ib/7/2/7	446	4.50
	24	Control	3/67	820	13.48	IIIa/7/3/1	445	11.15
	,,	127	55/60	240	3.41	IIb/6/1/1	600	7.60
	,,	127	55/48	175	2.98	IIIb/6/3/8	743	12.80
	,,	379	56/19	326	3.56	IIIb/8/3/4	150	4.90
	,,	379	56/15	175	2.10	IIIb/8/5/1	275	7.35
	,,	514	57/14	418	4.50	Ia/6/2/5	248	4.40
	,,	514	57/28	294	5.11	IIIb/5/1/3	357	4.47
	,,	880	5/10	600	11.05	Ib/8/3/5	406	6.72
	,,	880	5/14	392	4.38	IIIb/7/2/7	462	9.85
	,,	1321	8/41	407	10.34	IIa/2/4/8	394	9.15
	,,	1761	10/29	270	2.75	IIIa/8/4/8	291	6.05
	,,	1761	9/20	245	3.75	IIIa/8/1/4	535	9.40
	48	Control	11/49	338	2.37	IIIa/10/3/6	440	9.05
	,,	127	59/48	398	5.95	IIb/5/5/5	285	3.40
	,,	127	59/11	232	3.16	IIb/5/2/10	697	7.30
	,,	319	60/28	206	2.47	Ia/2/5/9	172	7.00
	,,	319	60/27	105	1.68	Ia/2/2/2	134	8.00
	,,	514	61/3	228	1.92	Ib/3/5/3	498	5.10
	,,	514	61/21	151	1.43	IIIb/9/1/5	540	9.25
	,,	880	14/1	180	4.52	IIb/8/2/9	436	6.70
	,,	880	14/17	99	1.85	IIb/8/5/3	375	7.65
	,,	1321	16/22	363	2.03	IIb/7/3/1	387	4.27
	,,	1321	16/13	245	5.32	IIb/7/2/4	389	5.70
	,,	1761	17/20	375	4.85	IIa/8/1/3	489	5.70
	,,	1761	17/23	204	3.28	IIa/8/4/6	568	10.85
	72	Control	20/21	670	7.40	IIb/9/2/5	395	9.27
	,,	127	63/23	283	2.05	IIIa/3/4/8	689	14.55
	,,	379	65/54	292	4.05	IIa/10/3/3	245	4.55
	,,	379	64/41	180	2.80	IIa/10/2/2	422	8.60
	,,	514	65/10	271	3.28	IIb/10/1/2	152	4.00
	,,	514	65/5	163	2.10	Ib/11/4/6	535	7.95
	,,	880	21/22	675	5.91	Ia/12/2/2	442	6.10
	,,	880	21/18	195	3.92	IIIb/4/2/2	442	6.85
	,,	1321	23/38	258	5.32	IIIa/4/2/2	475	7.20
	,,	1761	25/64	531	3.81	IIb/12/1/2	350	6.80

TABLE 9 (*contd.*)

P^{32} P_1—1956 P_2—1957

Type and Locality	Dry and duration of soaking in hours	Control* and initial activity in μc	Selections No.	Best Plant		Selection No.	Best Plannt	
				No. of fruits	Weight of seeds in gms.		No. of fruits	Weight of seeds in gms.
Brown Sarson (E. Punjab)	Dry	Control	27/5	207	0.60	IIa/7/5/8	668	20.30
	24	Control	30/32	366	5.35	IIIb/10/5/1	431	16.25
	,,	124	68/21	289	5.00	IIb/8/4/1	208	8.85
	,,	124	68/4	108	1.30	IIIa/12/4/2	290	11.30
	,,	394	69/37	102	2.75	IIb/5/3/4	272	9.70
	,,	457	70/18	283	6.30	IIIb/12/2/3	411	11.97
	,,	457	70/24	267	2.85	IIb/7/3/1	340	13.62
	,,	880	32/18	409	4.00	IIa/5/4/5	264	9.45
	,,	880	32/11	334	7.20	IIa/5/2/2	264	11.35
	,,	1321	33/43	139	2.48	Ia/3/3/5	484	13.90
	,,	1761	35/22	364	4.20	IIIb/9/5/5	168	5.30
	48	Control	37/21	319	4.87	IIb/4/3/8	203	5.35
	,,	124	72/3	381	7.51	IIb/2/2/1	323	18.60
	,,	124	72/10	107	1.60	IIIa/5/3/6	540	20.29
	,,	394	73/9	131	1.10	IIIb/8/2/2	215	14.70
	,,	457	74/2	376	5.53	IIb/3/2/10	197	6.80
	,,	457	74/10	218	2.10	Ib/6/5/8	1042	16.02
	,,	880	40/11	224	2.48	IIa/4/1/2	260	3.50
	,,	880	40/17	168	1.20	IIa/4/4/1	334	11.45
	,,	1321	41/13	217	1.60	IIa/3/1/1	103	1.20
	,,	1321	41/22	153	2.89	Ia/5/1/6	395	7.57
	,,	1761	44/9	205	2.10	Ib/8/4/2	80	7.30
	,,	1761	44/21	109	2.60	Ib/8/2/2	354	8.80
	72	Control	46/18	372	4.55	Ib/10/2/10	475	13.06
	,,	124	76/25	221	2.32	IIIb/1/3/2	298	9.35
	,,	457	78/19	207	0.87	IIa/10/1/1	112	2.45
	,,	457	78/4	116	2.13	III/a/4/4/7	352	9.45
	,,	880	48/6	451	5.35	Ib/11/4/7	348	6.05
	,,	880	47/13	350	7.85	IIb/9/2/10	328	7.20
	,,	1321	49/14	455	4.80	IIIb/4/4/2	361	10.64
	,,	1321	49/7	125	1.96	IIa/11/5/2	127	15.70
	,,	1761	51/17	140	0.30	IIIb/2/3/1	184	8.17

* Soaked in distilled water.

It will be seen from the above table that *Rai* 5 dry control gave 446 fruits and 4.50 gms of seeds. Four selections one each in 514 μc/24 hours, 127 μc/48 hours, 1321 μc/48 hours and 514μc/72 hours, gave lesser number of fruits and seeds than the dry control. The rest gave higher weight of seeds than the dry control, irrespective of the number of fruits. The wet controls in 24, 48 and 72 hours durations gave 445, 440 and 395 fruits with 11.15, 9.05 and 9.27 gms of seeds respectively. In 48 hours duration, a selection in 1761 μc gave higher number of fruits and seed weight than the wet control. In 72 hours duration, a good number of selections gave higher number of fruits than the wet control.

Brown Sarson dry control gave 668 fruits and 20.30 gms of seeds. Only one selection of 457μc/48 hours treatment having 1642 fruits was higher than the dry control. In 48 and 72 hours durations, most of the selections gave higher fruit and seed yields than their respective wet control.

C.4.2.C.2. *Flowering behaviour of selections.* In *Rai* 5 treatments of the current season (P_1-1957) the selections in all the three activities 844, 1266 and 2110 μcs and control of 24 and 48 hours durations flowered 3 to 6 days earlier than the dry control. In the 72 hours duration, the activities 844 and 2110 μcs flowered 1 day earlier only.

In *Brown Sarson*, the selections in activities 844 and 2110 μcs of 24 hours and control and 844 μc of 48 hours, flowered 1 to 3 days earlier than the dry control.

C.4.2.C.3. *Pollen grain sterility and pollen diameter.* No significant change in the pollen diameter of selections of *Rai* 5 was observed, except in 1266 μc/72 hours treatment, where it was 1.25 μ greater than the dry control.

In *Rai* 5, the sterility in control and 844 μc of 24 hours treatment and 844 and 1266μcs of 48 and 72 hours treatments is higher than the control. This is significantly higher in 844 μc /24 hours and 1266 μc/72 hours treatments.

In *Brown Sarson*, there was no significant increase in the pollen sterility of selections. However all the treatments gave increase in pollen grain diameter, ranging from 1.0 to 2.0 μ.

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

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

C.4.3.1. *Sowing season.* The monsoon was late and hence the plants were affected due to drought, during their intital stages of growth. Attempts were however made to overcome this, by artificially irrigating the fields.

C.4.3.2. *Treatments with radiophosphorus* (P^{32}) *and radiosulphur* (S^{35}). Dry seeds of three varieties of *C. olitorius* viz : *Tall Mutant* (a high yielding X-rayed mutant isolated in 1947), *R-26* and *Chinsura Green* and two varieties of *C. capsularis* viz: *D* 154 and D 386, were irradiated with 0.5, 1.0, 1.5, 2.0 and 2.5 mc of P^{32} and S^{35} and were sown along with their respective controls at the Falta Experimental Station.

C.4.3.3. *Survival to maturity.* As in the previous years, no correlation was observed between the number of surviving plants and the treatments.

C.4.3.4. *Plant heights and basal diameters.* There were no significant variations in the different treatments with P^{32}, with regard to plant height and basal diameter. In the varieties *R*-26 and *D*-386, some of the treatments in S_1 generation (treated with S^{35}) had shorter plants than the controls and the differences were found to be statistically significant. No significant variations in plant heights and basal diameters were observed in the P_2 generation also.

C.4.3.5. *Branching.* As in the previous years, a large number of plants in the treated population of *C. olitorius* varieties had 2-6 branches emanating from the soil level, or near to it (Table 10).

TABLE 10

PERCENTAGE OF PLANTS SHOWING BRANCHING IN P^{32} TREATED POPULATION

Variety / Treatment	Tall Mutant	R—26	Chinsura Green
Control	0	0	0
0.5 mc	1.8	5.7	1.8
1.0 mc	2.4	11.6	19.1
1.5 mc	40.9	25.2	41.9
2.0 mc	37.6	21.2	16.3
2.5 mc	34.2	23.8	29.8

It will be noted from the above table that as high as 42% of the population had branches in the variety *Chinsura Green*, treated with 1.5 mc P^{32}, as against un-branched plants in the control and in the S^{35} treatments. Among the branched plants, the one with 6 branches isolated from *R-26*, treated with 1 mc of P^{32} yielded 167 gms of fibre and 450 gms of wood, as against 47 gms of fibre and 137 gms of wood in the best control plant.

C.4.3.6. *Flowering time.* As in the previous years, some early flowering types were again selected from the P_1 population of *Tall Mutant*, the earliness varying from 8 to 39 days over the control. The early flowering types which were selected earlier from X--irradiated (X_1) and P^{32} treated (P_1) populations, were found to maintain this character, in their subsequent generations. The earliness in mean time of flowering in the different selections from the X-irradiated population varied from 68-74 days in *Tall Mutant*, 32-41 days in *R-26*; while those from P^{32} treatments varied from 50-81 days in *Tall Mutant* and 48-65 days in *R-26*, as could be seen in Table 11.

TABLE 11

The difference in mean time of flowering (in days) in the different selections with respect to the control.

Variety	Source of isolation	Selection No.	Difference in mean time of flowering (in days)	Generation
Tall Mutant	X-irradiated population	T_1X	68±1.59	X_8
		T_2X	72±1.60	X_7
		T_3X	74±1.54	X_7
		T_4X	72±1.46	X_7
R—26	X-irradiated population	R_1X	32±1.84	X_8
		R_2X	41±1.76	X_4
Tall Mutant	P^{32}treated population	T_1P	74±1.68	P_3
		T_2P	81±1.51	P_3
		T_3P	50±2.67	P_3
R—26	P^{32} treated population	R_1P	48±2.44	P_3
		R_2P	64±1.71	P_3
		R_3P	65±1.81	P_3

These types, during their harvest time, yielded sufficient quantity of fully ripe seeds and good fibres, while only good fibers were obtained from the control plants.

C. 4. 3. 7. *Yield of fibre.* During the year under report, the yield of fibre in the five varieties of jute studied, were found to be 36, 27, 34, 27 and 31 maunds per acre in *Tall Mutant*, *R*-26 , *Chinsura Green*, *D* 154 and *D*-386 respectively.

C.4.3.8. *Morphological abnormalities.* As observed during the previous years, the treated (P_1) population showed various types of morphological abnormalities like fasciation of the stem, double leaves, forking of leaves, forking of petioles, epiascidia, proliferation of mid ribs, chimaera etc. These abnormal plant growths were observed in the seedling stage and when these leaves dropped off, they never reappeared in mature plants, excepting the fasciation of stem and the crinckled leaves, which in many cases continued till the harvest time. Morphological variations like the forking of leaves and/or crinckled leaves, often associated with reduction in size of flowers, apeared in the P_2 generation, but their P_1 parents were normal.

C.4.3.9. *Morphology and anatomy.* Critical morphological and anatomical studies in the abnormal leaves and petioles revealed that fission and fusion of vascular bundles took place either before or after unfolding of leaves. In many cases, the course of the vascular bundles were completely changed. Normally, the petioles were seen to have 3 or 4 vascular masses, which gradually fused to from a ring of vascular bundles and finally three main vascular masses were formed as the mid-vein and two prominent lateral veins on either side. In double leaves, there were two groups of 3 vascular masses, each with their xylem facing each other. With the exchange of vascular elements between these two groups, the vascular masses separated out and led into the blades, which also were found to be free at the top.

C.4.3.10. *Cytology.* Meiosis in many of the treated population was found to be mostly normal, excepting in the aberrant plants, where chromosomal disturbances like univalents, intervivalent connections and extra chromosomes during diakinesis and metaphase I, fragmentation, bridges—single and multiple, non disjunction of bivalents at anaphase I, were observed.

C.4.4. *Investigations on induced mutagenesis in paddy (Oryza sativa) using X-rays and radio-isotopes.*

This is a research project being financed by the Atomic Energy Commission, Government oi India, for investigating both the fundamental and applied aspects of irradiation in paddy, with X-rays and radioisotopes and is in the second year of investigation, during the year under report.

C.4.4.1. *Sowing season.* The season was fairly fabourable for the growth of paddy and hence the germination, stand and yield were comparatively good.

C.4.4.2. *Treatments.* During the year, fresh irradiations with X-rays, radiophosphorus and radiosulphur were again given to the seeds of *Rupsail* and *Patnai* varieties of aman paddy. In addition, the seeds collected from selected plants of previous year's irradiated population, were grown as X_2, P_2 and S_2 generations in order to isolate recessive mutations and to note segregations, if any.

The dosages of X-rays given were, 15,000, 30,000 and 45,000 r units, while for P^{32} and S^{35}, 0.8, 1.6, 2.4, 3.2 and 4.0 mc were the initial activities, in which seeds were kept immersed for 24 hours.

All these freshly irradiated seeds and those collected from the irradiated population of the previous year, were sown in seed-beds in June, and the seedlings transplanted to field in July, 1957.

C.4.4.3. *Survival.* With X-rays, there was a fall in the percentage of surviving plants with increase in dosage in both *Rupsail* and *Patnai*, but there was no correlation between the dosage and the percentage of survival, when the seeds were treated with P^{32} and S^{35}, although they were lesser in the treatments.

C.4.4.4. *Time taken for first flowering (in days) and mean time of flowering (in days)* : There was no marked variation in the irradiated population as regards the time taken for first flowering. A maximum earliness of 4 days over the control was however observed in 0.8 mc P^{32} treatment in *Patnai*.

In mean time of flowering also, there was no difference except for 45,000 r X-ray treatment in *Patnai* and 3.2 mc P^{32} treatment in *Rupsail*, where 2 days earliness over the respective controls was observed.

C.4.4.5. *Pollen grain diameter and sterility.* The diameter of the pollen grains were greater in practically all the treatments, but there was no correlation between the dosage and the size.

As regards the percentage of sterility of the pollen grains, there was an increase in sterility with increase in dosage of X-rays. Such correlation was absent in both P^{32} and S^{35} treatments, where the lowest dose given i.e. 0.8 mc induced maximum sterility.

C.4.4.6. *Plant height, main leaf height, number of tillers and panicles.* No significant variations were observed in the treated population with regard to plant height, main leaf height, number of tillers and number of panicles.

C.4.4.7. *Selections for grain yield.* It has been reported earlier that in investigations on induced mutation in *Rupsail*, *Latisail* and *Patnai* varieties of aman paddy, with X-rays, radiophosphorus and radiosulphur, radiophosphorus in some cases proved to be more useful from the point of view of the yield. Many of the P^{32} treated plants in P_1 generation, produced more panicles and yielded significantly more grains than the controls. But no marked variations were noticed in grain yield in X_1 and S_1 populations. With radioisotopes treatment it was observed that with increase in the duration of treatments, there was a decrease in yield of grains. As regards yield of straw, some of the P^{32} treated plants as well as plants treated with 20,000 and 40,000 r X-rays, gave greater straw yield than the controls. In general, the S^{35} treated plants yielded less than the control.

The individual selections showing high grain yield made in the previous year from *Patnai* (X-ray treated) and *Rupsail*, *Latisail*, *Patnai* (P^{32}-treated), were put to yield trial. Of the four selections from *Patnai* (P^{32}-treated) selections 3 and 4 yielded significantly more, when compared to the others. There were however no significant variations among the selections in the others.

C.4.4.8. *Non-lodging selections.* Several plants have been isolated from the freshly irradiated population as well as from previous years selections, which did not lodge.

C.4.4.9. *Morphological peculiarities. Albinism*—Albinism or absence of chlorophyll was recorded in the varieties *Rupsail* and *Patnai* in X_2 and P_2 generations, upto a maximum of 10.6% in the latter. All these plants died in the seed-bed at the third or fourth leaf stage and hence no detailed studies could be made.

C.4.4.10. *Complete sterility.* In the variety *Patnai*, one plant in 4.0 mc P^{32} treatment was completely sterile. This plant had partial chlorophyll deficiency at the seedling stage. At maturity, the stem was found to be very thin, with narrow leaves, showing chlorophyll deficiency in the mid-rib region. This plant remained as a dwarf, attaining a height of only 143 cms as against 190 cms in the control. The pollen sterility was as high as 24.2%, as against 1.9% in the control, and the P.M.C's showed irregularities.

C.4.4.11. *Semi-sterile.* A case of semi-sterility was also noted in (4·0) mc P^{32} treatment in the variety *Rupsail*, where out of 16 panicles, 12 had very few seeds.

C.4.4.12. *Small-grains.* In the P_2 population of *Patnai* two aberrant plants were noted which had smaller grains than that of the control. The husk colour was also changed, being brown instead of the straw colour of the control.

C.4.4.13. *Cytological investigations.* Meiotic abnormalities like non-disjunction, early separation of bivalents, etc. have been observed in the irradiated population. A critical study in the treatments with P^{32} revealed an increase in the frequency of chromosome aberrations with increase in the activity of P^{32} upto 3.2 mc, after which there was a sharp fall.

C.4.5. *Irradiation and cytogenetical studies in Tomato (Lycopersicon esculentum)*

Investigations on tomato had been undertaken two years ago to find out the cytological and genetical changes induced by X-rays, beta rays from P^{32} and S^{35} and ultraviolet.

C.4.5.1. *Mutants isolated earlier.* It has been reported in the previous year that irradiation experiments using S^{35}, P^{32} and X-rays on dry, presoaked and germinated seeds of *Tezier's Prim*, a stable cultivated variety of tomato, obtained from France, were effective in increasing variability in the progenies. The interesting variants reported in the previous year were:

(1) a pale coloured variant having bilocular berrys and a knob at the stylar end in the initial stages of the development of the fruit. (2) A fasciated plant, (3) brachytic plants in three different X_2 progenies, (4) a X_2 progeny with four unfruitful plants and twentysix fruitful plants. Except for two brachytic plants, which were isolated from 16,000 r treatment on dry seeds, the rest of them were obtained from 8,000 r treatment on dry seeds.

C.4.5.2. *Behaviour of mutants*

The behaviour of these mutants in the X_3 generation of the current year, is as follows. (1) The X_3 progenies of the pale coloured variant had brighter foliage. In those fruits with more than two locules, the knobs were absent. The meiosis was found to be normal, (2) The progenies of the fasciated plant was normal (3) Some of the brachytic mutants were found to breed true and no meiotic irregularities were observed, (4) The X_2 progeny having

four unfruitful plants showed structural changes in the chromosomes—two chains, one with 6 chromosomes and the other with 4 chromosomes were present in the first meiotic division. The progeny of some of the fruitful plants segregated into fruitful and unfruitful plants.

During the year under report, S_2, P_2 and X_2 as well as some X_3 and $U.V_3$ generations were also grown. In the first generation, no meiotic irregularities were observed in the treated population of S^{35} and P^{32}. But the treated plants had slightly greater pollen grain sterility than the control plants. In P_2 and S_2 generations, a large number of variants, including lethals, semi-lethals and unfruitful plants, were seen. In a P_2 progeny where 86 seeds germinated, 17 were yellow green seedlings, which did not develop after cotyledonary stage.

It has been reported earlier that the pollen grain sterility increased with increase in dose of X-rays, ranging from 2,000 r to 48,000 r on dry seeds. At 40,000 r, the pollen grain sterility of some of the plants reached 100%. During meiosis, the treated plants above 24,000 r showed structural changes in the chromosomes. Out of 45 plants examined, two plants had a quadrivalent and five plants had inversion bridges and fragments at meiosis. The meiosis of the control plants was normal. A X_2 progeny with fruitful and unfruitful plants again occurred from 4,000 r treatment on the presoaked seeds.

C.4.5.3. *New variants observed during the current year.* (1) A high yielding selection with 103 fruits, 313 locules and a total fruit weight of 5253 gms was obtained from a X_2 progeny of the 4,000 r treatment on dry seeds. As against this, the best plant of the control had 62 fruits with 198 locules and a total fruit weight of 3362 gms. The performance of this selection during the X_3 generation could not be included, as the plants are still in the field. (2) A true breeding mutant with quicker growth and earliness in flowering was isolated from 8,000 r treatment on dry seeds.

(3) A number of dwarf mutants have been isolated in the X_3 generation. The X_1 plant, grown from dry seeds treated with 8,000 r X-rays and its X_2 progenies were normal. These mutants had erect main stem, measuring about 21 cms in height only. They had 4 or 5 small branches and crowded leaf fronds.

C.4.5.4. *Relative effectiveness of the mutagenes.* Of the four mutagenes tried so far, viz. X-rays, P^{32}, S^{35} and ultraviolet, the potentialities of inducing variants in tomato appear to be as in the following order, X-rays-P^{32},–S^{35}–ultraviolet.

C.4.6. *Radiation genetics in Ground-nut.*

The work on the radiation-genetics in Ground-nuts (*Arachis hypogea*) was started during the year under report with the object of isolating economic mutants.

C.4.6.1. *Types and Treatments.* Six different varieties of ground-nuts obtained from various sources selected for these experiments, were treated with X-rays and beta-rays from P^{32} and S^{35}. The types and the details of the treatments are included in the following table :

TABLE 12

Variety	Source	Treatment		
		Radiation	Dose	Duration
T.M.V. 3	Tindivanam, Madras	X-Rays	15,000 r 17,000 r 20,000 r	—
AK 10	Akola, Bombay	,,	15,000 r 17,500 r 20,000 r	—
T.M.V. 1	Tindivanam, Madras	Beta-rays (from P^{32})	3.0 mC	48 hrs. 96 hrs
		,,	6.0 mC	48 hrs. 96 hrs.
AK	Akola, Bombay	,,	1.5 mC	48 hrs. 96 hrs.
		,,	3.0 mC	48 hrs. 96 hrs.
T.M.V.	Tindivanam, Madras	,,	2.5 mC	48 hrs 96 hrs.
Coimbatore Kernel	Coimbatore (Local)	Beta -rays (from S^{35})	3.0 mC	48 hrs. 96 hrs.
			6.0 mC	48 hrs. 96 hrs.
Improved Small Japan	Coimbatore	,,	3.0 mC	48 hrs. 96 hrs.
			6.0 mC	48 hrs. 96 hrs.

C.4.6.2. *Experimental results.* From the seeds thus treated, the percentage of germination, the survival and growth of the progenies were studied. Interesting variants from the fundamental view point have been isolated, of which special mention may be made of a 'sectorial chimaera', in the X-ray treated *T.M.V.* 3 and two plants with red-stem, isolated from the S^{35} treated *Coimbatore Kernel* variety. Besides these, plants with increased vegetative activity and/or higher yield have also been isolated, which will be studied through the subsequent generations.

Studies on the yield of the treated progeny, seed and pollen sterility, have also been made and the data are under statistical analysis.

Radio-autographic study of the seeds treated with P^{32} for 48 hrs. showed that the maximum accumulation of the isotope takes place in the plumule region of the embryo. Detailed investigation on the distribution of isotope at the different stages of growth of seedlings with the aid of radio-autographic technique, is proposed to be undertaken.

Cytological investigations on the nature and frequency of chromosomal abnormalities in roots and pollen mother cells are in progress, to find out whether there exists any dose-response relationship and how far the cytological abnormalities are responsible for the pollen sterility.

C.4.7. *Radiation mutation in Sunn-hemp (Crotalaria juncea)*

The work started on radiation mutations in *Crotalaria juncea* during the 1956-57 season, was continued for the second year during the year under report.

C.4.7.1. *Treatments.* Pure line strains of *C. juncea* were exposed to 5,000, 10,000 and 15,000 r doses of X-rays. Some seeds were also exposed to beta radiation, by treating them with two initial activities of P^{32}, viz. 1 mC and 3mC respectively.

C.4.7.2. *Results.* The percentage of germination and the survival of the progeny have been investigated. A large number of morphological abnormalities like heavy lateral and base-branching, fasciation of the stem, stages in the bifurcation of the leaves, sectorial chimaeras and floral abnormalities, have been recorded.

The data with regard to the height, basal diameter and the maximum number of basal branches, have been collected and are being analysed, for the test of significance.

A few plants which grew abnormally tall in the X_1 generation and a single plant with crinkled leaves have also been isolated for studying their behaviour in the subsequent generations.

The seeds collected from the P_1 and S_1 progenies of 1956-57, were bulked and sown in the field for the second generation investigations. A very large number of morphological abnormalities like tri- and tera cotyledony, schizocotyledony, supression of the apical bud, polyembryony, leaf and stem abnormalities have been recorded. A few other morphological variants have been isolated and it is proposed to carry them over to the next generation to study the inheritance of some of these characters.

C. 5. Chemical mutagenesis

C.5.1. *Effects of certain hormonal herbicides on the meiosis of Crotolaria juncea*

A part of the work on the investigations of the responses of plant chromosomes to the treatment with hormone type of selective herbicides started in 1956-57, has been completed during the year under report.

C.5.1.a. *Experiments.* These investigations were conducted by injecting four different herbicidal products viz.: amine and butyl ester of 2,4-D, M.C.P.A. and2, 4-5T, by means of a hypodermal syringe in the inflorescence axis of *C. juncea.* Three different concentrations of each of the chemicals namely, 250, 500 and 750 p.p.m. have been used and their effects recorded in terms of metaphasic and anaphasic abnormalities. In the inflorescences treated with 1,000 and 2,000 p.p.m. concentration of the chemicals, the buds dried before the completion of the meiosis and hence the meiotic abnormalities could not be studied in these. It has been found that within the range of the treatment, the effect of the chemicals is proportional to the concentration.

C.5.1.b. *Result.* The relative effectiveness of the four chemicals has been compared and it has been found that M.C.P.A. is most effective on *C. juncea* and 2, 4, 5T, the least.

A large number of meiotic abnormalities like bridges, fragments, stickiness of bivalents, destruction of the spindle, laggards and extrusion etc, have been recorded. A rare instance of a bridge persisting from first to the second anaphase has also been recorded.

The present observations suggest the possibility of exploiting the property of these chemicals to disturb the normal chromosome cycle, in checking the seed setting in the weeds if the time of flowering of weeds does not coincide with that of the crop.

C.5.2. *Cytological responses of plants to Aminotriazole*

Aminotriazole or 3-AT is one of the recent herbicides. In the course of investigations, it has been found that following treatment with this chemical, the growth of the plants is partly or wholly stopped. The present investigations were, therefore, undertaken to study the effect of such a treatment on the cytology of plants.

C.5.2.a. *Experiments.* Three day old seedlings of *Pisum sativum* were treated with 100, 200, 1,000 and 5,000 p.p.m. for 2 durations of 2 and 24 hours in each concentration of aqueous solutions of Aminotriazole. Some of the seedlings were recovered 24 and 48 hours respectively, in running water.

C.5.2.b. *Result.* The frequency of cell divisions and the different stages, as well as the frequency of abnormalities in these root tips have been investigated. It has been found that in very high concentrations, aminotriazole acts as a mitostatic substance. Besides, it also delays the time of mitosis.

A large number of cytological abnormalities like bridges, sticky chromosomes, late separation etc, have been recorded. However, the spindle mechanism has not been found to be disturbed due to the treatment with aminotriazole, which shows that it is not a spindle poison and therefore differs in this fundamental property from the 2, 4-D group of chemicals.

Recovery in running water does not seem to have restored the mitotic activity of the plant. Thus, it has been suggested that one of the main factors of its phytoxocity is the stoppage of growth due to mitostatic action.

C. 6. Modifying factors in induced mutagenesis

C.6.1. *Investigation on the physiological factors on induced mutagenesis in paddy*

The work on the physiological factors in induced mutagenesis was started in 1956, to find out the influence of the various factors like varying levels of moisture content, active and inert gases like oxygen, nitrogen and carbon dioxide, on the frequency of mutations and/or cytological and morphological abnormalities.

C.6.1.a. *Previous findings.* During the previous year, the relationship between the different percentages of moisture content of the seeds and varying doses of X-rays were investigated in paddy. From this study it was concluded that the effect of various doses of X-ray is not directly proportional to the doses, but that there is an optimum dose for the maximum effect. But the various degrees of moisture content have been found to vary the effects of each dose of irradiation.

C.6.1.b. *Experiments.* During the year under report, the work has been extended to the study of the modifying effects of the various gases on the action of X-rays.

The treatments were made in a specially designed container so as to enable the exposure of the materials to X-rays in an atmosphere of different gases, in one of which, the irradiation was given just after the gases were introduced and in the other after allowing the seeds to remain for 24 hours. From the seeds thus treated, the germination, vegetative activity, meiotic and mitotic abberrations and pollen sterility were studied.

C.6.1.c. *Germination and survival.* The germination percentage of the various treatments and the control, differed to a certain extent. When compared to the control, it was found that there is an increase of 6% in survival for 2,500 r and 5,000 r treatments, irrespective of the gases and the duration of exposure. In the 7,500 r and 10,000 r treatments, the survival percentage was lower than the previous ones, but slightly higher than in the 'control'. Among the gases such as oxygen, nitrogen and air, the highest survival was recorded in those treated with oxygen. While considering the effect of the various durations of exposure to gases, it has been found that the percentage of mortality is lower in case of shorter duration of the treatment.

C.6.1.d. *Growth.* Weekly record of the height of the plants was taken in different treatments. From the statistical analysis of the data it was found that the mean height of plants X-radiated in air is significantly lower than that of the plants grown from the seeds irradiated in either nitrogen or oxygen. The differences in the heights of the plants obtained from the seeds irradiated in nitrogen or oxygen are not very different. Besides, the variation in the heights due to different doses of irradiation, is statistically significant.

Again, the interactions between doses and gases (including air) has been found to be significant in the different types of gases.

C.6.1.e. *Pollen grains.* Similarly, the fertility of pollen in the different treatments was studied. It has been found that the fertility of pollen due to different treatments is highly significant in each of the gases.

Broadly, there is a regular decrease in the 5,000 r treatment with increasing duration of exposure and a gradual increase in most other cases.

C.6.1.f. *Yield.* The yield of paddy per plant in each treatment was also subjected to statistical analysis. The increase and decrease in yield due to various doses of irradiation show an uniform pattern in the two gases namely, nitrogen and oxygen. The yield decreases slowly up to the 5,000 r treatment, after which its becomes irregular. From the present observation it appears that among all the gases, oxygen gives the highest yield. However, for the different doses, the yield does not follow a definite pattern.

Considering all the factors together, it seems that among all the gases, oxygen has the maximum effect on the growth, pollen fertility and yield of paddy. The effect of air and nitrogen is lower than that of oxygen. This suggests that active gases like O_2 have a tendency to increase the variations in plants, whether physiological or genetical, as compared to the inert gases like nitrogen. The performance of air, being the mixture of both oxygen and inert gases, occupies the middle position.

Generally, a shorter duration of exposure to the gases brings about less variation than a longer duration. But the relation of different doses under the same or different gases, does not follow any specified pattern.

C.6.1.g. *Cytological studies.* Besides the above investigations, studies on the mitotic and meiotic abnormalities induced as a result of gases and /or irradiation, were also undertaken for each treatment.

a) *Mitosis.* For mitotic study, root tips were fixed in acidified alcohol and sqashed in iron—acetocarmine. Most of the study was done from squash preparations. A number of abnormalities were recorded both at anaphase and metaphase. The anaphasic abnormalities included bridges and/or fragments in the same cell, laggards and failure of spindle formation. There were a number of instances with more than one bridge in the same cell.

A rare occurrence of two bridges interlocked at the equatorial region was observed at anaphase in one treatment with 10,000 r X-rays, after exposing the seeds to oxygen for 24 hours.

The metaphasic abnormalities included fragments, non-congression and orientation of the chromosomes, variation in the diploid number, tandom satellites and occurrence of looped chromosomes, most of which have also been observed in the previous experiments with varying degrees of moisture content.

b) *Meiosis.* Meiotic studies were done from the temporary squash preparations made in iron aceto-carmine.

The main abnormalities were found mostly in the first division. They included variation in the number of bivalents, failure of pairing resulting in univalents, unequal size of the two chromosomes of the same bivalent, multivalent formation and interbivalent connections at the metaphase stage. At the anaphase stage, a number of abnormalities like bridges, laggards, unequal distribution of chromosomes at the poles etc. were observed.

C.6.1.h. *Morphological abnormalities.*

There were a number of chlorophyll mutants, which did not survive. Five dwarf mutants were observed in X-ray treated plants in air and oxygen. The subsequent behaviour of these mutants in the next generation is being studied.

C. 7. Cytological and cytotaxonomic studies

C.7.1. *Cytotaxonomic position of Crotalaria Brownei, Bert.* ex. DC.

The cytological and cytotaxonomical studies in genus *Crotalaria* have been continued during the year under report. Critical karyotype investigations on two species, viz. *C. saltiana* Andr. Bot. Rep. and C. *Brownei*, Bert. ex. DC. have been made and it has been found although their somatic chromosome number is the same, they differ from each other in the morphology, the length of the chromosomes and the number of medianly and submedianly constricted chromosomes.

On the basis of these observations, it has been concluded that *C. Brownei* Bert. ex. DC should be recognised as a separate species and should not be merged with *C. saltiana*, as suggested by the taxonomists (Ref. Index Kewensis).

The chromosome number of *C. Brownei* ($2n = 16$) has been reported for the first time. Two pairs of chromosomes have been found to be satellited, which produce nucleoli.

C.7.2. *Cytotaxonomic studies in the species and varieties of cotton (Gossypium sps).*

The present investigations on the somatic chromosomes of the species and varieties of cotton were started with the aim of finding out the inter relationship of the species and origin of cultivated types, on the basis of the chromosome morphology. The following species and varieties have been taken up for the preliminary investigations.

1. *G. braziliense* Macf. (or *G. barbadense* L. var. *braziliense* H. & G.). (Tree Cotton).
2. *G. arborium* L. var. *Sanguineum*. Hassk.

G. braziliense :

The 2n chromosome number of this species is 52. The somatic complements is composed of 2 pairs of satellited and one pair of secondarily constricted chromosomes. The nucleolar study, using Feulgen—Fast Green technique was undertaken to investigate the nature of the satellited and secondarily constricted chromosomes. It has been found that there are six small nucleoli at the early prophase, suggesting that all the three pairs are nucleolar in nature.

A large number of cytological abnormalities like bridges, laggards, hyper and hypo-polyploid cells etc. have been recorded.

G. Sanguineum

This species has a 2n complement of 26 chromosomes, of which 2 pairs are satellited. There is a difference in the length of SAT-stalk and the size of knobs between the two pairs. The prophase study has revealed the presence of 4 small nucleoli showing that 4 satellited chromosomes are also nucleolar in nature. No cytological abnormalities have been recorded in this species.

Further investigations on the karyotypes of *G. hirsutum* L. var. *Andrews* and other varieties of *G. arboreum* like *K-I*, and 7036 etc., are in progress.

C. 8. Plant Anatomy

C.8.1. *Anatomical studies of fibres in jute*

It was reported earlier that considerable differences existed in the number of ultimate fibre cells in three varieties of *Corchorus olitorius* L., namely *Chinsura Green*, *R.* 26 and *Tall Mutant*, the last one being a higher yielding X-ray mutant of R-26.

During the year under report, anatomical studies of ultimate fibre cells have been made in some unusually thick plants of *D*-154 and *D*-386, belonging to *capsularis*. These plants were observed both in the control and in the P^{32} treated population. Onc ounting the ultimate fibre cells it was found that the thicker plants had more fibre cells than the normal plants in the ratio of 2.2 : 1. The yield of fibre and wood of these plants was also found to be more in the ratio of 2.0 : 1 and 2.5 : 1 respectively, to the normal plants.

C.8.2. *Anatomical studies of leaf teratology*

C.8.2.a. *Helianthus* sp. Terminal stalked ascidium (cup) and forked leaf have been found to occur abnormally in this species. The phyllotaxy is spiral, but in some instances, two or three leaves arise abnormally from the same node. This abnormal phyllotaxy is noted in two or more consecutive nodes at the lower region of some plants. Fasciation of stem was also observed.

The stalked ascidium originates from the terminal node, which is more swollen than the parts lying below and above this node. As seen in serial transverse sections, the vascular bundles are arranged here broadly in a ring. Gradually towards the tip, the ascidial stalk is found to enclose the terminal bud of the axis. Later, the terminal bud becomes completely separated from the ascidial stalk, but remains engulfed in a narrow cavity, which is surrounded by the ascidial stalk. The engulfed bud ends a little above the base of the ascidial stalk, the remaining longitudinal part of which encloses a narrow central cavity. This cavity widens upwards and forms the inner cavity of the cup above. The vascular bundles, by gradual orientation, form ultimately the two mid-ribs of the cup. In some rare cases, one or two small stalked leaves are found to grow from the engulfed bud through the central cavity and such leaves are therefore, always surrounded by the basal region of the ascidium. Morphologically and anatomically, the cup is an epiascidium.

C.8.2.b. *Salvia* sp. Terminal stalked ascidia and forked leaves have also been found in the garden plant *Salvia* sp. This plant shows opposite and decussate phyllotaxy. Very rarely, some leaves of this plant are abnormally forked and the forking is mostly found in one leaf, but the opposite leaf from the same node remains normal.

Detailed morphological and anatomical studies of the above materials are in progress.

C.9. Physiological anatomy and changes induced by herbicides

During the year under report, investigations were made on (1) physiological anatomy of some common species of *Cyperus* of West Bengal, with reference to their ecology and (2) the selective action of herbicides.

C.9.1. *Studies on physiological anatomy*

The following twelve species of *Cyperus* have been investigated. *C. malaccensis* Lam, *C. procerus* Rottb., *C. elatus* L., *C. aristatus* Rottb., *C. mitis* Steud, *C. rotundus* L., *C. cyperinus* Suringar, *C. tenuispica* Steud, *C. platystylis* Br., *C. exaltatus* Retz, *C. imbricatus* Retz and *C. corymbosus* var *Longispiculatus* Kuntz. During these investigations the following points of general interest, have been noticed.

(a) *Stellate parenchyma*

It was present in the leaves and inflorescence axes of a number of species under study. It has been found that in the early developmental stages of the tissue, the cells are smaller with inconspicuous intercellular spaces. As the particular organ of the plant grows, the growth of this tissue does not keep space with the surrounding tissues and consequently, they are slightly pulled out from all directions, resulting in the formation of long arms.

(b) *Wavy wall of the epidermis*

Epidermal cells with radial walls showing a wavy contour is a common feature in the leaves of *Cyperus* and an attempt was made to study the cause of this undulation. It has been

found that the cells are straight-sided when young and as the leaf grows to attain full dia-mensions, the walls become gradually undulated. Cutinization seems to play a large part in this phenomenon, as it was found that they are taking place at the time of cutinization and that the cavities of undulated cells are roofed with patches of cuticle.

(c) *Bulliform cells.*

Presence of bulliform cells in the leaves, is another common feature in all the species investigated. Considerable variation exists in the plant kingdom with regard to their origin and distribution. In *Cyperus*, bulliform cells are modified epidermal cells, occurring on the upper surface of the leaves along the mid-vein. It has been found that in *Cyperus*, the unfolding of the leaves is by the timely and simultaneous development of these cells, which have only the same size of other epidermal cells before unfolding begins.

(d) *Diaphragms :*

Perforated diaphragms, one to several layers thick, that cross the air passages at intervals were noticed in aerial shoot of many of the species investigated. No appreciable difference could be noticed as to the presence or structure of diaphragms between the immersed and aerial parts. Experiments were also conducted to study the nature of diaphragms in the same species grown in water and under dry conditions, which also showed no apparent change in their presence or structure. Diaphragms in *Cyperus* act as stiffening plates and keep the plant from flooding the air spaces. Since diaphragms with and without cross-bundles were observed, the view that they serve as supporting structures of cross bundles, cannot be taken as a function of all diaphragms.

(e) *Mechanical tissue*

The mechanical tissue is distributed in the various parts of the plant in a manner to bring about a high degree to functional efficiency. One of the most difficult aspects of this subject is the problem as to how it happens in the initial differentiation of tissues, before mechanical problem arises, as the mechanically effective tissues are laid down in such a manner that they can meet the mechanical needs of the adult plant with a high degree of efficiency. A study of different species of *Cyperus* in different habitats shows that the distribution of mechanical tissues is fairly constant within the species. It has been concluded that the question of heredity cannot be ruled out in this respect, though in the quantitative development, the forces to which they are subject to, might play a prominent role.

(f) *Vascular bundles :*

The vascular bundles of the genus not only show decided variation in their general form, but a given type of bundle also exhibits variation from amphivasal to collateral or from collateral to amphivasal, with intermediate stages at certain regions of the plant. A possible line of specialization of the bundles has been drawn in which the collateral form whose xylem is composed of tracheids, forms the most primitive and amphiviasal bundles with vessels in the xylem forming the most specialized ones.

(g) *Vascular system of roots :*

Roots of *Cyperus* provide an excellent material to show the size-structure correlation in the vascular system. It has been found that the number of xylem points increases with

increase in size of the root stele, keeping the ratio fairly constant in each species. It has been concluded that the number of xylem points depends upon the space available for development, as it has been found that larger the meristem, the larger is the root stele and also number of xylem points.

(h) *Air space :*

Air spaces varying from small intercellular spaces to large air chambers are of wide spread occurrence in the aerial and under-ground regions of *Cyperus*. The results of the present investigations show that stretching and consequent collapse are involved in the formation of large cavities in the aerial parts of the plant, while starvation and lack of oxygen seem to be the two factors invoived in the formation of cavities in the roots. In *Cyperus* inhabiting in water and whose photosynthetic tissue is well above the water level, a transference of oxygen from the outer air through the lacuna may be expected during darkness.

C.9.2. *Selective action of herbicides*

An approach is being made to explain anatomically the difference in the degree of susceptibility of plants to the hormone type of herbicides. This is in continuation of the work done during the last year, when certain anatomical responses of *Croton sparsiflorus* Morung to 2, 4-D were reported. Work was further carried out in *Ludwigia parviflora* Roxb. *Croton sparsiflorus*, *Cyperus rotundus*, and *Imperata arundinacca* Cyrill and the chemicals used were, amine 2,4-D, M.C.P.A. and Weedone 2, 4-5T. From the study so far made, the following conclusions have been arrived at.

(1) Concentrations of different herbicides which are lethal to *Ludwigia* did not produce much appreciable changes in *Croton* and *Cyperus* and that concentrations which were even herbicidal to *Croton* promoted the growth of *Imperata*.

(2) It has been found that greater the degree of formative disturbance induced by a substance, the more effective is the substance as a herbicidal agent and that the susceptibility of a plant to a chemical can be judged from the degree of cell proliferation induced by it.

(3) Death of a plant is brought about mainly by the destruction of the phloem. Pholem may be destroyed by the crushing of the tissue, consequent to meristematic activity of the surrounding tissues or by the exposure of the phloem to the driect action of chemical after the cortex has been destroyed. Thus the easily susceptible nature of the dicot plants in general may be ascribed to the peripheral position of the phloem and the presence of the meristematic tissues or tissues which can easily become meristematic.

(4) The more resistant nature of monocots seems to be due to the scattered arrangement of vascular bundles and the absence of meristematic tissues. The nature of cell wall and distribution of stomata are also responsible. The presence at the apical meristem of the growing shoot of strong scales, is a safeguard to the growing point from the action of herbicides in the case of *Imperata*.

C. 10. Weed Control

This is a research project financed by the Indian Council of Agricultural Research, for the eradication of weeds by the application of hormone type of selective herbicides and is in the fifth year of investigation, during the year under report.

Investigations were conducted earlier to find out the most appropriate conditions of application of herbicides viz., morning, evening, sunny, rainy and cloudy conditions etc. Investigations on the retention of toxicity of the various herbicides in the soil and the extent of drift hazards through various agencies in some crops were reported. This year, more detailed investigations on the flowering aspects were undertaken.

(1) Investigations on the most appropriate stage of application.

(2) The economics of chemical weed control.

C.10.1. *The most appropriate stage for the application of the herbicides*

Detailed experiments were conducted to find out the most suitable stage and time of application in suitably planned lay-outs.

As paddy is the main crop of West Bengal, experiments were conducted on transplanted 'Aman' paddy. The following four chemicals viz: Amine, Ester, Sodium Salt of 2, 4-D and M.C.P.B. were used in 1,000, 2,500 and 5,000 p.p.m. concentrations of the active ingredient. The applications were made at early, (about 6 weeks after germination), pre-flowering, (11 weeks after germination) and flowering, (12-13 weeks after germination) stages, at an average application rate of about 80 gallons/acre. The data with regard to the maximum height of the plants and the percentage of pollen sterility were taken from 10 plants selected at random from each of the sub-plots. Besides, the mean yield for each one of the treatments at the different stages of growth was also taken. All the data were subjected to statistical analysis. It has been found that all the chemicals have a definite effect on the paddy. Considering the age of the treatment and growth of the plant the most deleterious effect of the herbicidal treatments on paddy, is at its early stage of growth, less at the preflowering and least at the post-flowering stage. It has been further found that the height of paddy when treated at the early stage, is significantly less than the other two stages. On the contrary, when the yield of the plants is taken into account, it has been found that the maximum loss in yield takes place when the treatment is made at the pre-flowering stage and that this loss in yield is statistically significant.

A number of ear abnormalities have been recorded in case of plants treated at the pre-flowering stage. The abnormalities include the supperssion and arrest of the ear, epinasty of the ear, irregular development of seeds, stiffening of the flag leaf, etc.

On the basis of these observations, it has been suggested that the most appropriate stage for the application of herbicides in paddy is the early stage of its growth. However, if the difference in the time of the flowering of the crop and the associated weeds is remarkable, herbicides can as well be applied at the pre-flowering stage of the weed.This will lower the seed setting in the weeds and thereby bring about an effective control during the subsequent seasons.

C.10.2. *The cost of treatment in relation to yield*

The second line of work, namely. the economic aspect of chemical weed control, was also taken up during the year under report. Planned experiments according to "randomized block lay-out" were conducted in the "Aus" variety of paddy with the following four chemicals :

a) Na-salt of 2, 4-D

d) Na-2, 4-Dichlorophenoxy ethyl sulphate.

The treatments were made at the rates determined on the basis of the requirement of the associated weeds, as ascertained during the previous years: The data with regard to height and yield of 10 plants selected at random from each sup-plot were taken. The total yield of each sub-plot was also separately recorded. These figures were compared with the corresponding figures for the two check (control) plots, one of which was weeded six times during the season and the other, not weeded at all.

When considering the yield, it has been found that the highest yield was obtained in the hand-weeded plots, not only because of the least competition with the weeds, but also due to cultural operations which accompany normal weeding. The next highest yield was obtained in plots treated Na-2, 4-D-ethyl sulphate. On statistical analysis it has been found that the differences in yield between hand weeded and the other plots are significant.

Similarly, the cost of weeding involved both in the chemical and conventional methods was also compared and it has been found to be highest in the latter. On comparing the cost and yield relationship in different treatments, it has been concluded that the use of Na-2, 4-D ethyl sulphate is most economic of all the chemicals.

The tillering performance of the plants in different treatments was also compared and it has been found that the high yield of the hand-weeded plots is the function of greater tillering of plants in these plots.

D. MICROBIOLOGY

D. 1. Production of antibiotic substances by microorganisms

1. *Production of a broad-spectrum antibiotic from Streptomyces spp.* Ac_{358}.

The streptomyces strain was isolated from the soil and compost collected at the India Botanical Gardens, Shibpur, West Bengal. This strain was studied in detail on the consideration of its remarkable antibiotic activity.

Several media were used for the production of antibiotics by Ac_{358}. Of them nutrient broth supplemented with Czapek Dox media was most effective as both antibacterial and antifungal activity were noticeable in the antibiotic spectrum. Addition of 0.2% malt extract to all media as nutrient supplement resulted in promoting growth and antibiotic production as well. The cultures grown here were all by the stationary flask culture method and the time taken to attain maximum titre was 7 days. In preliminary experiments the 7 test organisms were used and the results are given below in table 1.

TABLE 1

ASSAY OF ANTIBIOTIC —Ac_{358} PRODUCED BY STATIONARY FLASK METHOD

Test organism	Diameter of the Zone in mm culture filtrate of Ac_{358}
1) E. coil	30
2) V. cholerae	30
3) Staph. aureus	28
4) Eb. typhosa	32
5) Fusarium vasinfectum	20
6) Alternaria solani	10 ?
7) Asp. niger	10
8) Curvularia sp.	20

? Doubtful results

Antibiotic production by Shake flask culture method

A reciprocating type flask shaker machine was designed and made in the Bose Institute. The whole assembly can be operated continuously for 4-5 days with 24 (250 or 500 cc capacity) conical flasks which is the maximum load. In the following experiments (i) the period of fermentation was 4 days, (ii) temperature was maintained at 25°-26°C and (iii) the medium used was nutrient broth plus C-D with 0.2% malt extract. The results are given in table 2 which shows that the fermentation improved significantly. There was a definite rise in the antibiotic titre in shake flask cultures. The results obtained here are given in table 2.

TABLE 2

ASSAY OF ANTIBIOTIC Ac_{358} PRODUCED BY SHAKE FLASK METHOD

Test organisms	Dia. of the Zone of inhibition in mm-culture filtrate of Ac_{358}
1) E.coli	35
2) V. cholerae	30
3) Staph aureus	35
4) Eb. typhosa	38
5) F.vasinfectum	20
6) Alt. solani	10?
7) Asp. niger	20
8) Curvularia sp.	20

? Doubtful results .. ..

On comparing the results of assay given in table 1 & 2 it will be seen that time required for complete fermentation was less by 3 days and titres of antibiotic increased definitely at least in 4 cases in the shake flask cultures.

Purification of the active principle elaborated by Ac_{358}

The fermentation of the antbiotic was done by submerged method and the fermented broth was harvested after 4 days. The mycelium was separated by filtration and adsorbed on activated charcoal using SB(X) grade. The filtrate was shaken with 20% charcoal at 25-26° C for 4 hours and filtered. The filtrate was tested for residual antibiotic potency and was found to contain no antibiotic substances showing that adsorption of the antibiotic material on charcoal was complete.

The elution of the active material from charcoal with organic solvents,—butanol, butyl acetate, ethanol, ether and methanol was practically ineffective. The next step was to treat the charcoal with 0.1 N acetic acid before elution with butanol (saturated with water). Following this procedure the adsorbed active substances came into butanol during elution. The butanol was evaporated and an aqueous solution of the antibiotic was finally prepared and assayed. The recovery of the active material was approximately 75%. Other experiments were done in which the fermented broth was shaken with all the above organic solvents and it was observed that except with butanol the partition did not occur in the other solvent phase. The partition here, however, was incomplete. The results are given in table 3.

TABLE 3

COMPARATIVE ASSAY OF THE ANTIBIOTIC Ac_{358}

Test organisms	Untreated culture filtrate	Butanol Ext.*
1. Staph. aureus	20	10
2. V. cholereae	15	10
3. E. coli	15	10
4. Eb. typhosa	25	17

* The butanol was removed by evaporation and an aqueous solution was made for assay.

2. *Studies on the effect of bacterial antibiotics on soil microorganisms*

The effect of four known bacterial antibiotics e.g. bacitracin subtilin, polymyxin B, and circulin along with the antibiotic substances of two *Bacillus subtilis* strains on the changes in the composition of soil microflora and the stability of the substances in soil have been studied. The fungi, actinomycetes and bacteria were affected depending on the nature of the antibiotic and the period of incubation. Bacitracin could be recovered from soil in fractions after 48 hours of contact and polymyxin B and circulin after 96 hours while subtilin remained firmly adsorbed in soil shortly after treatment. The partially purified antibiotic produced by the *B. subtilis* strain (B_7) in the Laboratory was added to the soil. There was 20% recovery of the active substance after 8 days. The antibiotic preparation of another strain of *B. subtilis* (B_{344}) was similarly added to the soil, and it was adsorbed less firmly than the product of (B_7). However, significant amounts of the antibiotic could be recovered after 8 days of treatment. In all cases the antibiotics disappeared more rapidly in non-sterile soils than in soils sterilised by heat-treatment.

Studies on the antibiotics produced by the soil bacteria —B 393

During the course of a survey of the antiobiotic-producing bacteria occurring in Indian soils, a pigment producing bacteria-B 393 with marked antibiotic activity against both gram-positive and gram-negative bacteria was isolated. The crude preparation of the active principles could be obtained by extracting the culture medium with an equal volume of chloroform and evaporating the active substance to dryness. The active principles could be further resolved by chromatography of the alcoholic solution of the residue on Brockmann's alumina. The chromatogram was then developed with 100 ml. benzene followed by 50 ml each of 10% and 20% acetic acid in benzene. At least three pigments violet, blue, and yellow—were obtained each characterised by antibiotic properties against *Staph. aureus*.

3. *Production of Streptomycin inhibitory substances by Polyporus ostreiformis.*

It was reported that some antibiotic-inhibiting substances could be isolated from a bacterial strain—*Bacillus pyocyanus*. Similar investigations were done here with a number of higher fungi. They were grown on 2% malt extract solution for 10 days after which the culture filtrates were assayed to test their antibiotic inhibitory property against dihydrostreptomycin sulphate.

Agar cup method of antibiotic assay was followed usually except in that the medium inhibitory concentration of dihydrostreptomycin sulphate was added in every case to the nutrient agar seeded with *Staphylococcus aureus*. Because of the addition of the above antibiotic the growth of the seeded bacteria on agar plates was checked. The culture solution of *P. ostreiformis* was put into the agar-cup of plates seeded with *Staph. aureus* and a zone of bacterial growth surrounding the agar-cup is formed. This is the so-called "Exhibition Zone" which is just the reverse of "Inhibition Zone".

In addition, two other cultures of higher fungi were tested for their antibiotic-inhibitory property described as above. (*Daedalea flavida*, and *Polystictus hirsutus*.) Of these, only the metabolic filtrate of *P. ostreiformis* was found to possess remarkable power of inhibiting the antibiotic property of dihydrostreptomycin sulphate.

A peculiar feature of the metabolic filtrate of *P. ostreiformis* (grown on 2% malt extract solution) was that it was acidic (*p*H 2.0–2.5). When the *p*H was gradually raised by addition of $NaHCO_3$ solution the power of inhibition of the metabolic filtrate was gradually reduced and at 7.0 the antibiotic inhibitory property completely disappeared. The *p*H reversed (with the addition of dil. HCl) the antibiotic-inhibitory activity of the metabolic filtrate was again revived.

It has been observed earlier that the acidity of metabolic filtrates of some *Polyporus* spp., contain organic acids. The antibiotic inhibitory tests were, therefore, done with conc. solutions of pure organic acids and similar "exhibition zones" were observed round the agar-cup described above. Several preliminary chemical tests were done to find the nature of the organic acids which might be present in the metabolic filtrates of *P. ostreiformis*. The experimental results show presence of certain amount of oxalic acid in the culture filtrate. Further work is now on progress.

D. 2. Techniques of UV irradiation

1. *Dose measurement of UV irradiation*

The microorganisms being irradiated with ultraviolet ray of various wave lengths, a germicidal-action curve may be plotted. The action of specific wavelengths on specific microorganisms was studied and the optimum killing wave length for microorganisms was found at 2650 Å. However, in the low pressure (not exceeding 10^{-4} atmosphere) UV lamp, the mercury arc is converted directly into radiation of wavelength 2537 Å and at this wavelength 85% of the maximum germicidal effect on most microorganisms is produced which is possible at 2650Å. The dosing of ultraviolet radiation was a difficult problem for a long time due to the diversity of radiation of ordinary UV lamps. The lamp used by ourselves was a low pressure 4 watts G.E. lamp. In this lamp the rays emitted are practically monochromatic (85%) and measurement of dose is simplified by eliminating undesirable radiation.

The Dosimeter used in the experiments was made by the Institute Du Radium of Paris under the guidance of Prof. R. Latarjet. The photocell sufficiently sensitive to visible radiation is made of selenium along with a particular fluorescent substance. The electrical response is measured by a portable apparatus (microamperemeter). Here the dose is measured under suitable conditions of light and temperature . It is stable in action and its response is sensitive in function, linear to the flux it receives. We have been working out the doses given from a standard curve prepared in Dr. Latarjet's Laboratory. A diaphragm is provided with on the top of the cell for reducing the intensity of the radiation by 1/5th as it was necessary sometimes to vary the doses of UV radiation. Dose of ultraviolet is measured in units-ergs/mm²/sec which is plotted on abscissa against microampere reading on the ordinate.

2. *Effect of pre-incubation on the mutation frequency of* UV *irradiated Asp. niger spore suspension*

In the following experiments the spore suspension of *Asp. niger* was incubated at 29°C ± 1° for 0,2,4 and 6 hours. Irradiation was given according to the scheduled dose (100

ergs/mm²/sec) and dilution plates were made. It was observed that treatment for 4 hours at 29°C showed the maximum frequency of mutation. The various factors known to have modifying effect on the mutagenic frequency of UV irradiation, were pre-and post-incubation at low and high temperatures. Further experiments on this aspect are now being continued.

3. *Effect of pH on the mutagenic effect of UV irradiation*

The treatment of the spore suspension of *A. niger* at various *p*H levels (2-8) showed that the rate of killing reached a peak at *p*H 8.0 while at lower levels there was no significant effect. Control experiments showed that alkaline condition (*p*H 8.0) of the spore suspension did not affect the survival rate.

4. *Effect of temperature on UV irradiation*

The change of frequency of mutation is dependent on several factors. The effect of temperature was, therefore, studied and the irradiation experiments were done at 15, 20, 25, 30 and 35°C. It was observed that the frequency increased with the rise of temperature. The dose level is kept constant in all cases of treatment and at 35°C the highest mutation frequency was attained.

5. *Effect of photo reactivation on irradiated spore suspension.*

The effects UV radiation on microorganisms is known to have been counteracted if the irradiated organisms, under proper conditions, are exposed to rays of longer wave length upto 4500Å units. It was found that the greatest effect occurs for the wave length between 3500 and 4500 Å units. The reversal of the mutagenic effect of UV radiation on *Aspergillus niger* was studied in the following experiments. The experiments were conducted in direct sunlight, diffused light in the room and with a 20-watts Phillips Daylight lamp for 30 minutes in each case. The number of survivors increased as a result of exposure to light and mutation percentage decreased significantly for direct sunlight.

D. 3. Studies on biochemical mutants of A. niger

Aspergillus niger strain V_{35} and its colour mutants U_3 An 21 and C51 were selected as materials for study. A 4 watts germicidal lamp, (General Electric U.S.A.) emitting 85% of the radiation at 2537Å was used. The intensity of UV radiation was measured with a dosimeter of selenium photo cell. Most of the experiments were done at a dose 100 ergs/mm²/sec for 3–24 minutes. Experiments were done on standardising doses of UV with a dosimeter elsewhere. The irradiated spore suspension of *Asp. niger* was plated in complete medium which had the following composition : Casein hydrolysate 1.5 gm; yeast ext 1.0 gm; peptone 1.5 gm; glucose 20.0 gm; hydrolysed nucleic acid solution-3.0 cc; vitamin mixture solution—1.0cc—distilled water 1000 cc and *p*H adjusted to 6.0. In complete medium the majority of the nutritional factors are present and the mutants are therefore expected to grow in it without any sign of deficiency.

1. *Technique of isolation of biochemical mutants*

The isolation of mutants were done satisfactorily by the total isolation method of Fries*. The survivors of irradiated spores developing on complete medium after UV irradiation were picked up by a special type of V-shaped needle and the inoculum was transferred onto minimal agar medium. While inoculating the agar plates were placed on a squared paper each having an area of 4 cm². The inocula were carefully transferred to the corners of each sq and the plates were allowed to incubate at 30°C.

Those having nutritional deficiency usually fail to grow on minimal† media and therefore all non-growing colonies of probable mutants were picked up and transferred again to complete media for further studies.

2. *Characterisation of biochemical mutants*

When there is feeble or no growth of an inoculum on minimal medium it is presumed that mutation has occurred and the organism is unable to synthesise some of the vital cellular constituents essentially required for its growth. The nature of the deficiency is now determined by subculturing the probable mutants into the following media given in table 1 :

TABLE 1

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

The well-known auxanographic technique was adapted all through the above experiments and the following results were obtained. Out of a total of 4047 colonies picked from irradiated spores, finally 72 showed feeble or no growth in minimal medium. The definite proof of deficiency was demonstrated on further analysis with pure chemical constituents of amino acids, vitamins etc., in all cases except in two which were believed to have occurred

* Fries, N. (1948) Physiol. Planta. **1** : 330

† The medium contains all the mineral salts plus glucose which is essentially Czapeck Dox composition.

due to some unknown factors. In table 2 the results obtained showing mutation deficiency in biochemical groups are given.

Growth requirements	No. of strains isolated
(a) *Amino acids*	17
1. Arginine	2
2. Arginine/Proline	1
3. Arginine/Ornithine	1
*4. Arginine/Ornithine/Adenine/Hypoxanthine	1
5. Arginine/Proline/Ornithine	1
6. Arginine/Proline/Citrulline	2
7. Leucine	4
8. Methionine	5
9. Methionine/Cysteine/Cystine	5
10. Lysine	1
11. Isoleucine	1
12. Glycine	2
13. Histidine	1
14. Phenylalanine	
(b) *Vitamins and growth factors*	4
p-Amino benzoic acid	1
p-Amino benzoic acid/folic acid	5
Nicotinic acid	4
Pyridoxine HCl	2
Aneurin HCl	1
Biotin	2
Choline chloride	1
Inositol	
(c) *Nucleic acid constituents and unknown factors*	5
Adenine/Hypoxanthine	1
Cytosine	2
Unknown factors	

*An interesting case of biochemical mutant worthy of detailed study

D. 4. Industrial fermenter

1. *Standardisation of the* 10-*litre stainless steel Pilot Plant fermenter.*

The Pilot Plant fermenter was given a series of charges to standardise the working conditions of the apparatus. The whole assembly was sterilised with running steam under pressure generated by a medium-sized autoclave. In a single charge 6.5 liters of the medium was used. After the sterilisation of the medium was complete, cold water was circulated through the jacket and the medium was cooled down to 25°C. Air from the compressed air circuit was let on and all precautions were taken to ensure sterility of the broth during fermentation.

The medium was inoculated with an inoculum prepared under the submerged method. The inoculum was pooled in a sterile flask having a rubber stopper with an outlet tube of glass. The mycelial suspension was introduced into the fermenter vessel by connecting through a sterilised rubber tube. The temperature of the apparatus was recorded by a thermometer and was controlled by circulation of water through the jacket.

The fermentation was complete in most cases within 72 hours and samples were collected at intervals of 8 hours. The fermenter vessel is provided with an outlet tube the nozzle of which was kept warm all the time to prevent any possible contamination. At the end of fermentation, broth was harvested by increasing the pressure inside the vessel instantaneously.

D. 5. Biochemistry of Streptomyces Spp.

1. *Studies on the acid-base indicating properties of a new strain of Streptomyces spp.*—Ac_2(B).

A strain of *Streptomyces* spp. isolated from the soil of the Bose Institute Garden was found to have strong acid-base indicating properties. It did not exhibit antibiotic properties on perliminary screening. When inoculated in Czapek-Dox agar slants it produced pink-red colouration on agar which changed to blue with age. The mycelial colour of the culture also changed from white to pink and finally to faint blue.

The acid-base indicating properties of the pigment was rather strong. The colour of the pigment changed to pink red in acid and blue in alkali. The neutral region of *p*H, however, showed violet colouration. It has noticed that the pigment was produced more readily and diffused abundantly into the agar medium. An attempt was made to grow the culture of streptomyces Ac_2(B) in Czapeck-Dox liquid medium both by the stationary and shake culture methods at 25°C, *p*H-6.0, 6.4, 6.8 and 7.2. In shake cultures the growth and pigment production was very small while in stationary cultures violet pigment was produced at *p*H 6.8 and 7.2.

It was observed that hydrochloric acid (2N) when added to the harvested metabolic filtrate a pink-red mass precipitated out and the filtered precipitate was highly soluble in NaOH with the production of blue colour. The acid ppt. was soluble in alcohol with any change of colour. The experiments on isolation of the aforesaid pigment have been undertaken and effect of buffer on pigment synthesis is being carried out.

D. 6. Studies on Counter Current Distribution

1. *Role of Salting-out effect in countercurrent distribution*

Salting-out effect in countercurrent distribution was reported previously in the course of separation of a mixture of antibiotics obtained in our laboratory from the bacterial strain B_7 isolated in this laboratory. An exhaustive study of this interesting phenomenon was performed both from theoretical and experimental point of view.

The two immisicible phases constituting the solvent system in which a solute to be distributed in the countercurrent apparatus are made mutually saturated prior to the experiment, so that during actual operation only the distributing solute may be partitioned, rendering the solvent composition unaltered.

In actual practice, however, it is seen that the solute, in addition to being partitioned, affects the mutual solubility of the two solvent phases by a sort of salting out effect and this effect becomes more and more prominent as the concentration of the solute is increased. When this results in an appreciable change of composition of the solvent system accompanied with a change of volume ratio of the two phases, interesting results may follow, as has actually been found in our laboratory.

This interesting phenomenon has been studied in a series of countercurrent distribution of the dyes methylene blue (E. Merck) and neutral red (E. Merck) of various initial concentrations in a system of 1%HCl and butanol (E. Merck).

Change of relative volume of the solvent phases due to salting-out effect suggests a possibility of a component, having a negligible partition ratio or having no partition at all, of proceeding with the lower phase accompanied with the upper phase dur ng each transfer and giving a distribution band and thus somewhat defeating its separation from a component which is distributed by partition phenomenon. Distribution of an impurity associated with netural red was thus explained by salting-out effect.

As it is evident from the experimental results, in case of a solution, concentrated enough to result an appreciable amount of salting-out effect, the composition and the relative volume of the phases are changed during each transfer and the solute cannot be expected to distribute throughout the tubes with a constant partition ratio. Thus the countercurrent distribution band becomes somewhat 'skewed' as termed by Craig.

The salting-out effect phenomenon has another interesting feature. It is known that buffer of suitable *p*H in case of acids and bases and suitable carriers or salting in agents are successfully used in many cases to provide an optimum partition ratio. These carriers, though previously equilibrated with the system in absence of the solute to be distributed, have a possibility to be distributed between the system in presence of the solute whose concentration is not too low. Thus, apart from suffering change of partition ratio, there might be a possibility of error in gravimetric estimation of the distributed material by evaporating an aliquot from each of the tube, if only the weight of the residue from the same aliquot of the solvent system is substracted each time. But this effect is not practically detectable in case of dilute solution. Experimental results with different dyes and other systems corroborated the theoretical findings.

2. *An exact formula for the determination of partition coefficient in countercurrent distribution*

The primary problem facing the worker, who deals with the countercurrent distribution technique is to find out the exact value of the partition coefficient of the distributed material without the help of which the calculation for the theoretical distribution curve is quite impossible.

The partition coefficient as measured before the distribution is that of the inhomogeneous solute, and is consequently not usable in the theoretical determination. According to Craig, the material in the tubes near the peak of the experimental distribution curve, however, should be the most nearly homogeneous, except in the case of inhomogeneity with an identical or nearly identical partition coefficient.

Craig showed empirically the relation of the tube N where peak of the distribution band attains, with the partition coefficient of the material distributed, K and the number of transfers, n, as

$$N = \frac{nK}{K+1} \quad \text{[J. Biol. Chem. } \mathbf{155}\text{, 519 (1944); Ibid., } \mathbf{168}\text{, 687 (1947].} \quad \ldots \quad (1)$$

From equation (1) K is calculated for the pure substance, as

$$K = \frac{N}{n-N} \quad \ldots \quad (2)$$

This empirical formula is only an approximate one and becomes exact only if K is equal to 1 or if there are an infinite number of transfers. But the difficulty arises in case of a finite number of transfers where partition coefficient deviates considerably from 1 on either sides. Here the calculated K value deviates considerably from the actual K value and the fitting of the theoretical distribution band with the experimental one is not perfect.

Subsequently Nichols [Anal.Chem. **22**, 915(1950)] presented a mathematical deduction of the above formula and pointed out the approximations involved in the deduction. But his procedures are not free from criticism and the actual problem was not solved thereby.

Attempt has been made in this laboratory for the deduction of a generalised formula involving K which will be always valid for finite number of transfers and at all values of K. To achieve this, equation (3) was differentiated with respect to r, after using Stirling's approximation, the derivative was set equal to zero and solved for K

$$T_{n,r} = \frac{n!}{r!(n-r)!} \cdot \frac{1}{(K+1)^n} \quad K^r \quad \ldots \quad (3)$$

(where $T_{n,r}$ = Fraction of the original solute distributed in the r-th tube at n transfers and K = partition coefficient).

$$K = \frac{r}{n-r} \cdot e^{-\frac{r-\frac{1}{2}n}{r(n-r)}} \quad \ldots \quad (4)$$

where r is the tube where peak of the curve is attained, i.e. N in equation (1) and (2). It can be easily shown that equation (4) approaches equation (2) as either K approaches 1 or n approaches infiinity. The utility of equation (4) for a finite number of transfers and at K having values much deviated from 1 on either sides is now under experimental study for a number of systems including succinic acid in amyl alcohol-water and succinic acid in amyl alcohol-phosphate buffer. Preliminary results are quite satisfactory.

3. *Physico-chemical interpretation of variation in the nature of the countercurrent distribution band with the partition coefficient of the distributed material : Bionomial distribution.*

Theoretical treatment of variation in the nature of the countercurrent distribution band with the change of partition coefficient of the distributed material where a Gaussian form of distribution is applied, is already reported (vide Annual Report—1955-56). But as the countercurrent distribution actually follows the mahematics of binomital expansion, Gausian distribution being an approximate form only applicable where a large number of transfers are applied, mathematical treatment should also be applied to the binomial expansion to have a complete idea about the problem.

But there is some difficulty in doing this, as, unlike probability curve, binomial distribution curve is discontinuous in nature. So the following assumptions are made :

1) The number n is very large and r is also not very small so that Stirling's approximations may be applied to them.

2) The change of r, n and K are infinitesimally small, so that differentiations of Tn, r in equation

$$Tn, r = \frac{n!}{r!(n-r)!} \cdot \frac{1}{(K+1)^n} \quad K^r \qquad \ldots (1)$$

with respect to r, n and K are applicable.

Differentiating equation (1) with respect to K, equating derivative to zero, K may be solved as

$$K = \frac{r}{n-r}$$

As the second differential has a negative value, it is expected that if different values of Tn, r are plotted against different partition coefficients, taking r as parameter, each curve will show a maximum value of Tn, r at $K = \frac{r}{n-r}$. The nature of such curves as represented by Williamson and Craig [J. Biol. Chem. **168**, 687; (1947)] for 8 transfers and by Slaunwhite [Anal. Chem. **23**, 687 (1951)] for 24 transfers is thus explained.

As for finite number of transfers binomial distribution curve is unsymmetrical in nature it is not possible to draw an analogy of binomial distribution curve with the perfectly symmetrical Gaussian distribution curve. However, the analogy at the maxima in both of the cases may be shown in the following way (cf. Annual Report 1955-56).

r is replaced by $\frac{nK}{K+1}$ in binomial equation ... (1)

$$Tn, r_{max.} = \frac{n!}{\left(\frac{nK}{K+1}\right)!\left(n-\frac{nK}{K+1}\right)!}\left(\frac{1}{K+1}\right)^n \cdot K^{\frac{nK}{K+1}} \qquad \ldots (2)$$

where Tn, r max, indicates the fraction of the material distributed in the tube where maximum concentration of the binomial distribution curve is reached.

Taking logarithm on both sides, expanding factorials upto 4 terms and simplifying,

$$\ln Tn, r_{max.} = \frac{\beta}{n} - \frac{\beta(K+1)}{nK} - \gamma - \frac{\beta(K+1)}{n} \qquad \ldots (3)$$

where β and γ are constants. Differentiating equation (3) with respect to K, equating the derivative to zero, and simplifying,

$$K = 1 \qquad \ldots (4)$$

As the second differential is positive it is expected that, if a series of $Tn, r-r$ curves are drawn for different values at K, the peak of the Tn, r-r curve will be the minimum at $K = 1$ as compared with those of the Tn, r curves at $K \neq 1$.

E. ZOOLOGY

E. 1. Effect of Sulpha-drugs on the metamorphosis of tadpoles (Bufo melanostictus)

It has been reported that the sulpha-drugs, viz., Sulphaguanidine, Sulphanilamide and other sulphonamide compounds, when administered to rats particularly but also to mice and dogs, produce hyperplasia and hypertrophy of the thyroid gland with a resulting low basal metabolic rate. In tadpoles, the thyroid gland is mainly responsible for their metamorphosis which can be checked by the removal of the gland. It was presumed that administration of the sulpha-drugs (chemical thyroidectomy) might inhibit the metamorphosis of tadpoles by inhibiting the synthesis of thyroid hormone and with this idea studies were made on the effect of sulpha drugs on the metamorphosis of Bufo melanostictus.

The tadpoles (Bufo melanostictus) were collected from local tanks and kept in glass jars containing sufficient water (tap water) and hydrilla for a few days. Then they were treated with sulpha-drugs, viz., *Sulphaguanidine*, *sulphanilamide*, *sulphathiazole*, *sulphapyridine*, *sulphamezathine* and *sulphadiazine*, 0.25 gm of each drug was thoroughly ground in mortar and added to 500 ml. of tap water in which 10 tadpoles were kept. Hydrilla was also given for food as well as for oxygenation of water. In such a way six groups, each containing 10 tadpoles, were treated with the respective sulpha-drugs and kept for investigation. The water was changed and fresh treatment was made at every alternate day. A control group was also run. The results of the treatments of sulpha-drugs on the tadpoles are summarised below :

E.1.1. *Sulphaguanidine*

It is very interesting to note that the sulphaguanidine, though reported to be an antithyroid drug, stimulates the metamorphosis and the morphological changes during metamorphosis are exactly the same as that produced after thyroxine treatment, the only difference is that the time period is longer in the former than that in the latter. In sulphaguanidine groups the metamorphosis started at about 7-th day of the first treatment and all the tadpoles completely metamorphosed within 20 days. It was found that when sulphaguanidine was applied to very young tadpoles, the metamorphosis completed within 20 days but the tail was not absorbed and they remained under water upto 25 days after metamorphosis. They all died within 26 days. This was exactly the same morphological change as was reported as tailed frog after thyroxine treatment.

E.1.2. *Sulphanilamide*

Sulphanilamide stimulates the metamorphosis. Metamorphosis starts at about 15-th day and it takes about one month to be completed in all larvae.

E.1.3. *Sulphathiazole*

It also shortens the period of metamorphosis. All the tadpoles metamorphose completely within one month. Initial changes are observed within 15 days in some tadpoles.

E.1.4. *Sulphapyridine and sulphamezathine*

No marked stimulation of metamorphosis is obtained after the application of these drugs. But the metamorphosis is not inhibited, the tadpoles gradually transform into froglets within normal period during which the control tadpoles too undergo transformation.

E.1.5. *Sulphadiazine*

No inhibition of metamorphosis of tadpoles is observed, rather the treated tadpoles metamorphose earlier than those of control group.

It was expected that the metamorphosis of tadpoles might be inhibited after treatment with sulpha-drugs. But from the above results it may be said that the sulpha-drugs under investigation, inspite of their antithyroid property, can not inhibit the metamorphosis of tadpoles. Sulphaguanidine, Sulphanilamide, Sulphathiazole and Sulphadiazine shorten the period of metamorphosis, sulphaguanidine being the most effective in initiating the transformation of the tadpoles into froglets.

E. 2. Effect of Insulin on the metamorphosis of tadpoles (Bufo melanostictus)

Studies on the effect of insulin on the metamorphosis of tadpoles have been undertaken but no conclusive result can be reported at present. However, it has been observed that the tadpoles, when kept in insulin solution (80 units per litre), grew in size and they metamorphosed earlier than the control tadpoles.

E. 3. Effect of Methyl-thiouracil on the metamorphosis of Rana tigrina

Methyl-thiouracil, an antithyroid drug, produces an enlargement of the thyroid gland associated with hypo-thyroidism in rats. It has been reported by other that in tadpoles, hypo-function of the gland, caused by the application of thiourea or thiouracil, the metamorphosis is retarded. (Gordon, A. S., *et al*, Nature, 152, 504-5; 1943). But it has been found in our experiments that methyl-thiouracil, inspite of its antithyroid action, cannot stop the metamorphosis of Rana tigrina; rather it may be said that when the tadpoles are kept in 1000 ml. water containing 0.1 gm of methyl-thiouracil, their metamorphosis is initiated earlier. This is a very confusing and contradictory result and the investigation will be continued further to ascertain the action of this drug on the metamorphosis of tadpoles of different species.

E. 4. Further studies on the induced metamorphosis of tadpoles by thyroxine after penicillin treatment

Previously it has been reported that metamorphosis can be initiated in the penicillin treated immature tadpoles of Bufo melanostictus by a small dose of thyroxine but their gills

and tails are retained. Efforts have been made for prolongation of their lives under water as long as possible , but it has not been possible to keep them alive for more than 22 days.

Since the previous year attempts are being made to rear the tailed frogs with suitable food materials. Series of experiments have been conducted with tailed frogs of induced metamorphosis by supplying them with decomposed hydrilla infested with numerous animalcules. The results of some of these experiments have already been published in a short note (Sci. and Culure, Vol. 23; pp. 380-382, 1958). Since the latter part of the previous year up to this time, series of experiments have been conducted with tailed frogs to keep them alive for a longer time. In addition to decomposed hydrilla leaves, vitamin B_{12} and hydrolysed casein were added to the water, so that this nutrient solution might be absorbed in the body. The accompanying table gives a summary of the results obtained by decomposed hydrilla, vitamin B_{12} and hydrolysed casein which increased the survival period of tailed frog than that reported previously.

TABLE

Groups	Total number of tadpoles taken	Number of tadpoles completely metamorphosed with retention of tail (tailed frog)	Survival time of tailed frog
1	20	20 (within five days)	6 died within 23 days 5 „ „ 27 „ 7 „ „ 36 „ 2 „ „ 38 „
2	50	50 (within 9 days)	35 died within 10 days 7 „ „ 20 „ 7 „ „ 26 „ 1 „ „ 36 „
3	15	15 (within 7 days)	4 died within 14 days 3 „ „ 20 „ 2 „ „ 23 „ 6 „ „ 31 „
4	30	30 (within 4 days)	3 died within 4 days 4 „ „ 12 „ 6 „ „ 18 „ 7 „ „ 20 „ 5 „ „ 31 „ 5 „ „ 36 „

E. 5. Physiological studies on Amoebae

In connection with the investigations conducted in this laboratory concerning the structural peculiarities of the inner part (contractile vacuole) of the pulvinated motile organs of the sensitive plant, comparative microscopic observations on locomotion and contractility of a common type of amoeba (A. proteus) are being made since the previous year. For

observation, specimens are collected from the tank of the Institute and cultured in petri-dish containing a semi-liquid jelly-like substance made by lacerated hydrilla plant with few grains of rice and a bit of salt. After a day or two a bit of decomposed material infested with amoebae obtained from stagnant pool, is then transferred to the petri-dish. More cultures are grown within three or four days. It can be readily seen under the cover glass and by changing the focus, all parts can be clearly seen under high magnification. When it is in motion the plasmasol, plasmagel, granules, contractile and food vacuoles can be easily seen. But the reversible sol-gel transformation could not be observed. At the time of progress, the vacuoles and granules flow freely along with the flow of plasmasol. Some preliminary observations were made by exposing the organism in different intensities of light. Sudden increase of light generally stops its movement and if light rays are thrown horizontally the amoeba generally elongates itself and produces pseudopodia in forward direction only. With weak solution of neutral red introduced under cover glass, it spreads out. But for a few minutes the organism does not take any stain. Then the cell becomes red all over and becomes rounded, streaming slowly decreases. With high dilution the vacuole takes a pinkish colour and movements are slightly increased.

In view of the recent advances in biological investigations relating to the influence of nucleus upon cytoplasm of a differentiating cell by transplanting nucleus from cell to cell, it is proposed to work on this line with Amoeba proteus and Amoeba discoides, stocks of which are easily available in the Institute tank. For manipulation of the minute cell–contents, micro-manipulators have been installed in the laboratory and preliminary arrangements are being made for starting the work as early as possible.

F. WORKSHOP

During the year under report (1957-58) the fabrication of Apparatus and small components, and also the repair works were carried out according to specifications received from the different departments of the Institute. The total number of requisitions for such works was 547 against 477 in the previous year. Out of these about 90 percent were completed during the year and the rest are in course of completion. The following is a list of major works undertaken:

1. For I.G.Y. programme:—All components of a neutron monitor unit were made. A neutron pile according to I.G.Y. specification has been assembled for a trial run. Three B F_3 metal counters were made according to the same specifications. For meson telescope, metal counters and other equipments were constructed. One electrically operated camera similar to the one made in the previous year which is working satisfactorily at Mayapuri Research station, is under construction.

2. Two medium size electron collection ionization chambers having polysterene gaskets and insulators were assembled.

3. The consturction of a Rectangular cloud chamber of dimension $3' \times 2'\text{-}6'' \times 1'\text{-}6''$ and its accessories are in progress.

4. Remodelling of a cylindrical cloud chamber.

5. For Carbon dating work a large proportional counter with 20 anticoincidence metal counters and other components were constructed and assembled in a suitable stand.

6. Construction of two liquid Scintillation counters.

7. For Neutron Generator one Mild Steel T shaped tube, component of the discharge system, metal clamps for the same and other components of vacuum system were constructed.

8. Photo-multiplier casings.

9. Accessories for Ultra-sonic investigation Apparatus.

10. Paper Electrophorasis Apparatus made from Perspex sheet.

11. The construction of a Mechanical Cell disintegrator is in progress.

12. Accessories for Gas choromatography apparatus.

13. Monocular Microscope tubes.

14. Apparatus in connection with Acharya Jagadish Chandra Bose Birth Centenary Exhibition.

In the glass blowing section, besides repairs and construction of glass apparatus, a large number of G.M. counters were made. One counter filling system has been constructed and fitted up in Mayapuri Research station, Darjeeling.

Regular maintenance and repairs of motors, fans, air conditioning units, refrigerators and pumps were carried out.

In Carpentry section large number of furniture and various works in connection with research and maintenance were made.

At Shamnagar and Falta the regular maintenance of Diesel engines and Pumps were carried out.

This year one Universal Grinding Machine and a Radial Drilling Machine have been purchased and installed in the Workshop.

12 Air Conditioning units and 7 refrigerators have been obtained from Directorate of Disposals through the Ministry of Education, from New Delhi. They are being overhauled for the use in the laboratory of the Institute.

LIBRARY

The Library consists of 4,375 volumes (2,176 books and 2,199 bound periodicals). During the year under report 219 volumes (107 books and 112 bound periodicals) were added to the stock against 301 during the previous year. The total number of periodicals received was 205 (inland 59 and foreign 146) out of which 111 were subscribed and the remaining 94 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 greatly indebted to Dr. J. N. Ray of the Calcutta Chemical Co. Ltd. for his valuable gift of a number of important books and periodicals.

Though the use of the Library is primarily meant for its research staff only, it was very frequently consulted by many research workers and scientists from other institutions because of its well-arranged selected acquisitions and efficient service.

Due to the increase in the number of research staff in the Institute and the growing number of outside research workers who use the Library the volume of work in the Library has increased considerably; to cope with the situation the Library staff has been strengthened by the appointment of an Assistant Librarian. Further, arrangement has been made to keep the Library open for 61 hours a week—from 9 A.M. to 8.P.M. on weekdays and from 9 A.M. to 3.P.M. on Saturdays—for providing better reading facility to readers.

The pressing demands of the readers for more books and journals in the Library could be partially met by the addition of 31 new periodicals in the current subscription list of periodicals and arrangement has been made to procure a large number of important American publications worth about $2 ,500.00 on different branches of science out of the Wheat Loan Interest Fund. It is felt that further additions of important books and periodicals are extremely desirable to meet the requirements of readers as well as to build up a well stocked up-to-date research library.

II. PUBLICATIONS

Volume 21 (1956-57) 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. Other publications during the year under report include (1) Report of the Bose Institute for 1956-57 and (2) The Nineteenth Acharya Jagadish Chandra Bose Memorial Lecture on "India and the Atomic Age: her natural resources for nuclear power production" by Dr. D. N. Wadia, F.R.S. with the Director's Report.

APPENDIX 'A'

I. Governing Body :

Dr. Debendra Mohan Bose
Sri Sudhansu Mohan Bose
Sri Kedarnath Chatterjee
Sri Sudhir Kumar Mandal
Prof. Sisir Kumar Mitra, F.R.S.
Sri Abani Nath Mitter
Dr. Basanti Dulal Nag Chowdhury
Prof. Bhupati Mohan Sen

II. The Council :

Sri S. M. Bose
Sri K. N. Chatterjee
Sri S. K. Mandal
Prof. S. K. Mitra, F.R.S.
Sri A. N. Mitter
Prof. B. M. Sen
} Nominees of the Governing Body

Dr. D. M. Bose, Director, Bose Institute (Ex-officio)
Prof. M. S. Thacker, Secretary to the Govt. of India, Ministry of Education and Scientific Research (Nominee of the Central Government)
Sri Jitendra Nath Lahiri, M. P. (Representative of the Loka Sabha)
Prof. P. Parija, Vice-Chancellor, Utkal University
Dr. K. Ahmed Chowdhury, Professor of Botany, Aligarh Muslim University
} Scientists nominated by the Govt. of India.
Sri Nandadulal Sreemany (Representative of the Calcutta Corporation)

III. Finance Committee :

Prof. B. M. Sen, Chairman (Representative of the Council)
Sri S. Natarajan, Accountant-General, West Bengal (till 13.5.57)
Sri A. K. Mukherjee, Accountant-General, West Bengal (from 14-5-57)
Dr. D. M. Bose (Ex-officio)
Dr. S. K. Roy, Actg. Registrar, Secretary

IV. Building Committee :

Dr. D. M. Bose (Ex-officio)
Sri K. N. Chatterjee (Representative of the Council)
Sri A. N. Mitter (Representative of the Governing Body)
Dr. S. K. Roy, Actg. Registrar, Secretary.

APPENDIX B

Director : Dr. D. M. Bose, M.A., Ph.D., F.N.I.

Actg. Registrar : Dr. S. K. Roy, M.Sc., Ph.D. (Lond), D.I.C.

Department of Physics :

Head of the Dept. : The Director
Research Assistant : Dr. T. C. Bhadra, M.Sc., D.Phil.
AEC Junior Research Fellow : Sm. Nilima Basu, M.Sc.
Research Scholar : Sm. Anjali Chowdhury M.Sc. (from 8.7.57)
Attached worker : Prof. H. P. Dey, M.Sc., P.R.S.

Department of Chemistry :

Head of the Department : Dr. P. K. Bose, D.Sc., F.N.I.
Research Fellow : Dr. A. Sen, M.Sc., Ph.D. (Leeds)
Dr. D. P. Burma, M.Sc., D.Phil.
Dr. S. P. Sen, M.Sc., D.Phil.
National Research Fellow : Dr. A. K. Barua, M.Sc., D. Phil.
(Govt. of India)
Research Scholars : Sri D. P. Chakraborty, M.Sc., (upto 25.8.57)
Sri S. Pattabi Raman, M.Sc.
Sri Nirmal Kr. Sinha, M.Sc., (from 12.4.57)
Sri. K. Venkateswara Rao, M.Sc. (from 15.9.57)
Sri S. K. Chakraborty, M.Sc., (from 5.1.58 to 28.2.58)
Sri A. K. Banerjee, M.Sc. (from 1.8.57)
Junior Research Fellow : Sri D. P. Chakraborty, M.Sc., (from 26.8.57)
(N.I.S.)
Sir J. C. Bose Fellow, Calcutta University : Sri Jyotirmoy Datta, M.Sc., (from 1.9.57)
Govt. of India Senior Scholars : Sri Jyotirmoy Datta, M.Sc. (upto 31.8.57)
Sri K. V. Rao, M.Sc. (upto 14.9.57)
Sri S. K. Chakraborty, M.Sc. (upto 4.1.58)
Govt. of India Junior Scholar : Sri A. K. Banerjee, M.Sc., (upto 31.7.57)
Attached workers : Dr. S. N. De, M.B., D.T.M., Ph.D. (Lond)
Dr. P. N. Das, M.B.
Dr. K. K. Mukherjee, M.B.B.S.
Sri S. K. Chakraborty, M.Sc.
Dr. C. R. Raha, M.Sc., D. Phil.
Sm. Archana Neogi, M.Sc.

Department of Botany :

Head of the Dept. : Dr. K. T. Jacob, M.A., Ph.D. (Lond)
Research Fellow : Dr. S. K. Roy, M.Sc., Ph.D. (Lond) D.I.C
Statistician : Sri A. K. Roychowdhury, M.Sc.

Research Assistants : Sri B. K. Datta
Sri A. T. Guhathakurtha
Sri A. K. Adhikary, M.Sc.
Dr. S. B. Sarkar, M.Sc., Dr. rer Nat. (from 6.7.57)
Sri V. S. Sarma, M.Sc. (from 6.1.58)
Research scholars : Dr. B. B. Biswas, M.Sc., D. Phil.
Sri V. N. Gadgil, M.Sc.
Sri V. S. Sarma M.Sc. (upto 5.1.58)
Sm. Maharani Chakraborty, M.Sc. (from 1.8.57)
Mary & Richard Keating Student : Sm. Chitra Ghosh, M.Sc. (upto 14.8.57)
National Research Fellow : Dr. Sunanda Bose, Filosofic Licenciat (Lund)
(Govt. of India) (on study abroad)
Govt. of India Junior scholar : Sm. Mary Korah, M.Sc. (upto 14.11.57)
Govt. of India Senior scholar : Sm. Mary Korah, M.Sc. (from 15.11.57)
Attached Worker : Sri D. K. Chakraborty, M.Sc.
Sm. Madhuri Datta, M.Sc.

Dept. of Microbiology :

Head of the Dept. : Dr. P. N. Nandi, M.Sc., Ph.D. (Lond) D.I.C.
Research Assistant : Sri A. K. Mishra, M.Sc. (from 1.1.58)
Sri P. P. Mukherjee. M.Sc. (from 1.3.58)
Research Scholars : Sri A. K. Mishra, M.Sc. (upto 31.12.57)
Sm. Mira Purkayastha, M.Sc. (from 1.6.57)
Sm. Asha Lata Paul, M.Sc. (from 24.2.58)
Govt. of India Junior Scholar : Sm. Mira Purkayastha, M.Sc. (upto 31.5.57)
Attached worker : Sri Narendra Nath Shee

Dept. of Zoology :

Research Assistant : Sri. G. C. Bhattacherjee
Govt. of India Senior Scholar : Sri A. K. Medda, M.Sc. (upto 1.5.57)
Attached worker : Sri A. K. Medda, M.Sc.

Workshop :

Superintendent : Sri M. L. Chatterjee
Superintendent of Electricals, Electronics, Refrigeration : Sri S. R. Ghosh

Library

Librarian : Sri P. K. Chakraborty
Asst. Librarian : Sm. Asoka Dhar.
Accountant : Sri A. N. Chowdhury
Asst. Accountant : Sri M. L. Dutta
Sri P.C. Chakraborty

STAFF APPOINTED UNDER GRANT-IN-AID SCHEMES

A. Board of Research on Atomic Energy :

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

Scheme No. 1-A Cosmic ray Investigation :

Dr. S. D. Chatterjee, D.Sc. (*Honorary*)
Dr. M. S. Sinha, D. Sc. (*Research Officer*)
Sri I. L. Chakraborty, M.Sc. (*Junior Research Asstt.*)
Sri S. N. Sen Gupta, M.Sc. -do-
Sri Chittaranjan Mallick, M.Sc. -do- (from 11.2.57 to 12.12.57)
Sri Nirmalendu Chowdhury (*Junior Research Asstt.*) (*from* 15.3.58)

Scheme No. 1-B. Nuclear Physics Investigations :

Sri A. M. Ghosh, M.Sc. } (On study leave)
Sri N. K. Ganguli, M.Sc. }

Scheme No. II Neutron Generator Investigation :

Sri Bimalendu Mitra M.Sc. (from 17.6.57)

Scheme No. III Investigation on the induced mutagenesis in Crop plants using X-ray & Radioactive Isotope.

Sri Amal Kumar Bose, M.Sc.
Sri Balaram Mozumdar, M.Sc. (from 8.1.58)

B. Indian Central Oilseeds Committee :

Inducing Mutants with economic characters in til, rape, and Mustard by X-ray.
(*Investigator-in-Charge :* Dr. K. T. Jacob)
Dr. U. K. Rai, M.Sc., D. Phil. *Botanist*
Sri R. N. Mukherjee, M.Sc., *Asstt. Botanist* (upto 23.7.57)
Sri G. Gopalan Nair, M.Sc., -do- (from 23.8.57)
Sri N.C. Das, M.Sc., *Chemical Asstt.* (upto 31-7-57)
Sri Dev Kumar Basu, M.Sc., *Chemical Asstt.* (from 31.8.57).

C. Indian Council of Agricultural Research :

Control of Weeds (*Investigator-in-charge :* Dr. K. T. Jacob)
Dr. M. G. Srivastava, M.Sc., D.Phil.; *Asstt. Botanist.*
Sri A. P. Mani, M.Sc., *Asslt. Botanist.*

D. Indian Central Jute Committee :

'Radiation Mutation in Jute'
(*Investigator-in-charge :* Dr. K. T. Jacob)
Sri Rabindra Kumar Basu, M.Sc., *Cytologist.*

E. Council of Scientific & Industrial Research :

Studies on the production of antibiotic substance by Streptomyces spp.
(*Investigator-in-charge :* Dr. P. N. Nandi)
Sri Saroj Bandhu Ghosh, M.Sc., *Junior Research Asstt.*
Sri Patit Paban Mukherjee, M.Sc. -do- (upto 28.2.58)

F. National Institute of Science, I.C.I. Fellowship :

(*Investigatr-in-charge :* Dr. D. M. Bose)
Sm. Mridula Datta, M.A.

Gift :

Dr. J. N Ray made a valuable gift to our Institute Library of Books, Journals and Periodicals on Chemistry.

LIST OF PUBLISHED PAPERS 1957-58

Title of the note	Authors	Where published	Reference
Tracer studies on Sulphur metabolism in plants. The path of sulphate reduction.	B. B. Biswas & S. P. Sen	Science & Culture	Vol. 22 pp.697-9
Some growth regulating substance of barley leaves.	S. P. Sen	Jour. Ind. Bot. Soc.	Vol. 36 pp. 312
Triterpenoids IV. Isolation of new triterpenes from Barringtonia Acutangula Gaertn.	P. C. Maity & A. K. Barua	Science & Culture	Vol. 22 pp. 516
Triterpenoids V. Triterpencs from Luffa Cylindrica Roem.	A. K. Barua & P. K. Bose	Trans. Bose Inst.	Vol. XXI
Triterpenoids VI. Sugar constituents from Luffa Cylindrica Roem.	A. K. Barua	Science & Culture	Vol. 23 pp. 154
Triterpenoids VII. Triterpenes from the lark of Prunes nepalensis Koch.	A. K. Barua & P. C. Maity	-do-	Vol 23 pp. 155
Triterpenoids VIII. Triterpenes from the lark of Erratamea coronaria stapf.	S. P. Raman & A. K. Barua	Joun. Ind. Chem. Soc.	Vol 34 pp.912
Triterpenoids IX. Isolation of acid sapogenins from the Allizzia leb—Benth.	A. K. Barua & S. P. Raman	Science & Culture	Vol. 23 pp. 435
Induced Metamorphosis of Tadpoles.	A. K. Medda & G. C. Bhattacherjee	-do-	Vol 23 pp. 380–2
Destruction of salvinia through Herbicides.	K. George	Trans. Bose Inst.	Vol. XXI
Chemical control of weeds in the Paddy fields of West Bengal.	K. George	-do-	-do-

Title of the note	Authors	Where Published	Reference
Loss of Turgor in the pulvinus of Mimosa pudica due to excitatory contraction.	B. K. Dutta & A. Guhathakurtha	Trans. Bose Inst.	-do-
Investigations in the Physiological factors in induced mutageneis.	M. Korah	-do-	-do-
On the measurement of Ultrasonic Power radiated from a quartz crystal Transducer into liquid medium Part I.	T. C. Bhadra	-do-	-do-
On the measurement of acoustic energy desnity and Streaming velocity to determine the absorption Co-efficient by the Streaming method Part II.	-do-	-do-	-do
Inducing mutants with economic characters in Til, Rape and Mustard by X-rays.	U.K. Rai & K. T. Jacob	-do-	Vol. XXI
Cytological Response of plants to Aminotriazole.	M. G. Srivastava	-do-	-do-
Phosphorus metabolism in higher plants. Effect of Fluoride on the incorporation of P^{32} in excited tissues.	S. K. Roy & Madhuri Dutta	Bull. Nat. Inst. of Science (Isotope posium)	in press
Cascade decay of a heavy K-meson	S. N. Sengupta & M. S. Sinha	Phil. Magazine	Vol. 2, No. 19, 936
Associated production of aΣ° and Kμ2	C. R. Mallik & M. S. Sinha	Nuvo Cimento	1958
Vacuoles and movement of the pulvinus of *M. pudica* : The contractile vacuoles.	M. Datta	Nature	Vol. 179, pp.253-54
Thioctic acid and photosynthetic fixation of carbon dioxide	B. B. Biswas & S. P. Sen	Nature	Vol. 181 pp. 1219