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REPORT

OF

THE BOSE INSTITUTE

FOR

1965–66

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

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REPORT OF THE BOSE INSTITUTE
1965—66

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

The Institute has at present four main departments of research, Physics and Biophysics, Chemistry including Plant Chemistry, Physical and Biochemistry, Botany, including Plant Physiology, Plant Biochemistry, Cytogenetics, Plant Breeding, Microbiology including Soil Microbiology and Genetics of Microorganisms. The income of the Institute is derived from endowment funds, from Government of India grants and from grants-in-aid for support of different research projects. The management of the properties and investments of the Institute is vested in a Governing Body, a Board of Life Trustees; and the management of the affairs of the Institute is vested in a Council, in which some representatives of the Governing Body, nominees of the Government of India, Government of West Bengal, Director General, C.S.I.R., representative of the Lok Sabha, Accountant General, West Bengal and some representative Scientists are included.

Obituary

The Bose Institute suffered a grievous loss by the passing away on May 16, 1965 of Shri Kedar Nath Chatterji, one of the senior most member of the Council. Shri Chatterji was a member of the Governing Body since 1948 and its Secretary since May 1962. In 1948 he was elected by the Governing Body as a member of the Council of the Bose Institute. He served the interest of the Bose Institute with ability and conscientiousness.

Governing Body

Three meetings of the Governing Body were held during 1965-66. Some of the important items considered by the Governing Body are given below.

Approved the Budget Estimates of the Governing Body for 1965-66.

Adopted condolence resolutions on the death of the following members of the Governing Body.

i) Shri S. C. Mukherjee died May 1, 1965. He was a member of the Governing Body since March 1927; and of the Council for some years commencing from 1946;

ii) Shri Kedar Nath Chatterjee died May 16, 1965. He was a member of the Governing Body since 1948, its Secretary since 1962. He was a member of the Council since 1948.

Dr. B. D. Nagchowdhuri was elected Secretary, Governing Body in the place of Kedar Nath Chatterjee.

The Governing Body elected Dr. Sivatosh Mookerjee a member of the Governing Body and nominated him a member of the Council.

The birth centenary of Lady Abala Bose was celebrated in the hall of the Bose Institute on August 18, 1965.

The Governing Body nominated Dr. B. D. Nagchoudhuri a member of Bose Institute Finance Committee.

The following sub-committees were constituted—

i) utilisation of the Acharya Bhavan,

ii) administration of the J. C. Bose Centenary Endowment Fund,

iii) administration of J. C. Bose Centenary Publications.

The Governing Body has handed over the Fiat car of Acharya J. C. Bose to the Birla Industrial and Technological Museum for exhibition in the Transport section.

Council

Three meetings of the Council of the Bose Institute were held during the year.

Some of the important items considered by the Council during the year are given below :

i) appointed Dr. B. C. Kundu, Ph.D., F.N.I., as Joint Director, Bose Institute, with effect from 16th July 1965.

ii) nominated Prof. B. M. Sen, Member of the Council as a member of the Finance Committee.

iii) granted Dr. Sunando Bose, Head of the Department of Botany leave for one year with effect from 23-8-65 to work as a Visiting Scientist in the Institute of Genetics, Lund.

iv) accepted the recommendation of the Governing Body, Bose Institute, (a) appointing Dr. B. D. Nagchowdhuri, as a member of the Finance Committee as its representative, (b) nominating Dr. Sivatosh Mookerjee as member of the Council.

v) permitted Dr. P. K. Bose, D.Sc., F.N.I., to work as an Emeritus Scientist of C.S.I.R. in the Department of Chemistry.

vi) invited Prof. P. Maheswari, F.R.S., to deliver the J. C. Bose Memorial address on 30 November 1965.

vii) revised the rules of the Bose Institute Contributory Provident Fund.

viii) approved the Budget Estimates for 1966-67.

Grants

The following grants were received during the year 1965-66.

(a) *Non-recurring grants* :	(i) Govt. of India, Ministry of Education	Rs.	2,85,000·00
(b) *Recurring grants* :	(i) Govt. of India, Ministry of Education	Rs.	10,85,000·00
	(ii) Govt. of India for Research Training Scholarship ...	Rs.	27,629·00
	(iii) Governing Body, Bose Institute ...	Rs.	34,182·84

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

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

Sponsoring Body	*Schemes*		*Amount received*
Council of Scientific and Industrial Research :	(i) Studies on semi-large scale of production of Antibiotic No. 1 ...	Rs.	3,150·00
–do–	(ii) –do– No. 2 ...	Rs.	3,000·00
–do–	(iii) Fellowship—Dr. A. De ...	Rs.	1,926·67
–do–	(iv) Metabolism of amino sugar ...	Rs.	2,500·07
–do–	(v) Gas chromatography ...	Rs.	4,652·00
–do–	(vi) Physical chemical investigation on milk proteins ...	Rs.	7,300·00
–do–	(vii) Emeritus Scientist—Dr. P.K.Bose	Rs.	26,966·00
–do–	(viii) Pool Officer—Dr. S. Bose ...	Rs.	1,500·00
–do–	(ix) Chemical Examination of medicinal plant ...	Rs.	6,800·00
	(x) Chemical and plant taxonomy ...	Rs.	6,750·00
–do–	(xi) Study of frequency power spectrum	Rs.	10,784·17
–do–	(xii) Sudies on Ionospheric ...	Rs.	15,143·20
–do–	(xiii) Enzymatic synthesis of RNA ...	Rs.	12,800·00
–do–	(xiv) Phytin synthesis of Nucleic Acid...	Rs.	6·081·85
–do–	(xv) Sr. Fellowsrhip—R. Roychowdhury	Rs.	3,150·00
–do–	(xvi) Effects of ultraviolet light on DNA	Rs.	4,500·00
–do–	(xvii) Jr. Fellowship—Miss Malati Mazumder ...	Rs.	3,138·65
–do–	(xviii) Studies on the chemical constitution I ...	Rs.	13,600·00
–do–	(xix) –do– II ...	Rs.	2,250·00
–do–	(xx) Pool Officer—Dr. (Mrs.) P. Sinha...	Rs.	1,500·00
–do–	(xxi) –do– Dr. S. Chanda ...	Rs.	1,500·00

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–do–	(xxi) –do– Dr. S. Chanda ...	Rs.	1,500·00

Sponsoring Body	*Schemes*	*Amount received*
Dept. of Atomic Energy	(i) DAE (Shri A. M· Ghose)	... Rs. 57,115·02
–do–	(iii) DAE (Dr. B. B. Biswas)	... Rs. 5,966·00
–do–	(iii) DAE (Dr. S. Bose)	... Rs. 7,061·00
PL—480 Project	(i) VLF—Propagation	... Rs. 59,800·00

A grant of Rs. 10,000/- was received from the C·S.I.R. for the purchase of equipments/ and apparatus for a Micro-analytical Laboratory in the Bose Institute under the supervision of Dr. P. K. Bose, Emeritus Scientist.

Visitors

The following visited the Institute during the year—(i) Prof. R. L. M. Synge, N.L., F.R.S. of the Rowett Research Institute, Aberdeen, U.K.; (ii) Dr. Gray Felsenfeld, National Institute of Health, Bethesda, U.S.A.; (iii) Dr. A. M. Shams-el-Din, Director, Laboratory of Electro-chemistry of the National Research, Council U.A.R.; (iv) Dr. Willium, Professor of Histology, British Institute of Science; (v) Lord Bessborough, Opposition Spokesman in the House of Lords on Commonwealth Affiairs on Science and Technology; (iv) Dr. K. R. Lewis, University of Oxford, U.K.; (vii) Prof. J. H. Quastel, F.R.S., Professor of Biochemistry, McGill University, Montreal, Canada; (viii) Dr. C. Marton, National Bureau of Standards, Washington, D.C.; (ix) Mr. Avdo Humo, Minister Zuogoslovacia; (x) Dr. Richard Biebl, Prof. Plant Physiology, University of Vienna, Austria; (xi) Dr. Wellginhn, Plant Biochemist, German Democratic Republic,; (xii) Prof. T. Asdeoff, Max-Planck Institute, G.D.R.; (xiii) Prof. E. N. E. Willmer, Department of Physiology, Cambridge, U.K.; (xiv) Prof. Beynsen, International Quiet Sun Year.

Acharya Jagadis Chandra Bose Memorial Lecture

The twenty-seventh Lecture of the series entitled "Botany and the Food Problem of India" was delivered by Prof. P. Maheshwari, F.R.S., Head of the Department of Botany, Delhi University, on November 30, 1965. The Lecture is being published.

Acharya Jagadis Chandra Bose Endowment Lecture

The sixteenth to eighteenth Endowment Lectures of the series were delivered during the year :

i) Prof. N. K. Bose, F.N.I., ex-Director, Anthropological Survey of India, on 'Recent trend in Social Anthropological Research in the U.S.A.'

ii) Shri A. K. Roy, F.N.I., Officer-in-Charge, Rain and Cloud Physics Research, National Physical Laboratory, New Delhi, on 'Physics of Cloud Modification'.

iii) Prof. J. H. Quastel, F.R.S., Director, McGill Unit of Cell Metabolism, Montreal, Canada, on 'Molecular Transport at Cell Membranes'.

Special Lectures

i) Dr. Gray Felsenfeld, National Institute of Health, Bethesda, U.S.A., on 'Physio-chemical studies of Nucleic Acid Structure'.

ii) Lord Bessborough, on 'Scientific Britain'.

iii) Dr. W. R. Piggot, Visiting Professor to the Institute of Radio Physics and Electronics on 'Science and Government in the U.K.—a review of the D.S.I.R.

iv) Dr. K. R. Lewis, University of Oxford, on 'Chromosome polymorphism'.

Degree Awarded

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

i) Shri Arun Kumar Chakraborty—Department of Botany.

ii) Sm. Susweta Biswas—Department of Botany.

iii) Sm. Anima De—Department of Botany.

iv) Shri Deb Kumar Mukherjee—Department of Chemistry.

v) Shri H. Bhattacherji—Department of Physics.

Study Abroad

i) Dr. Ranjit Kumar Roychowdhuri, Senior Research Fellow, C.S.I.R. has been awarded a post-doctoral Fellowship by the University of Texas, Austin, U.S.A.

ii) Dr. Arun Kumar Chakraborty, Senior Research Fellow, C.S.I.R., has been awarded a post doctorial Fellowship by the University of Baylor, Baylor, U.S.A.

iii) Dr. Ajit Kumar Medda, Research Fellow in Animal Physiology, has been awarded a post doctorial fellowship by the Massachusetts Institute of Technology, U.S.A.

Grants for Study abroad

Grants of Rs. 2,000·00 each out of Sir J. C. Bose Trust Fund were made available to Dr. R. K. Roychowdhuri, Dr. A. K. Chakraborty and Dr. A. K. Medda.

New appointments and awards

The following appointments were made during the period under review.

A. *Research Fellow* :

Shri Balaram Mojumdar, M.Sc.—in the Dept. of Botany with effect from 1-4-65.

B. *Research Scholar* :

1. Shri Swapan Kumar Ghosh, M.Sc.—in the Dept. of Chemistry with effect from 1-9-65.
2. Shri Utpal Chatterji, M.Sc.—in the Dept. of Botany with effect from 9-11-65.
3. Shri Sukumar Gupta, M.Sc.—in the Dept. of Botany with effect from 15-12-65.
4. Sm. Sukla Sengupta, M.Sc.—in the Dept. of Botany with effect from 1-1-66.
5. Shri Satyanarayan Chongdar, M.Sc.—in the Dept. of Physics with effect from 1-3-66.

C. *Govt. of India Research Training Scholar* :

1. Shri Sisir Kumar Chattopadhyay, M.Sc.—in the Dept. of Microbiology with effect from 5-1-66.
2. Shri Sunil Kumar Paul, M.Sc.—in the Dept. of Chemistry with effect from 1-11-65.

D. *PL*-480 *projects on VLF Propagation—Research Assistant* :

1. Shri P. V. Monoranjan Rao, M.Sc.—in the Dept. of Physics with effect from 1-6-65.
2. Shri Himangshu Bhattacharjee, M.Sc.—in the Dept. of Physics with effect from 1-6-65.
3. Shri Salil Kumar Banerjee, M.Sc.—in the Dept. of Physics with effect from 1-7-65.

E. *D.A.E. Research Projects—Junior Sceintific Officer* :

1. Shri Asoke Das, M.Sc.—in the Dept. of Botany with effect from 10-8-65.
2. Shri Netai Chandra Mondal, M.Sc.—in the Dept. of Botany with effect from 15-10-65.

F. *Council of Scientific and Industrial Research* :

a. *Senior Research Fellow* :

i) Dr. Anima De in the Dept. of Botany with effect from 11-11-65.

b. *Junior Research Fellows* :

i) Sm. Anjali Mukherji, M.Sc., in the Dept. of Botany with effect from 15-3-66.
ii) Shri Pranab Kumar Sanyal, M.Sc., in the Dept. of Chemistry with effect from 28-9-65.
iii) Shri Buddhadev Paul, M.Sc., in the Dept. of Chemistry with effect from 1-11-65.
iv) Shri Santimoy Banerjee, M.Sc., in the Dept. of Chemistry with effect from 14-12-65.
v) Sm. Manju Dutta, M.Sc., in the Dept. of Chemistry with effect from 14-12-65.
vi) Shri Sachin Kumar Ghosh, M.Sc., in the Dept. of Chemistry with effect from 1-8-65.
vii) Shri Bejoy Krishna Chowdhury, M.Sc., in the Dept. of Chemistry with effect from 2-8-65.
viii) Shri Saktipada Basak, M.Sc., in the Dept. of Chemistry with effect from 3-8-65.
ix) Shri Animesh Roy Chowdhury, M.Sc., in the Dept. of Botany with effect from 1-6-65.

G. *Placement of Pool Officer :*

i) Dr. (Mrs.) Asoka Ray.
ii) Dr. N. K. Sinha.
iii) Dr. Sankar Mitra.
iv) Dr. A. N. Bhaduri.
v) Dr. Biswambar Saha
vi) Dr. Sunirmal Chanda.
vii) Dr. S. B. Ghosh.
viii) Dr. (Mrs.) Purnima Sinha.

RESEARCH ACTIVITIES—NON-TECHNICAL SUMMARY

PHYSICS

1. Nuclear Physics

(*a*) *Gamma Rays*

1. It is well known that gamma counters, like scintillation counters of various types, are not equally sensitive to gamma ray photons of different energies. It has been shown theoretically that a perforated cylinder of aluminium of suitable length placed coaxially before a plastic scintillator makes the sensitivity of the detector independent of photon energy. The conclusion was tested experimentally using artificial sources. The agreement between theory and experiment was found satisfactory.

2. The photoelectric cross-section of lead for 0.84 Mev gamma rays was measured by the sphere transmission technique developed in the laboratory. The result was used to determine the ratio of photoelectric effect for electrons in the K-shell to that in the other atomic shells. It was found that this ratio is different from the values for 0.66 and 1.25 Mev photons. This is in agreement with our previous observation.

3. (*a*) An empirical formula has been derived for the coherent scattering cross-section of gamma rays in the Mev range for heavy elements. The formula agrees remarkably well with the experimental values and can be used readily to calculate the coherent scattering probility for small momentum transfer.

(*b*) It has been shown by theoretical calculation that the measurement of polarisation during scattering is a more sensitive test for the existence or otherwise of the Delbruck scattering phenomena.

(*b*) *Neutron Physics*

1. Measurements have been made of the absolute $(n, 2n)$ cross-sections of a few light and medium heavy atomic nuclei for 14.8 Mev neutrons. The neutron flux has been measured by an absolute method developed in the laboratory while the positron activity has been measured by a slow-fast coincidence circuit.

2. Response of organic scintillators for fast neutrons has been studied from the theoretical point of view. It has been possible to explain the high energy portion of the recoil proton spectrum quantitutively using this theory. The results have been used to develop new methods for measuring fast neutron intensity under various experimental conditions. The theory has been verified by using plastic scintillators of different shapes and sizes.

3. Non-elastic cross-sections of various nuclei for 14.8 Mev neutrons have been measured using the Bose Institute neutron generator. New approaches has been made for correcting various sources of errors which occur in this type of measurements. The results have been compared with other experiments as well as with theoretically predicted values.

(*c*) *Low Intensity Measurements—Diffusion in liquids*

A new method of measurement of the self-diffusion coefficient in liquids developed in this laboratory and reported last year was applied for the measurement of (*i*) diffusion coefficients of aqueues phosphoric acid solution into the medium water for a wide range of concentration, the advantage of radioactive tracer technique allowing the measurements to very dilutes solutions in which case conventional optical method is not accessible ; (*ii*) self-diffusion coefficients of phosphoric acid solutions for a number of concentrations.

II. Ultrasonic and Microwaves

1. Measurements on the cavitation threshold in liquids are in progress. These will provide information regarding the tensile strength of liquids. By cavitation is meant rupturing of liquids by producing negative pressure of magnitude greater than that of the vapour pressure of the liquid at that temperatures. This means that we are determining how much energy is to be supplied to the liquid system in order to produce the phase transition i.e., from liquid to vapour phase. From the practical point of view, the knowledge of cavitation helps in the matter of designing ship's hull and the under-water communication system.

2. Present-day knowledge of crystals indicates that crystals grown by taking utmost care, are not free from imperfections. Ultrasonic attenuation and velocity measurements have been undertaken to provide data on the nature of the imperfections in the crystals. Along with the development of crystal growth techniques, material development will be possible.

III. Radio Wave Propagation and Atmospherics

Using the pulse-transmitter of 8 KW peak power constructed last year and the pulse-receiver with the moving-film camera system, continuous and simultaneous photographic records of the ionospheric echoes of the *first* and the *second* orders were taken on certain frequencies during the morning, evening and night hours for the determination of the apparent reflection coefficient and total ionospheric absorption. Records were also taken during the total solar eclipse on 23.11.65 and during the partial solar eclipse on 20.5.66.

As there was difficulty in recording the ionospheric echoes during day-time due to considerable noise from the various electrical machineries in operation in the neighbourhood, a new pulse-transmitter with a peak power of 50 KW has been constructed. It has a pulse-width of 100μsec and a repetition frequency of 50 c/s and works on three different frequencies.

With the duplicate automatic wave-from recorder, constructd in the laboratory, oscillographic records of electric field-changes during the electrical discharges within the clouds were taken. They showed the complex nature of the field-change patterns. In some of the records there appeared sharp pulses of relatively small amplitude confirming the existence of returns in multiple-stroke discharges within the clouds. The similarity of the field-change patterns recorded during the cloud discharges (except at the initial stage) with those obtained during the cloud-to-ground discharges was also evident in some records.

A new technique involving the use of a reactance-tube frequency-modulator and a saw-tooth generator has been employed in recording *directly* the amplitude of any particular frequency in the spectrum of a sferic.

To avoid uncertainty regarding the frequency spectrum of the sferics at the source, it has been decided to adopt the two-station method of obtaining the amplitude distribution in the VLF-range, one station being in Calcutta and the other at Banaras. The location of the sferic source at very large distances would be found by the method suggested by Croom-. The characteristics of VLF-progagation over large distances are thus correctly known.

CHEMISTRY

Protein Chemistry

Further studies on physico-chemical properties of bovine haemoglobin have been carried out with the help of electrophoresis, sedimentation, spectrophotometry and alkali denaturation. Its molecular weight has been found to be 66,000 and like human haemoglobin it undergoes dissociation at acid pH's and in presence of high NaCl concentration. Its isoelectric point of 6.8 is about the same as that of human haemoglobin.

Hydrogen ion titration curves of goat β-lactoglobulin and buffalo β-lactoglobulin have been studied in greater details. These proteins have been shown to undergo conformational changes at pH 7.5 like cow β-lactoglobulins A and B.

Studies have been commenced on interactions of detergents with β-lactoglobulins. In the case of sodium lauryl sulphate (anionic) binding presence of two types of complexes are indicated in sedimentation patterns as shown by the formation two peaks. With cetyl trimethyl pyridinium bormide only one peak is formed indicating the presence of a single complex.

Plant Chemistry

A thorough chemical investigation of the fruits of *Barringtonia acutangula* known to be powerful larvicides and fish poisons has been carried out. It has been possible to isolate the active principles in pure state and the chemical nature of some these compounds has been elucidated. The active principles have been found to be glycosides. The aglycones obtained by acid hydrolysis of these glycosides, are polyhydroxy triterpenes and angelic acid esters of some of these triterpenes. The physiological properties of these compounds are being studied.

Chemical structure of one of the active principles of the plant *Lantana camara*, which cause severe photosensitization in sheep has been fully elucidated and study on another active constituent is almost complete.

Chemical investigations on some antibiotics isolated from *Streptomyces sp.* have been carried out. The antibiotic isolated from a mutant strain of *Streptomyces sp.* Ac 460 and having both antibacterial and antifungal activity has been characterized as member of the actinomycin group. It has been possible to obtain rubidin, and antibiotic isolated from

Streptomyces strain A , in a pure and crystalline form. Much progress has been made in the study of its chemical constitution.

A number of new triterpenoid sapogenins having very novel skeletal structure have been isolated from the plant *Mollugo spergula* which is known in W. Bengal as ghima.

Studies on chemical taxonomy in relation to the family *Rutaceae* has resulted into complete structure elucidation of three carbazole derivatives, glycozoline, glycozolidine and murrayanine by degradation and synthesis. The structure of new β-substituted furanolactone, mahoganin, bearing a taxanomic and biogenetic significance has been unveiled. The antifungal action of carbazole derivatives has been further studied. Some carbazole derivatives related to the natural products have been synthesised for testing their antifungal action.

Biochemistry

Enzymatic phosphorylations of sugars have been investigated in some detail using both bacterial and animal systems with a view to understanding permeation process. The results of this investigation suggest that in *E. coli* the permeation process of sugars may be regulated by a membrane bound particle containing a novel type of phosphate transfer system.

2, 4-Bis (arylamino) pyrimidines, a new class of synthetic broad spectrum antimicrobial compounds, have shown significant nucleic acid antagonistic properties. Whether these compounds may be of use in chemotherapy is now being investigated in details.

Analytical Chemistry

The technique of thin layer chromatography has been adopted for separation of different varieties of carbazole. Some success has already been achieved in. Studies on the alkaloid contents in the bark of Jute plants by thin layer and paper chromatography have been made.

Investigation of the fatty acid content of four commercial butter samples has been carried out by gas chromatography with some interesting results.

Studies on protein contents of different varieties of maize seeds by paper electrophoresis have been made.

An elegant and easy liver function test has been evolved based upon the paper electrophoretic pattern of the serum proteins and serum transaminase activities in various types of liver diseases including cancer.

BOTANY

Plant Physiology

Investigations on the propagation of action wave through the sub-petiole of *Mimosa pudica* revealed instanteneous sappearance of positive stimulus at a distance indicating that these are probably produced by some hydromechanical distrurbance due to contraction of the pinnules.

The effect of application of load on the pulsatory movement of the leaflet of *Desmodium gyrans* has been worked out. It was observed that the abaxial side of the pulvinule plays

the dominant role in the pulsatory activity of the leaflet. The fast movement in the abaxial direction is independent of the weight of the leaflet and has been considered to be due to active contraction of the cells in the lower side of the pulvinule.

A comparative study of the electrical responses of pulvinule and other parts of leaflets of *D. gyrans* indicated that with downward movement of leaflets the electrical sign developed in the pulvinule was negative due to its contraction while the electrical positivity at the midrib and lamina was produced by hydrostatic impact from the contractile pulvinule.

Cytogenetics and Plant Breeding

Treatment with X-rays and P^{32} on different organs of the same plant revealed that pollen mother cells are more radiosensitive than root tip cells.

Three radiation induced mutants of aus paddy viz. S. 1, S. 240 and S.235 were put to micro yield trial at Falta and Shamnagar Experimental Stations and grain yield of S.1 was found to be significantly more than the control in both the locations.

S.1 and its control were again treated with different doses of X-rays. With equal doses applied, the percentage of survival was more in the treatments than the control.

Inheritance studies on the induced neck leaf mutant of aus paddy indicated the mutant characteristic to be recessive.

Cytological and cytotaxonomical studies in the family Malvaceae were continued. Chromosome morphology of two species each of *Malvaviscus* and *Hibiscus* including two varieties of *H. mutabilis* have been worked out.

Cytotaxonomical studies on seventy four varieties belonging to *Capsicum annuum, C. frutescens, C. pendulum, C. pubescens* and *C. sinense* have been completed. From karyotypic and meiotic analysis, it has been suggested that structural alteration of chromosomes and gene mutations played important roles in the speciation of the genus *Capsicum* with *C. annum* as the most primitive species.

Anatomy and Taxonomy

Investigations on shoot apex organisation and leaf histogenesis of *Mimosa pudica* has been studied upon collections in summer and winter. The apical region displayed structural variation with change in season and periodicity of growth and development of the plant.

Floral anatomical studies on fourteen plant members under five tribes of the sub-family Papilionaceae have been made with a view to determining their phylogenetic status. The genus *Crotalaria* has been considered to be most primitive in having ten free stamens and two sets of gaps in the stele for perianth and stamen traces ; while *Cicer* appears to be the most evolved genus showing total absence of disc.

Cytological, anatomical and palynological studies in several members of the family Boraginaceae have been carried out to establish their taxonomic position in the family. The chromosome numbers of *Cynoglossum wallichii* and *C. lanceolatum* are reported for the first time.

Anatomical, cytological and palynological studies on the genus Nyctanthes along with some members of the familites Oleaceae Loganiaceae and Verbenaceae have been made in order to evaluate its correct taxonomic position. In general, *Nyctanthes* shows more resemblance to the members of Oleaceae rather than to those of Verbenaceae. The alkalodal constituents of *Nyctanthes* which appear to be indoleacceous provide evidence in support of its affinity to the members of Loganiaceae.

Palynology

Investigations on pollen morphology with special reference to taxonomy in the families Convolvulaceae, Solanaceae and Casuarinaceae have been undertaken.

Biogeochemical studies on peat samples collected from Birati revealed presence of water soluble protein suggesting possibility of having free amino acid from the sample.

Tissue Culture

Work on suspension culture of normal tissue cells was carried out following the method as used for gall tissue. In a very small number of cases embryo-like structures were observed. But there was no further growth on transferring these cells to solid substratum.

Success has been achieved in establishing cultures from embryo and endosperm tissues of *Abelmoschus esculentus ;* stem tissue of *Cestrum nocturnum Nigella sativa, Murraya* koenigii ; rhizome tissue of *Alocassia indica* and *Rhoeo discolor* and petiole tissue of *Cycas revoluta.*

Plant Bio-chemistry

The changes in the relative amount of different inositol phosphate with different periods of soaking of Mungbean seeds have been studied. A novel enzyme Phosphotransferase which can transfer phsphate from phytin to GCP with the formation of GTP has been isolated from germinated mung seeds and purified. Co-factor requirements of such reaction and mechanism of such enzymatic transfer of phosphate have been elucidated.

Triplet GGG has been established to be a codon for glycine, the study being made with m-RNA obtained by enzymatically extending existing RNA of the chloroplast by attachment of poly-guanylic acid at the end.

Interactions of phosvitin, a protein isolated from hen's egg-yolk, with different substances were separately measured turbidometrically. Equipalent weight of phosvitin was found to be different ranging from 8-280. the theoretical value being 120. Phosvitin knocked off DNA from DNA poly-lysine complex.

Histone and poly-lysine inhibit RNA polymerase activity but inhibition is restored when phosvitin is added with polylisine in the former assay system.

The nature of biosynthesis of basic proteins and RNA in different stages of soaking have been studied. Experiment with radioactive phosphorus showed the presence of m-RNA after 4 hours of germination. Growth hormone treated tissues showed an increase in RNA content even in the m-RNA level.

A high phosphatase activity has been detected in the leaves of *Desmodum* sp., which increases in dark and decreases in presence of light.

Attempts have been made to study the mechanism of gram staining. The uptake of dye (crystal violet) by gram hegative bacteria is higher than that of gram positive ones and in both the groups the uptake is reduced in presence of different alcohols. Retained colour after washing with sodium thisulphate is extractable by organic solvents from stained bacteria. The suggests that permeability barrier is not the only factor for this. Pretreatment with cetyl pyridinium chloride changes the gram character of *E. coli.*

MICROBIOLOGY

During the year under report investigations were carried out on the following lines :— Antibiotic production, Induced mutation in microorganisms, Microbial genetics, Studies on Rhizobium bacteriophage, Ecology of air microorganisms, Industrial fermentation, Transformation in microorganisms and Physiology of microorganisms.

Biosynthesis of polyene antibiotic

A polyene antibiotic Ac_2 435 produced by a strain of *Streptomyces* was produced inliquid culture, extracted with solvents and purified. The antibiotic could be produced in a medium containing ammonium sulphate as the sole nitrogen source. At pH 4.9 the highest titre of the antibiotic was recorded on the 8th day of incubation. The strain could be mutated for higher production of the antibiotic. The course of synthesis of the active substance wa followed.

Antibiotic production by Streptomyces strain Ac_5 658

The fermentation of the antibiotic was done in cotton seed meal which is now extensively used as a substitute for corn steep liquor. The purification of the active antibiotic substance was done through several steps and the final product was analysed by thin layer chromotography and found to contain 3 fractions of which a major spot was found to be active. The active substance was ninhydrin positive.

Screening of anticancer antibiotics

Anticancer compounds occur as a metabolic product in the cultures of *Streptomyces* spp. A rapid method of screening the activity was adopted and the active filtrates were further tested against Sarcoma 108 (Foley) and Hela cells (Human cervix carcinoma). After the presumptive tests the active substances were tested for toxicity to confirm the possibility of the development of potential chemotherapeutic agents.

Multiple resistance to antibiotics

Sputum samples of three patients, who were known to have been suffering from lung tuberculosis and not responding to streptomycin and other anti-tubercular drugs, were bacteriologically tested and the isolated cultures were tested against various well-known anti-

biotics. The sputum did not contain any *Myco. tuberculosis* but strains of *Staph. aureus* and unidentified gram-negative bacteria. All the isolates of *Staph. aureus* were highly resistant to streptomycin and penicilin. They were found to be sensitive to chloramphenicol.

Modifying effects of chemicals on UV irradiation

Modifying effects of chemicals on UV irradiation were studied in *Aspergillus chevalieri* using nucleic acid, casein hydrolysate and vitamins. Treatments were given during irradiation before irradiation and after irradiation. Treatment of spores of *A. chevalieri* with nucleic acid hydrolysate, per cent killing was significantly higher than that of UV alone. Postirradiation treatment with the above chemicals modified the injurious effects of UV and pre-irradiation treatments showed reduced rate of killing of spores.

Segregation of heterokaryotic colonies produced by the recombination of different auxotrophs of the Streptomyces strain Ac 460.

Heterokaryons were produced by mixing two kinds of auxotrophs in the limiting medium. Heterokaryotic colonies resemble either one member of the auxotroph pair having marker in respect of superficial colour and colony appearance of having no resemblance to any member of the auxotraph pair. Some heterokaryons do not show any segregation into parental types and others have been found to produce diffusible pigment. Antibiotic activity has been observed in a few heterokaryotic colonies.

UV-induced mutation in pigment-forming Streptomyces strain Ac_1 805

Selected strains of *Streptomyces* spp. having antibiotic properties together with pigment producing capacity were subjected to UV irradiation. Strains from irradiated population were grown in media having glucose as the sole source of carbon. Active filtrates of the cultures were tested for thermostability and the antibiotic activity was found to be lost at 60°C in most strains except one in which the activity is retained even when heated at 100°C for 10 minutes. The spores of such selected strains were treated with UV rays and isolation of mutants was done. Out of 680 mutants 105 morphological and 6 biochemical were mutants.

Modifying effects of thioures on UV irradiation

Protecting power of thioures against the effect of ultraviolet irradiation was tested using conidiospores of five species of *Aspergillus*. From the studies on the effect of modifying factors it was found that pre-and post-treatments with thioures had effects in protecting the spores of fungi against the injurious action of ultraviolet rays. Use of the same concentration of thiourea during irradiation resulted in completely eleminating the killing effect of UV irradiation in all the species of *Aspergillus*. In another experiment, it was found that the survival percentage decreased in *Aspergillus niger* with 2 hrs. of pre-irradiation treatment.

Studies on UV induced mutation in *Penicillium vermiculatum* 77.62

Mutation experiments with UV irradiation have been continued and doseresponse relationships have been determined. A large number of mutants having deficiences in nucleic acids, vitamins, amino acids and various combinations of these as also sulphate nitrate non-reducers have been isolated. Antibiotic activity recokoned as a genetic marker of a few mutants of *Penicillium vermiculatum* and this was tested with *Staphylococcus aureus* and *Escherichia coli* by the cup assay method. With mutation the genetic marker tended to be variable.

Investigations on the conditions favouring sporulation were undertaken. Modified C-D medium with 20% glucose was found to encourage sporulation favourably. Effect of the medium and effects of temperature, humidity, and light on sporulation were studied. Of these factors, light was found to be the most important in inducing sporulation under normal laboratory conditions.

Studies on UV induced mutation with *Penicillium purpurogenum* Stoll strain P

A genetically pure strain of Penicillium purpurogenum was selected for mutational experiments with UV rays adopting suitable technique. Of the biochemical mutants induced the more important ones are deficient in amino acids, vitamins and nucleic acids.

Studies on induced mutations of *Sordaria bosensis*

Detailed assay of primary selection of mutants from colonies cultured on agar plate on treatment of ascospores with X-rays were done. The deficient mutants responded to (*a*) biotin, (*b*) biotin/choline/PABA, (*c*) choline, (*d*) choline/riboflavin/pyridoxine/biotin and (*e*) choline/biotin.

Field trial experiments on legume inoculation with phage-resistant and phage non-resistant Rhizobium spp.

Investigations were done on the growth and nodulation of legumes as a result of seed inoculation. *Arachis hypogea*, *Trigonella foenum graecum*, *Lathyrus sativum*, *Phaseolus radiatus* and *Cicer arietinum* were species tested. Various nodulation responses were found to be linked up with the height of plants and dry wt. of shoots and roots.

Results obtained in the field trial experiments of *Arachis hypogaea* were remarkably different from the other four legumes. Here the number of nodules increased until the 49th day and a wide difference between two inoculated sets, non-resistant was most significant in this case.

In *Cicer arietinum*, the nodules were absent in the plants of uninoculated plots and in this case. In *Cicer arietinum*, the nodules were absent in the plants of uninoculated plots and there was a sharp difference in the growth between the plants in the uninoculated and inoculated plots. The dry wts. of shoots and root were greater in the inoculated than those of the uninoculated ones and the increase was nearly twofold.

Ecology of air micro-organisms

A survey of the fungal units occuring in air has been carried out for the last three years. Among the varios fungal genera *Cladosporium*, a common air-bourne fungus showed a sharp seasonal variation. They are totally absent during the summer and rainy season. With the fall of temperature in winter *Cladosporium spores* appear on the plates exposed to air.

Cellulose decomposing ability of microorganisms isolated from air samples viz. *Ceratocystis paradoxa*, *Sordaria hypocorpoides* and *Stachybotrys* sp. were tested for their ability to grow on cellulose available from filter paper.

Industrial fermentation by strains of *A. niger*

A group of 35 strains of A. niger was freshly isolated after screening 209 samples and their gluconic acid producing capacity was determined. Specially constructed growth vessels were devised for the fermentation of gluconic acid, and production of gluconic acid was correlated with the increase in the pressure of air in fermenter vessels.

Transformation in *A niger* strain (V_{35})

In transformation experiments the following genetic markers were chosen for the donor or recipient strains (*a*) conidial colour (*b*) size and shape of the colony (*c*) pigment production and (*d*) specific nutritional requirements. The occurrence of intermediate characters amongst the transformed recipient strains was observed.

Solubilization of insoluble phosphates by rhizosphere fungi

A detail survey of phosphate solubilizing fungi was made. Phosphate solubilizing capacity was tested according to the method of Katznelson and Bose (1959) and also by the estimation of phosphorus by photoelectric colorimeter (Fiske & Suba Rao 1925). Rhizospheres of legumes in general contained higher percentage of phosphate solubilizing gungi than non-rhizosphere soils.

Microbiological investigations on a Pellagra-like disease caused by infected rice in the district of Nadia

Surface scrapings of rice grains revealed the presence of fungal structures e.g. spores and mycelial bits which gave rise to well-formed colonies. It is probable that a thermostable fungal toxin might be the causative factor of the pellagra-like disease caused by the intake of infected rice in the Nadia district of West Bengal. Different fungi isolated from the infected rice are—*Heterosporium* sp., *Aspergillus* sp., *Mucor* sp., *Penicillium* sp., *Masiniola* sp. and *Chaetomium* sp.

A. PHYSICS

I. Nuclear Physics

A. GAMMA RADIATION

1. *Uniform Spectral Sensitivity Counters for Low-Energy Photons.*

A photon counter whose efficiency is independent of the energy of gamma radiation incident on it is a powerful tool in the metrology of radionuclides. Conventional counters are useless for this purpose, especially when the same counter has to be used for the measurement of photons with energies varying over wide range. Previous investigations carried out in the laboratory has shown that by using a suitable aluminium filter with a NaI/Tl scintillation counter it is possible to obtain uniform spectral sensitivity photon counter covering a region of several Mev in energy beginning with a threshold of about 250 Kev. Photons of lower energy are strongly absorbed by the solid filter and hence the threshold value cannot be pushed much further below this, without sacrificing uniformity of response. We have now developed a perforated aluminium filter coupled paraxially to a plastic scintillation counter. It has been shown theoretically that for paraxial rays, the counter has uniform sensitivity for photons in the energy range starting from the lowest limit of operation of the scintillator to at least 1·5 Mev. A geometrical procedure has been developed to determine the optimum perforation ratio of the filter. Theoretical value of the root-mean-square variation of the efficiency in the entire range of operation of the counter has been shown to be less than 1%. Using solid filters, instead of perforated filters, simplified procedures has been developed for use with sources which are either constant or decay at known rate. The performance of the filter has been checked by using Hg^{203}, Na^{22}, Cs^{137}, Mn^{54} and Co^{60} sources. The results agree with the calculated values within the limits of the experimental error which is $\pm 2\%$. Non-paraxial behaviour of the system is under investigation.

2. *Photoelectric Cross-Section.*

It was reported previously that our previous measurements on the atomic photoelectric cross-sections by the spherical symmetry method developed in the laboratory indicate that the ratio of atomic to K-shell photoelectric cross-sections vary with energy even for photons above 0·5 Mev in energy. To check this point further, sphere transmission measurements were made by using a Mn^{54} source (0·84 Mev) and thin lead absorbers. The results support our previous conclusion, although due to low source strength used in this experiment, desired statistical accuracy could not be obtained. We are repeating the measurements using a stronger source.

3. *Coherent Scattering of γ-rays.*

(a) Development of an empirical formula for the estimation of coherent scattering intensity at low momentum transfer. When the momentum transfer q involved during

the scattering process is small ($0 < q < mc$), coherent scattering intensity is essentially composed of Rayleigh scattering. In this range of momentum transfer, the only theoretical treatment available is that of form-factor approximation, the limitations of which have become evident from experimental as well as theoretical investigations. In view of the fact that coherent scattering intensity is appreciable only at low θ when q is low and that in many experimental situations (*e.g.*, nuclear resonance scattering) one requires accurate estimation of the coherent scattering intensity, an empirical formula based on the accurate cross-section data reported from this and other laboratories, has been developed for the purpose.

In formulating the general expression, the following two experimental trends were taken into consideration. Firstly, for a given element coherent scattering cross-sections can be represented, to a first approximation at least, as a function of q only, apart from the polarisation factor $(1+\mathrm{Cos}^2\,\theta)$. Secondly the Z-dependance of coherent scattering intersity may be expressed by a simple power-law relation of the type $Z^{n(q)}$, where the exponent $n(q)$ increases with increase of q. The final expression, obtained after a trial and error procedure, is of the form

$$\sigma(\theta) = \tfrac{1}{2}(1+\mathrm{Cos}^2\,\theta)r_0^2\, Z^{a+b_q+c_q^{1/2}}$$

when compared with the experimental data, satisfactory agreement is obtained for $Z = 50$ and 29 upto $q = 0.5\,mc$ and in the case of $Z = 82$ upto $q = 1\,mc$.

(b) *Polarisation of coherent scattering of 2.2 Mev γ-rays in lead.*

Polarisation of coherent scattering intensity in the case of 2.2 Mev γ-rays scattered from Pb has been theoretically computed at different angles ($20° < \theta < 180°$) from the reported theoretical work on Rayleigh and Delbruck scattrering at this γ-rays energy. The principal motive behind the work has been to examine whether polarisation measurements may be worthwhile to establish the existence of Delbruck scattering in the low energy range. It has been found that from this point of view, polarisation is generally a more sensitive parameter to the existence of Delbruck scattering than coherent scattering cross-section. In particular for ($30° \leqslant \theta \leqslant 80°$), polarisation data with and without Delbruck scattering differ by several factors, so that polarisation measurements with 2·62 Mev γ-rays may be significant in establishing Delbruck scattering.

B. NEUTRON PHYSICS

1. *Determination of absolute (n,2n) cross-sections.*

Absolute determination of $(n, 2n)$ reaction cross-section of the above nuclei has been performed, by employing activation analysis technique. 14·8 Mev neutrons have been obtained from Bose Institute's neutron generator, by accelerating deuterons to a potential of 150 Kilovolts and using tritium-adsorbed targets. Determination of absolute cross-section necessitates the (i) absolute determination of flux of neutrons and (ii) the absolute determination of saturation activity of the product nuclei. Absolute flux of neutrons have been measured by a method developed in our laboratory, described in the next section.

The method is based on the study of the characteristics of the biased plastic scintillators, irradiated by 14 Mev neutrons. Absolute flux has been determined to an accuracy of the order of $\pm 3\%$ of error.

Absolute determination of the saturation activity of the irradiated products was performed by detection of annihilation radiations from the β^+-active end products from above nuclei, by the method of coincidence counting.

Irradiations were performed by placing the samples in the forward geometry, at a distance from the tritium target so that the angular spread of the incident neutrons on them does not make a change of energy of neutrons more than ± 100 Kev around 14·8 Mev. The natural samples of high purity, were irradiated for sufficiently long time to ensure generation of saturation activity. Then they were transferred to the counting system. The samples were in the shape of discs and were held between two 7·8 mm thick aluminium "converters" of positrons. The scintillation counters are 2″ photomultipliers, employing $2'' \times 1\frac{3}{4}''$ NaI(Tl) crystals as γ-detectors. The coincidence circuitry has been tested and counters calibrated by employing a prepared Na^{22} source of known strength. Conventional electronics *viz.*, fast and slow concidence circuits, amplifiers, single-channel pulse height analysers and scalers have been used.

The following are the results obtained, which are presented in a tabular form :

TABLE 1

σ $(n, 2n)$ FOR 14.8 MEV NEUTRONS

Nuclei	Product	half life	σ(n, 2n) in mb
F^{19}	F^{18}	112 mnt.	49.4 ± 5.0
Cu^{63}	Cu^{62}	9.7 ,,	544 ± 25
Zn^{64}	Zn^{63}	38.3 ,,	174.4 ± 17.5
Ga^{69}	Ga^{68}	68 ,,	1013 ± 100
Ag^{107}	Ag^{106}	24.3 ,,	562.4 ± 56.3

2. *Response of plastic scintillators towards fast neutrons.*

Organic scintillators are extensively used as detectors of fast neutrons. However, the complex nature of pulse-height distribution, even for mono-energetic neutrons, has been a limiting factor in many fields of application of these scintillators.

Starting from Birk's semi-empirical relationship between specific ionisation and specific fluorescence in organic scintillators it has been possible to develop an analytical theory for the high energy portion of the pulse-height distribution curve for plastic scintillators. The results can satisfactorily account for the approximately linear energy pulse-height relationship observed experimentally. The results have been applied to develop a new absolute method for the measurement of mono-energetic neutron flux. Corrections have been made for anistropy of n–p cross-section in the c.m. system for high energy neutrons,

for the escape of neutrons from the ends of the scintillator as well as for the statistical broadening of the spectrum.

We have also developed a new method for the discrimation between elastically and inelastically scattered neutrons. From an analysis of the properties of the plastic scintillators indicated above, it can be shown that by conducting measurements on neutrons at two different biases, it is possible to get an accurate estimate of the elastic neutron intensity. A great advantage of this method is that it requires no knowledge of the elastic neutron spectrum for this measurement.

3. *Measurement of nonelastic cross-sections of nuclei for fast neutrons.*

The non-elastic cross-sections of nuclei for fast neutrons are conventionally measured by the sphere transmission technique. The extraction of the non-elastic cross-section from the transmission data however requires the evaluation of several correction factors which are often very large. The methods hitherto employed for the determination of these corrections are usually very complicated and require a knowledge of the total and differential elastic scattering cross section data. The latter is a particularly severe limitation especially in experiments with high-energy neutrons for which these data often do not exist. We have, therefore, developed a new method which can be employed to determine non-elastic cross-section from transmission data alone. The method has been tested by measuring these cross-sections for 14·8 Mev neutrons. Table II shows the results obtained by us along with the measurements made by previous investigators in the same energy region. The results completely justify our method. An analysis has also been made of these results in terms of the non-local optical potentials.

TABLE 2

NON-ELASTIC CROSS-SECTIONS OF NUCLEI FOR '14-MEV' NEUTRONS (IN BARNS/ATOM)

Element	Graves et al. 14.1 Mev.	Taylor et al. 14.1 Mev.	Flerov et al. 14.1 Mev.	Mac Gegor et al. 14.0 Mev.	Ball et al.	Present investigation 14.8 Mev.
C	0.601±.006	0.51±0.08	0.73±0.02	0.56±0.02	0.55±0.03 (14.0 Mev)	0.55±0.02
O			0.85±0.03			
Al	1.00 ±0.01	0.91±0.05	1.02±0.02	0.97±0.02		0.85±0.04
Cu	1.42 ±0.04	1.44±0.04	1.48±0.02	1.49±0.02	1.52±0.03 (14.0 Mev)	0.94±0.03
Cd	1.95 ±0.05		1.92±0.03	1.91±0.03		1.46±0.03
Sn	1.96 ±0.05	1.82±0.06	1.85±0.02	1.90±0.03	1.87±0.07 (14.5 Mev)	1.58±0.04
Ce						1.91±0.04
W			2.48±0.03	2.40±0.05		2.03±0.08
Pb	2.49 ±0.02	2.52±0.09	2.54±0.05	2.560±.03		2.44±0.06
						2.50±0.05

C. LOW INTENSITY MEASUREMENTS

Diffusion in liquids.

Employing the new method developed in this laboratory for the measurement of self-diffusion in liquids (*vide* previous year's report) the following measurements were carried out.

(a) Mutual diffusion measurements with phosphoric acid solutions : Pure radio-active phosphoric acid solution (P^{32} tagged) was obtained from A.E.E., Trombay. Diffusion-coefficients for the diffusion of aqueous phosphoric acid solution into the medium water were measured at 25°C for the concentration range 0·0003 *mc*–7 *mc*. The data obtained in the range 0·08–7 *mc* were compared with the results reported by Edwards and Huffman (Edwards and Huffman, *Journal Phys. Chem.*, 63, 1959). The latter set of results were obtained by the optical method and reported to be accurate within ±0·3%. The present set of data in this concentration range shows excellent agreement with those of Edwards and Huffman. However, with the radio-active tracer technique as developed in this laboratory, it was possible to extend the measurements to a considerably lower range of concentration. The dilute solution data are being analysed.

(b) Self-diffusion measurements with phosphoric acid solutions : For these measurements both solute and solvent were phosphoric acid solutions of the same concentration, the solute being distinguished from the solvent by the radioactive properties. Measurements have been complete (Temp.= 25°C) for the concentration range 0·03 *mc*–7 *mc*. Compared to the mutual diffusion-coefficients, which remain fairly constant for a large range of concentration, those for self-diffusion show, with increase of concentration, a monotonously decreasing pattern, the rate of decrease being relatively faster in the lower concentration range. Also, for the same concentration, self-diffusion coefficient is considerably less (by ≈ 10–20%) than the corresponding mutual diffusion-coefficient.

Further analysis of these data and comparison with theories are being made.

II. Radio Carbon Dating

The construction of the counting apparatus which will be used for carbon dating is complete. Final adjustments and tests are now being performed to make it leak-tight and light-tight.

Another new method for the synthesis of toluene is being tried. The method consists of the conversion of Benzoic acid into its Tosylhydrazide and subsequent reduction of the latter directly to toluene.

III. Ultrasonic and Microwaves

Acoustic and Radiation Induced Cavitation in liquids.

Experiments will be performed to demonstrate that acoustic cavitation could be nucleated by ionizing radiation and to obtain quantitative information regarding the process

and thereby throwing more light on the understanding of the working of the bubble chamber which is widely used at present for research in nuclear and particle physics. Further, the effect of the shock waves generated due to the collapse of the cavity bubbles on the phase transition of supercooled liquids will be investigated.

Much progress has already been made towards the fabrication of the glass apparatus. A five-litre glass sphere which will be used as an acoustic resonator, has been made. The sphere filled with the experimental liquids will be excited to radial mode of vibrations by means of magneto-striction transducer in the frequency 25 Kc/s to 50 Kc/s. Transducer will be cemented to the outside of the glass sphere by means of epoxy whose acoustic property is good. The cavitation threshold will be measured by a calibrated PZT5 ceramic transducer.

At present a 100 watt speech amplifier will be used to excit the transducer. A R–C oscillator will provide the required frequency.

Investigation of Solids by ultrasonic techniques.

A pulsed-oscillator is under construction to measue the absorption and the velocity in solids. The attenuation constant will be determined from the logarithmic decrement of the echo pulses. These experiments will be carried out over a wide range of temperature ~ 300°K to 70°K.

Study of paramagnetic resonance by means of microwaves and elastic waves.

The construction of the coils of the powerful electromagnet (10,000 gauss) is in progress in the workshop. Rotating table aad magnet yoke have already been made.

The apparatus for the generation of 3·0 cm microwave is ready for use. An oscillator (9–30 Mc/s) for generating ultrasonic waves is under construction.

IV. Radio wave propagation and Atmospherics

The following two CSIR-Schemes were in operation during the period under review :

(i) Studies in Ionospheric Absorption.

(ii) Study of the Frequency Power Spectrum of Lightning discharges (till 28-2-1966).

A project under PL-480 Aid Programme of the National Bureau of Standards, Boulder Laboratories, Colorado, USA, on VLF-propagation was sanctioned and the work started from the 1st June, 1965. The overall work on Radio Wave Propagation and Atmospherics is summarized below :

1. *Ionospheric Studies.*

Systematic investigations on ionospheric absorption are well underway, after having completed the entire experimental set-up necessary for such investigations. Continuous and simultaneous records of ionospheric echoes, on certain frequencies, of the *first* and the *second* order on a moving film were taken during the morning, evening and night hours for

the determination of the apparent reflection coefficient and total ionospheric absorption. The analyses of some of these simultaneous records and also of a few of the fading records which had been previously obtained by using an experimental arrangement consisting of three integrator circuits, two difference amplifiers and a divider circuit are nearing completion. Records were also taken during the total solar eclipse on 23-1-65 and during the partial solar eclipse on 20-5-66. The records showed the effects of the solar eclipse on ionospheric absorption.

As much difficulty was experienced in recording ionospheric echoes during day-time due to considerable background noise on account of the running of electrical machineries in the neighbourhood, a radio pulse-transmitter of nearly 50 KW peak power has been designed and its construction just completed. A short description of the new pulse-transmitter is as follows—

The newly constructed pulse-transmitter has a peak power of 50 KW (with scope of much higher power) and works on three different frequencies, using plug-in coils and vacuum condensers in the tank circuit, the lowest frequency being 2·2 Mc/s. It has a pulse-width of 100 microseconds and a repetition frequency of 50 c/s.

The non-availability of pulse-transformer and many other components has compelled us to make a cathode-pulsed type of oscillator. The oscillator tubes employed are a pair of C1150 and the cathode tubes are a pair of 715 B procured from disposal junks. The cathode tubes are driven from 4 EL 37 in parallel cathode-follower type arrangements to deliver positive pulse drive of 600 volts at 2 amps. This is enough for bottoming the 715 B's at a plate voltage of 1 KV. The final output is taken directly from the tank and fed to a dipole aerial.

2. *Electric Field-changes during Lightning discharges.*

With the duplicate automatic waveform recorder, constructed in the laboratory, various oscillographic records of electric field-changes during I–C (inside cloud) discharges were taken. Previous oscillographic records of these discharges taken a few years ago (1962) had shown similarities with the C–G (cloud to ground) discharges, in respect of both sharp and *quasi*-periodic pulses (which were called high-frequency components) and the low-frequency components, indicating the existence of return strokes and the *c*-field changes and also in respect of other features. The recent records of electric field-changes during I–C discharges have however revealed the complex nature of the field-change patterns similar to those observed by Kitagawa and Brook (1960). In some of the records there appeared sharp pulses of relatively small amplitude and we attribute these to the return in multiple-stroke I–C discharges. In some cases, these may be identified with what are called 'K-discharges' (names after Kitagawa). The similarity of the electric field-changes during the I–C discharges (except for the initial stage) with those during the C–G discharges with regard to the *c*-field and the *J*-field changes has been accepted by Kitagawa. Further work is necessary to elucidate the complex nature of the electrical discharges inside the clouds.

An electrostatic fluxmeter of the field-mill type has been completed for studying the slow field-changes before and after the return stroke in a C–G discharge.

Some theoretical results have also been obtained regarding (i) the relation between the corona discharge and the slow field-change, and (ii) the time-variation of the return-stroke current in a lightning discharge.

3. *Frequency Power Spectrum of the Lightning discharges.*

A drastic modification has been made in the experimental method of studying the frequency power spectrum of the lightning discharges. The previous method has to be abandoned due to various experimental difficulties. To obtain a *direct* record of the amplitude-spectra of the sferics, the following experimental technique has been adopted and the construction according to design is nearly completed :

In a reactance-tube circuit, the incoming sferic triggers a saw-tooth voltage into the grid of the reactance-tube which with a variable transconductance (g_m) causes the output capacitance to vary in accordance with the saw-tooth voltage. The varying capacitance is shunted across the tank-circuit of a Hartley oscillator. As this capacitance shunt varies, the frequency of the Hartley oscillator also varies. The incoming sferic is mixed with the varying frequency of the oscillator and the mixed voltage is detected by an i.f-stage, sharply tuned to the quiescent frequency of the oscillator. The total change in the oscillator frequency caused by the reactance-tube is adjusted to be about 50 Kc/s. The output after detection at any instant corresponds to the difference between the oscillator frequency at that instant and the i.f. In the absence of the sferic, this output would have been zero, as the i.f.-stage is sharply turned to the quiescent frequency. In the presence of the sferic, a finite detector output would be obtained which would correspond to the amplitude of the particular frequency component of the sferic being the difference between the oscillator frequency at that instant and the i.f. Thus the detector output would directly give the spectrum of the incoming sferic in the range 0–50 Kc/s.

4. *VLF-propagation.*

The energy spectrum of a sferic at the receiving and depends on (i) the energy spectrum of the same at the source, and (ii) the characteristics of the propagation path between the receiver and the lightning discharge which gives rise to such a sferic. It has been generally accepted that for those sferics which give smooth and continuous amplitude variations in narrow-band tuned amplifiers and which show at the same time successive ionospheric echoes after the return-stroke, the energy spectra are the same at the source, at least in the VLF range. Thus any change produced in the received spectra of these sferics should be associated with the propagation path only. This would enable the study of attenuation of VLF-waves with distance.

Considering the particular type of sferics for which the energy spectra at the source are supposed to be the same, and following the method of Fourier integral transformation for obtaining the energy spectra, the attention of VLF-waves over a frequency range from 3 to 15 Kc/s was studied last year, the distances of the sources of the sferics having been estimated from the time-interval of successive ionospheric echoes. Since the spectra of

the origin of the sferics of the particular type cannot be *strictly* considered identical, the two-station method of studying the propagation of VLF waves has been worked out. It has been proposed to have two stations, one at Calcutta and the other at Banaras. From the spectra obtained at the two stations, by the tuned amplifier method or by the Fourier integral transformation method, our object is to study the attenuation characteristics of VLF-waves by the method adopted by Jean, Taylor and Wait (1960).

The location of the sferic source situated at a distance greater than 1000 Km would be found by the method suggested by croom. The distance of the source is to be determined by the usual ionospheric reflection method, if the source is within a distance of 1000 Km and by the *quasi*-periodic delay method suggested by Hepburn (1957), if the source is at a distance greater than 1000 Km.

In order to locate the source, circles of radii equal to the distances of the source from the two stations must be drawn about the position of each station. The position of the source is given by one of the points of intersection of the two circles. Out of the two stations, if one station determines the direction of arrival of the sferic, the source of the sferic can then be located. Crossed loop-aerials with wide-band amplifiers at VLF are to be used as direction-finders of the sferics.

Regarding extremely low frequency (ELF) waves it has been theoretically shown that the corona currents after the return stroke could produce such waves.

B. CHEMISTRY

B.1. Protein Chemistry

B.1.1. *Studies on bovine haemoglobins*

Further studies (cf. Annual Report 1964-65) on bovine haemoglobins have been carried out with the help of electrophoresis, sedimentation and spectrophotometry.

Sedimentation : Sedimentation experiments have been carried out with different concentrations of bovine haemoglobin-B in phosphate buffer at *p*H 6·82, ionic strength 0·1 at 25°C. The value of $S_{20,w}$ decreases as the protein concentration is increased but appears to remain constant at higher concentrations in the region of 1·1 g/100 ml to 2·2 g/100 ml.

Experiments on the effect of *p*H on $S_{20,w}$ of bovine haemoglobin B and AB in the *p*H range 3·20 to 10·46 have shown that, like human haemoglobin, the value of $S_{20,w}$ of bovine haemoglobins decreases below *p*H 6 and above *p*H 9·0 indicating dissociation/denaturation at higher and lower *p*H values. The value of $S_{20,w}$ of bovine haemoglobin in phosphate buffer or NaCl of higher ionic strength decreases indicating dissociation of the molecule into subunits. This has also been indicated by Archibald experiments.

The molecular weight of bovine haemoglobin B calculated from combined sedimentation and diffusion data and from the results of Archibald experiment in phosphate buffer of *p*H 6·82, ionic strength 0·1 have been found to be 65,800 g/mole and 63,200 g/mole respectively. By Archibald method, molecular weight of bovine haemoglobin B in 2·0 M NaCl has been found to be 48,600 g/mole.

Electrophoresis : Free electrophoresis of bovine haemoglobin B in phosphate buffer in the *p*H range 5·90–7·76, ionic strength 0·1, shows that the material is heterogeneous at *p*H 5·90 and 6·40. Single peaks were obtained above *p*H 6·4. The isoelectric point of bovine haemoglobin B has been determined as 6·80.

Alkali denaturation : Alkali denaturation studies of bovine haemoglobin A, B, AB and foetal haemoglobin have shown that foetal haemoglobin is more susceptible to denaturation in alkali than A, B or AB. Of the three types bovine haemoglobin AB is least susceptible to denaturation.

Spectrophotometry : The specific extinction coefficients of bovine haemoglobin B have been found to be 8·47 at 576·5–580 *mμ* and 8·23 at 542·5–544 *mμ*.

Effects of *p*H, ionic strength and protein concentration on the absorption spectrum of bovine haemoglobin B have been studied. It has been observed that at high concentration of phosphate buffer, optical density at 576·5 *mμ* of the protein solutions falls and the absorption spectrum of the protein shows characteristic bands of ferrihaemoglobin in addition to those of oxyhaemoglobin. The effect is more pronounced at low *p*H and low protein concentration.

B.1.2. *Studies on milk proteins*

(a) *Hydrogen-ion dissociation curves of goat β-lactoglobulin.*

The acid-base titration curve of goat *β*-lactoglobulin has been studied in some details.

Direct titration of the native protein has been done in 0·15 M KCl covering the *p*H range 1·9–11. The titration curve has been found to be reversible in the acid range and reversibility at higher *p*H values is being checked. Principles of thermal titration and titration in presence of formaldehyde have been utilised to obtain information regarding the nature and number of different ionising groups present in the protein molecule. Titration of alkali-denaturated protein has also been done and results are compared with other titration results.

Direct titration studies of goat *β*-lactoglobulin indicate the maximum acid-binding capacity of the protein to be 41 (assumed mol. wt. 35,500). The number of groups titrated in the acid region and neutral region of the titration curve is 44 and 8 respectively. Thermal titration curve shows the presence of 44 normal carboxyl groups having a ΔH value close to 1500 Kcal/mole and 6 imidazole groups having a ΔH value of 7500–8000 Kcal/mole. In formol titration, the total number of epsilon-amino groups is determined as 29 and the total number of carboxyl groups is 46, two of which appear to be abnormal carboxyl groups having a ΔH value close to that of imidazole group.

Further work is in progress on the acid-base titration of this protein.

(b) *Hydrogen-ion dissociation curves of buffalo β-lactoglobulin.*

The acid-base titration curve of buffalo *β*-lactoglobulin has been continued (Ann. Rep., 1963-64). The thermodynamic analysis of the titration curve obtained at 0·15 ionic strength and at 25°C has been done in the acidic region. It has been found that the side-chain carboxyl group of buffalo *β*-lactoglobulin can be reasonably assigned to a value of 4·65 as the pK_{int} of these groups. A reasonable value of 0·039 for w at 0·15 ionic strength has been obtained. These values are close to those obtained by Nozaki *et al.* (1959) with cow *β*-lactoglobulin.

Preliminary results on titration curves of alkali denatured buffalo *β*-lactoglobulin showed that the difference in the titratable groups between this curve and the titration curve for the native protein is about 3·6 at *p*H 7·0. With cow *β*-lactoglobulin-B also the same difference has been obtained. This difference results from release of buried groups from the protein molecule during alkali denaturation and also from reduction in electrostatic interaction.

(c) *Purification of cow α-lactalbumin-A.*

Serum albumin and other minor components, generally precipitated with cow *α*-lactalbumin at *p*H 2, are separated by repeated dialysis at *p*H 4. The technique developed in the laboratory is detailed below :

The *α*-lactalbumin precipitate freed from *β*-lactoglobulin is dissolved in dil. NH_3 soln. (the *p*H of this solution is approximately 6·6) the total volume of the solution being 1/100th

of the volume of whey. An equal volume of sat. Am_2SO_4 at *p*H 6·6 is added to α-lactalbumin solution. A slighly coloured (brownish) precipitate is separated by centrifugation and the supernatant is saturated to 90% with Am_2SO_4. This precipitate is white and clean. It is filtered through Buchner funnel and the α-lactalbumin made into a thick paste is dialysed against water. After dialysis, the volume of α-lactalbumin solution should not exceed 1/200th of the volume of whey. This solution is then dialysed against NaAc/HAc buffer (*p*H = 4, $\mu = 0{\cdot}01$). This dialysis is repeated thrice to get almost pure α-lactalbumin containing traces of minor components which can be separated by column chromatography.

(d) *Interactions of detergents with cow β-lactoglobulin-B.*

Preliminary studies have been carried out with one anionic detergent, sodium lauryl sulfate (NaLS), and one cationic detergent, cetyl trimethyl ammonium bromide (CTAB).

Equilibrium dialysis in 0·1 M phosphate buffer has been employed as the main experimental technique for getting binding isotherm (no. of moles of detergent bound/mole of protein vs. log C, where C is the concentration of the free detergent in equilibrium with the protein-detergent complex). This method is supplemented by sedimentation velocity and difference spectrophotometric measurements. So far the results have been encouraging although measurements have been carried out at one *p*H only viz. 5·4.

A comparison of the binding of NaLS with that of CTAB indicates that the anionic detergent is bound more strongly than the cationic one, considering the fact that the alkyl chain in the latter is 4 carbons longer than that of the former and hence hydrophobic interactions are expected to be much stronger with the latter. This would indicate that the nature of the charged group and hence, electrostatic interactions are also of importance in this type of binding.

In the sedimentation velocity measurements also, an interesting difference is observed. Two distinct peaks of different sedimentation coefficients are obtained in the case of NaLS binding indicating the presence of two types of complexes with NaLS, the slow moving peak may be identified with the initial or statistical region of binding and the faster moving peak, with an 'all or none' type of binding. CTAB binding, however, results in the formation of only one peak, indicating the presence of a single complex.

B.2. Organic and Plant Chemistry

B.2.1. *Survey of Saponin bearing plants of India*

(a) *Barringtonia acutangula* Gaertn

In the previous report the isolation of a new triterpenoid sapogenol (now called barringtogenol B) besides barringtogenol C and D was reported. It was also mentioned that barringtogenol B contains an αβ-unsaturated ester function. On hydrolysis with alcoholic alkali under very mild conditions barringtogenol B furnishes a neutral alcohol and an acid. The alcohol has been identified to be barringtogenol C and the acid has been identified as angelic acid. Barringtogenol B is, therefore, an angelic acid ester of barringtogenol C.

The above finding has been confirmed by the mass spectra of barringtogenol B and its derivatives.

The products mentioned above were all isolated from the fruits of *B. acutangula* Gaertn. Recently attention has been focussed on the branch wood of the same plant. The branch wood of *B. acutangula* has been found to be rich in saponins which on hydrolysis furnishes a number of sapogenins, both acid and neutral. From the neutral part, one sapogenol has been isolated in a pure and crystalline state, m.p. 255–258°. Attempts are being made to isolate the other components of the sapogenin mixture in a pure state.

(b) *Duranta plumeri*

An acid sapogenin and a triterpene have been isolated from the leaves of *D. plumeri*. Work is in progress to characterise these products.

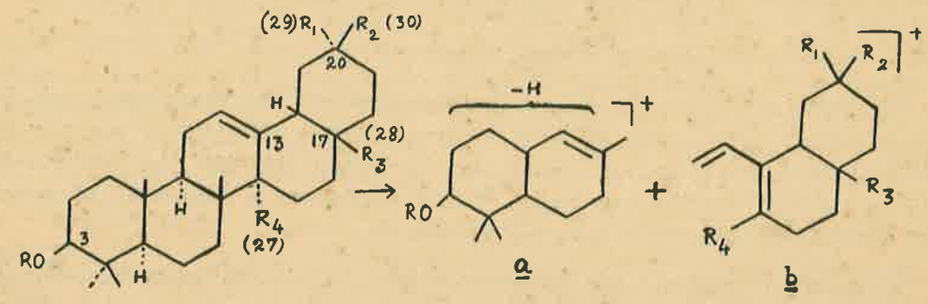
B.2.2. *Chemical examination of Mollugo spergula*

The isolation of two new sapogenins, spergulagenin A and spergulagenic acid from *Mollugo spergula* was reported earlier (vide Annual Report 1964-65). It has now been possible to isolate two other neutral sapogenols having m.ps 295–298° (dec.) and 274–278° (dec.) respectively, from the same plant. Further studies on the constitution of spergulagenin A and spergulagenic acid have also been made.

Previously spergulagenic acid was shown to be 3 β-hydroxy-Δ^{12}-olean-28, *x*-dioic acid by its conversion to oleanolic acid. The second carboxyl group was located at C-20 and it was considered to have α-orientation on the basis of the case of saponification. Further studies, however, indicate the β-orientation of the C-20 carboxyl group in spergulagenic acid which is now represented as (I).

The mass spectral analyses of acetyl dimethyl spergulagenate (III) and the triol (IV) obtained by lithium aluminium hydride reduction of dimethyl spergulagenate, clearly support the structure assigned to spergulagenic acid (I). The mass spectrum of acetyledimethyl spergulagenate (III) shows typical retro-Diels-Alder fragmentation pattern involving the 12 : 13 double bond leading to the ion species *a* (m/e 249; R = $COCH_3$) and *b* (m/e 306; $R_1 = R_4 = CH_3$; $R_2 = R_3 = COOCH_3$). In case fof the triol (IV) ion species *a* (m/e 207; R = H) and *b* (m/e 250; $R_2 = R_3 = CH_2OH$; $R_1 = R_4 = CH_3$) are formed. The mass spectra of acetyl dimethyl spergulagenate (III) and the triol (IV) clearly located the two carboxyl groups and the corresponding primary hydroxyl groups in that part of the molecule consisting of rings C, D and E. As mentioned earlier the conversion of spergulagenic acid to oleanolic acid left only one carboxyl group to be located for which three possible positions, C-27, C-29 and C-30 had to be considered. The position C-27 has been ruled out because acetyl dimethyl spergulagenate (III) depressed the melting point of acetyl dimethyl chincolate (VI) when admixed. Previously α-configuration (C-29) was preferred to β-configuration (C-30) for the carboxyl group attached to C-20 because of the comparative ease of saponification of the corresponding methyl ester and also because of the discrepancy in th ephysical constants of the dicarboxylic acid monomethyl ester (V), m.p. 292–294°; $[\alpha] + 96{\cdot}7°$,

obtained from dimethyl spergulagenate by partial saponification, and compound (V), m.p. 292–293°; $[\alpha]_D$+59°, a degradation product of queretaroic acid (Djerassi, *et al.*, *J.A.C.S.*, 78, 3783, 1956). Unfortunately authentic sample of compound (V) was not available for direct comparison by mixed melting point determination and TLC. Only recently (Tursch, *et al.*, *T. Letters*, 47, 4161, 1965) the structure of mesembryanthemoidigenic acid has been established as 3β, 29-dihydroxy-Δ^{12}-olean-28-olean-28-oic acid. An authentic sample of a degradation product (VII) m.p. 265–267°, of the above acid was available for comparison of mixed melting point with acetyl dimethyl spergulagenate (III), m.p. 285–287°. A clear depression in melting point was observed when the above compounds were admixed. Therefore acetyl dimethyl spergulagenate should be represented as (III) and not as (VII) and hence spergulagenic acid should have the structure (I).



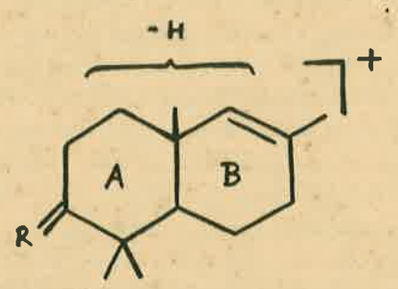
I. R = H; $R_1 = R_4 = CH_3$; $R_2 = R_3 = COOH$
II. R = H; $R_1 = R_4 = CH_3$; $R_2 = R_3 = COOCH_3$
III. R = $COCH_3$; $R_1 = R_4 = CH_3$; $R_2 = R_3 = COOCH_3$
IV. R = H; $R_1 = R_4 = CH_3$; $R_2 = R_3 = CH_2OH$
V. R = H; $R_1 = R_4 = CH_3$; $R_2 = COOH$; $R_3 = COOCH_3$
VI. R = $COCH_3$; $R_1 = R_2 = CH_3$; $R_3 = R_4 = COOCH_3$
VII. R = $COCH_3$; $R_1 = R_3 = COOCH_3$; $R_2 = R_4 = CH_3$

Spergulagenin A was previously shown to be a completely saturated pentacyclic triterpene having three hydroxyl and a keto group (vide Ann. Report 1964-65). Spergulagenin A triacetate forms an oxime, $C_{36}H_{57}O_7N$, m.p. 213–215° (dec.), which on treatment with acetic anhydride and sodium acetate furnishes a tetraacetate, $C_{38}H_{59}O_8N$, m.p. 207–208° (dec.). The NMR spectrum of spergulagenin A triacetate shows a sharp singlet at 2·17 δ for the three protons of an acetyl group ($CH_3.CO$) in addition to the singnals due to the protons of the three acetoxy ($CH_3.COO$–) group. Spergulagenin A triacetate on Wolff-Kishner reduction forms a triol, $C_{30}H_{52}O_3$, (M^+ 460), m.p. 246–248°, which furnishes a triacetate, $C_{36}H_{58}O_6$, m.p. 245–247°. The NMR spectrum of this triol triacetate does not show any signal at 2·17 δ for an acetyl group thereby confirming the presence of an acetyl group ($CH_3.CO$) in spergulagenin A. The mass spectra of both spergulagenin A and its triacetate show strong peaks corresponding to the loss of mass unit 43 ($CH_3.CO$). The mass spectrum

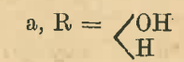
of the Wolff-Kishner reduction product of spergulagenin A shows peaks corresponding to the loss of $-CH_2CH_3$ instead of $CH_3.CO$.

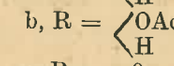
Spergulagenin A, on oxidation with CrO_3-acetone-H_2SO_4 furnishes a colourless crystalline compound, $C_{30}H_{44}O_4$, (M^+ 468), m.p. 279–282° (dec.), [α] +13·4°, which shows a terminal absorption at 205 $m\mu$ (ϵ 3,078). It does not develop any colour with tetranitromethane. On treatment with alkali it furnishes a product, $C_{30}H_{42}O_3$ (M^+ 450), m.p. 296–300°, $[\alpha]_D$−121°, which shows an absorption maxima at 239 $m\mu$ (ϵ 14,790), characteristic of an α,β-unsaturated ketone.

Mass spectra of spergulagenin A, its triacetate and its oxidation product show peaks at m/e 207, 249 and 205 corresponding to the ion species Ia, Ib and Ic respectively.



I

a, R = 

b, R = 

c, R = O

This type of fragmentation is very suggestive of 3-hydroxy-4, 4-dimethyl triterpenes. Mass spectral studies also show the absence of any other oxygen function in ring A and B. The nature of the other part of the molecule and the location of the remaining functional groups are yet to be established.

It may be mentioned here that in any known C-30 pentacyclic triterpene skeleton an acetyl ($CH_3.CO$) group can not be accommodated as such. As far as our knowledge goes, any triterpene embodying this system and having a C-30 formulation is yet to be reported. In this respect spergulagenin A appears to be a novel type of pentacyclic triterpene. Further work is in progress.

B.2.3. *Chemical examination of Mollugo hirta*

From the alcoholic extract of the defatted plant material a crystalline glycoside has been isolated which has been found to be homogenous by TLC. It is being further studied.

B.2.4. *Chemical investigation of Lantana camara*

As mentioned in the previous report, a systematic examination of the plant *L. camara*, which causes severe photosensitisation in sheep, was continued during the year under review. From the acid fraction of the active lantadene mixture obtained from the petroleum ether extract of the plant, four compounds were obtained. Preliminary studies on two of them, compound A and B (now called lantanolic acid and lantanilic acid respectively), have been reported earlier. Previously both lantanolic and lantanilic acids were isolated from the non-saponifiable part of the acid fraction obtained from the petroleum ether extract of the plant. It has now been possible to isolate lantanolic acid directly by chromatography of the acidic fraction of the crude petroleum ether extract.

Lantanolic acid has the molecular formula $C_{30}H_{46}O_4$, m.p. 306–309°; it forms a mono methyl ester, m.p. 197–198°. Previously methyl lantanolate was assumed to contain either an epoxide or 1,4-oxide ring, but latter findings did not corroborate it. Methyl lantanolate forms an oxime, m.p. 221–224°; this oxime on heating with acetic anhydride and fused sodium acetate furnishes an oxime diacetate, m.p. 187–188°. The formation of this oxime diacetate shows conclusively that lantanolic acid contains a keto and not an aldehyde group as in that case a nitrile would have been formed. Methyl lantanolate on reduction with potassium borohydride furnishes a diol, $C_{31}H_{50}O_4$, m.p. 232–234°, which on acetylation with pyridine and acetic anhydride forms a diacetate, m.p. 236–239°. Hydrogenation of methyl lantanolate with Adams' catalyst under normal conditions also leads to the same diol. The above diol diacetate on oxidation with selenium dioxide forms a compound showing triple UV-absorption maxima at 243, 249 and 260 $m\mu$, characteristic of Δ11 : 12, 13 ; 18 -dienes of the oleanane series, thus showing lantanolic acid to be a member of the oleanane group with a 12 : 13 double bond. In the mass spectrum of both this diol and the diol diacetate, there are peaks corresponding to M-31 and M-73 respectively, which establish the presence of a primary hydroxyl function in the diol. Methyl lantanolate, however, does not show any M-31 peak in the mass spectrum.

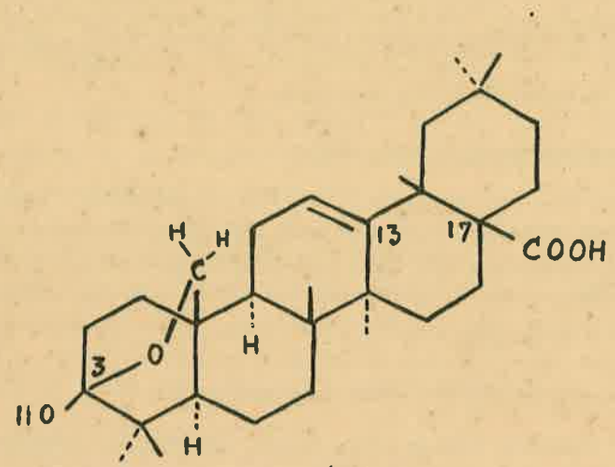
Lantanolic acid, like oleanolic acid, forms a mono bromo-γ-lactone, m.p. 251–252°. Its IR spectrum shows a band in the carbonyl region at 1755 cm^{-1} for a γ-lactone. This together with the fact that the rate of saponification of methyl lantanolate is similar to that of methyl oleanolate, suggest that the carboxyl group in lantanolic acid is in the C-17 position. It is also clear from a study of the mass spectrum of methyl lantanolate, which shows a fragmentation pattern typical of the oleanane and ursane series with a 12 : 13 double bond, that besides the carboxyl function, there are no other substituents in rings, C, D and E.

Methyl lantanolate on treatment with methanolic sulphuric acid furnishes a ketal, $C_{32}H_{50}O_4$, m.p. 226–229°, which on treatment with hydrochloric acid in tetrahydrofuran gives back methyl lantanolate.

On Wolff-Kishner reduction, methyl lantanolate forms a product, m.p. 172–174°, which contains a primary hydroxyl group as evident from the M-31 peak in its mass spectrum. This on Sarett oxidation forms a product, m.p. 212–220°, which on subsequent Wolff-Kishner reduction yields a product $C_{31}H_{50}O_2$, m.p. 169°–170°, which has been proved to be identical with 3-deoxy methyl oleanolate.

The diol obtained by potassium borohydride reduction of methyl lantanolate on Sarett oxidation yields a product, m.p. 222–227°, which gives positive reaction to Zimmermann colour test for a 3-keto group, whereas the product formed by Sarett oxidation of the Wolff-Kishner reduction product from methyl lantanolate does not respond to the Zimmermann colour test. Methyl lantanolate also does not respond to this test.

From the above facts it is evident that lantanolic acid contains a keto group in C-3 position and a primary hydroxyl function besides the C-17 carboxyl group. The absence of any M-31 peak in the mass spectrum of methyl lantanolate point to the possibility that the carbonyl and primary hydroxyl functions in lantanolic acid are not free but occur as hemi-ketal. On the basis of the above data, a tentative structure (I) is proposed for lantanolic acid.



(I)

Lantanilic acid, $C_{30}H_{46}O_5$, m.p. 293–296° (dec.), $[\alpha]_D$+155° (EtOH), forms a methyl ester, $C_{31}H_{48}O_5$, m.p. 227–229°. On heating with pyridine and acetic anhydride on water bath for 3 hr. methyl lantanilate gives a mixture of mono and diacetate which has been separated by column chromatography. The mono acetate $C_{33}H_{50}O_6$ and the diacetate $C_{35}H_{52}O_7$, have m.ps. 233–235° and 156–158° respectively. The monoacetate may be converted into the diacetate on prolonged heating with pyridine and acetic anhydride. Methyl lantanilate forms an oxime, $C_{31}H_{49}O_5N$, m.p. 196–199°. The IR spectrum of methyl lantanilate shows a band at 1745 cm^{-1}, besides the band at 1720 cm^{-1} for carbomethoxyl group. The band at 1745 cm^{-1} may be attributed to a five-membered ring ketone or an aldehyde. This band is absent in the triol, $C_{31}H_{50}O_5$, m.p. 216–218°, which is obtained by potassium borohydride reduction of methyl lantanilate. The triol forms a triacetate, $C_{37}H_{56}O_8$, m.p. 234–236°. The triacetate, on selenium dioxide oxidation gives a product which could not be obtained in a pure state but it shows triple absorption maxima at 243, 252 and 260 $m\mu$, characteristic of the Δ11 : 12, 13 : 18 -dienes of the oleanane group. The above product on

saponification with alcoholic caustic potash (2%) furnishes a triol, $C_{31}H_{48}O_5$, m.p. 261–265°, which shows UV absorption maxima at 243, 252 and 260 *mμ* (ε 27,224; 30,000 and 21,137) similar to the starting material.

Lantanilic acid forms a monobromo-γ-lactone, $C_{30}H_{45}O_5Br$, m.p. 248–250°, with bromine in acetic acid, thus locating the carboxyl group at C-17. Mass spectral fragmentation pattern of methyl lantanilate and many of its derivatives is similar to that of methyl lantanolate and its derivatives. Thus lantanilic acid appears to be a triterpene of oleanane series. The rate of saponification of methyl lantanilate with 20 per cent alcoholic caustic potash is rather high (50%) and suggests the presence of one hydroxyl group at the β-position (C-16 or C-22) with respect to the carboxyl group in lantanilic acid.

Methyl lantanilate forms a compound, $C_{32}H_{50}O_5$, m.p. 284–287° (dec.), on treatment with methanolic sulphuric acid. This product is either an acetal or ketal which is converted to methyl lantanilate on treatment with hydrochloric acid in tetrahydrofuran.

Further work on lantanilic acid is in progress.

B.2.5. *Chemical investigation of Lippia nodiflora*

From the petroleum ether extract of *L. nodiflora* (whole plant), a number of triterpene acids and a colouring matter have been isolated. The alcoholic extract furnished a low melting sterol, a triterpene, m.p. 263–266°, and a steroid glycoside, m.p. 260–265°, besides two phenolic glycosides.

Further work is in progress.

B.2.6. *Chemical investigation of Momordica charantia*

The plant, *M. charantia* belongs to the family *Cucurbitaceae*. The fruits of the plant are used in the indigeneous system of medicine. Because of its hypoglycemic properties, the fruits of the plant have been investigated by us.

The petroleum ether (b.p. 60–80°) extract of the fruit contains an appreciable amount of oil. The alcoholic extract of the defatted fruit has been found to contain an water-soluble alkaloid. From the non-saponifiable part of the alcoholic extract two highly crystalline non-nitrogeneous compound A and B have been isolated in pure state. Compound A, m.p. 218–220°, gives pink-blue colour in the Liebermann-Burchard test. Its IR spectrum shows a broad intense band in the region 3380–3390 cm^{-1} for a number of hydroxyl groups and there is no characteristic absorption in the carbonyl region. On treatment with acetic anhydride and pyridine it forms a highly crystalline acetate, m.p. 169–170°, which gives an intense yellow colour with tetranitromethane. The acetate shows a termal absorption at 205 *mμ*. Compound B, m.p. 131–133°, gives pink→green colour in the Liebermann-Burchard test and hence appears to be a sterol.

From the alcoholic extract another neutral crystalline compound, m.p. 140–145°, has been isolated directly without previous saponification. It does not respond to the Lebermann-Burchard test. It has been found to be homogeneous by TLC.

Further work is in progress.

B.2.7. *Chemical investigation of Clerodendrom infortunatum*

From the alcoholic extract of the dried roots of *C. infortunatum* two highly crystalline compounds, having m.ps. 142–145° and 262–267°, have been isolated. The former has been found to be sterol. Work is in progress to characterise these products.

B.2.8. *Chemical investigation of Chrysenthellum indicum*

A crystalline compound has been isolated from *C. indicum* (whole plant). It is being further investigated.

B.2.9. *Further studies on the constitution of entagenic acid*

The structure of entagenic acid was previously proposed as 3β, 21α, 22α-trihydroxy-Δ^{12}-olean-28-oic acid. This has been confirmed by mass spectral studies on methyl entagenate and other derivatives.

B.20.10. *Chemical investigation on some antibiotics isolated from Streptomyces sp.*

(i) *Chemical investigation on an antibiotic isolated from a mutant strain MT-10 derived from Streptomyces sp.* (Ac_{21} 460).

The antibiotic reported earlier (vide Ann. Report, 1964-65) has now been characterised as a mixture of three closely allied antibiotics of the actinomycin group. The orange-yellow colour, highly negative specific rotation and the absorption spectra (ultraviolet, visible and infra-red) of this compound show remarkable similarity with those of antibiotics of the actinomycin group. Presence of peptide chain has been shown by acid hydrolysis of the antibiotic followed by paper chromatography of the hydrolysate.

(ii) *Chemical investigation on rubidin*

Chemical investigation on rubidin, (Banerjee, A. K., Sen, G. P. and Nandi, A. Antibiotic Annual, 640, 1955-1956) an antibiotic isolated from a Streptomyces strain A_{12}, has been carried out. It has now been possible to isolate the antibiotic in a pure and crystalline state. It appears to have the molecular formula $C_{22}H_{20}O_{10}$ or $C_{22}H_{22}O_{10}$. It is a quinonoid type of compound and most probably contains four hydroxyl and two carboxyl groups. It is active against a number of gram-negative and gram-positive bacteria. Further work on the chemical constitution of rubidin is in progress.

B.2.11. *Preparation of some potential chemical mutagenic agents.*

In connection with our work on the synthesis of potential mutagenic agents, two new organo-phosphorus compounds have been synthesized.

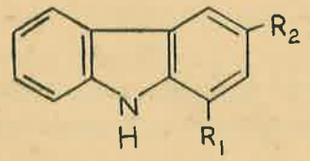
B.2.12. *Chemotaxonomic work in relation to the family Rutaceae*

(a) *Structure of murrayanine*

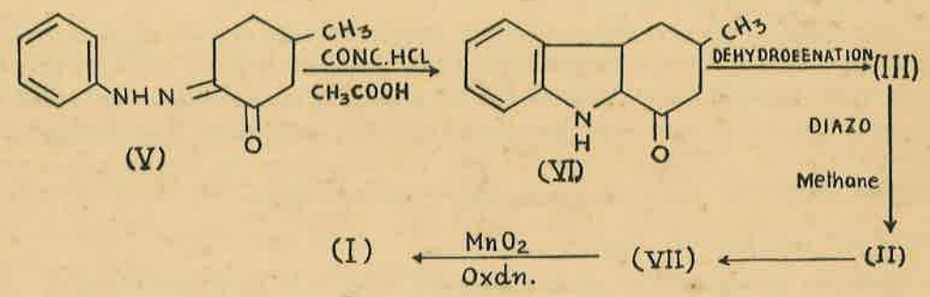
The structure of murrayanine was shown by Chakraborty *et al.* (*Tetrahedron*, 21, 681, 1965) as 1-methoxy-3-formyl carbazole. The location of the formyl group at position 3

of the carbazole nucleus was based mainly on the NMR data. Further studies on the structure of murrayanine have confirmed the previously proposed structure.

On Wolff-Kishner reduction, murrayanine (I) furnished a methoxy methylcarbazole $C_{14}H_{13}NO$ (II), an oily product. On demethylation, this compound furnished a phenol $C_{13}H_{11}NO$, (III) m.p. 158° (λmax 224, 242, 292, 330$m\mu$ with log ϵ 4·59, 4·69, 4·09 and 3·58 respectively). It could be acetylated to $C_{15}H_{13}NO_2$ (IV), m.p. 154° (λ max 238, 248, 257, 292 and 328$m\mu$ with log ϵ 4·65, 4·40, 4·55, 4·17 respectively). The structure of phenol (III) was determined by synthesis as 1-hydroxy-3-methyl carbazole as follows :



	R_1	R_2
(I)	OCH_3	CHO
(II)	OCH_3	CH_3
(III)	OH	CH_3
(IV)	$O.COCH_3$	CH_3
(VII)	OCH_3	CH_2OH



4-methyl cyclohexane 1 : 2 dione 1-phenyl hydrazone $C_{13}H_{16}N_2O$ (V), m.p. 209° was obtained when 2 hydroxy methylene-5-methyl cyclohexanone reacted with phenyl diazonium chloride under Japp-Klingemann condition. Cyclisation of (V) afforded 3-methyl 1-oxo-1,2,3,4 tetrahydro carbazole, $C_{13}H_{13}NO$ (VI), m.p. 197° (λ max 237, 308$m\mu$ with log 4·26 and 4·36 respectively). This compound (VI) on dehydrogenation with palladised charcoal afforded 1-hydroxy-3-methyl carbazole, $C_{13}H_{11}NO$ (III), m.p. 158° (λ max 224, 242, 292, 330$m\mu$ with log ϵ 4·59, 4·69, 4·09 and 3·57 respectively). The acetate of the synthetic phenol and the acetate (IV) were identical in all respects. The synthetic phenol (III) on methylation with diazomethane afforded the Wolff-Kishner reduction product of murrayanine (II). This settles the structure of Wolff-Kishner reduction product of murrayanine as 1-methoxy-3-methyl carbazole. 1-methoxy-3-methyl carbazole was converted to 1-methoxy-3-hydroxy methyl carbazole via 1-methoxy-3-methylene bromide carbazole. 1-Methoxy 3-hydroxy methyl carbazole, m.p. 127° on oxidation with active MnO_2 in CCl_4 afforded 1-methoxy-3-formyl carbazole, m.p. 168° identical with murrayanine. This confirms the previously proposed structure of murrayanine (I).

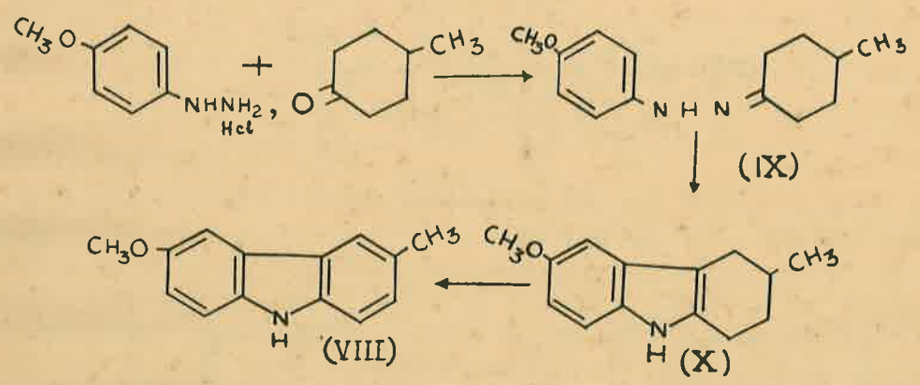
(b) *Structure of Murrayazoline*

Besides the previously isolated carbazole derivatives from *Murraya koenigi* Spreng, a new carbazole derivative, named murrayazoline, has been isolated. Murrayazoline is a paper chromatographically homogeneous compound. It has been assigned the molecular formula $C_{23}H_{25}NO$ (mass 331), m.p. 260°. The IR spectrum of the compound showed bands characteristic for –NH– function and aromatic system. The UV spectrum of the compound was similar to that of dihydrogirinimbine, tetrahydromahanimbine and 2-methoxy carbazole. It suggests the presence of a 2 oxygenated carbazole system in the compound.

NMR spectrum showed the presence of aromatic protons, aromatic C-Me and two sets of deshielded *gem*-dimethyl groups. NMR data shows absence of any vinyl protons. But the compound could be hydrogenated to its dihydro derivative $C_{23}H_{27}NO$, m.p. 95°, showing the presence of a tetrasubstituted double bond. On zinc dust distillation murrayazoline furnished carbazole, m.p. 225°. This shows that murryazoline is built on a carbazole skeleton. On chromic acid oxidation murrayazoline furnished acetone. On ozonolysis murrayazoline furnished a solid ketone which readily gave a 2 : 4 DNPH derivative. These data show that murryazoine has isopropylidine group in its side chain. Further work is in progress.

(c) *Glycozoline*

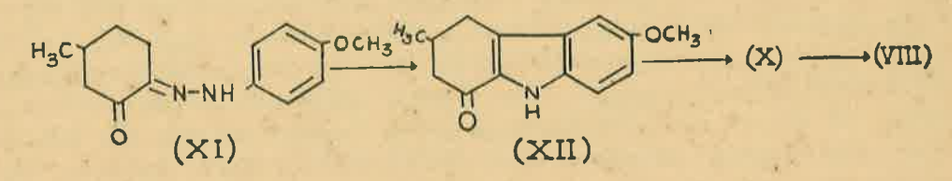
In a previous report the structure of glycozoline was reported as a 6-methoxy-3-methyl carbazole from the results of some degradation studies. Further investigations have established the structure of glycozoline as 3-methyl-6-methoxy carbazole (VIII). The phenol obtained from hydrobromic acid demethylation of glycozoline was tosylated. The tosyl derivative, on reduction with Raney nickel afforded 3-methyl carbazole, m.p. 203°. This clearly establishes the 3-methyl carbazole skeleton of glycozoline which has been synthesised as follows.



(i) *p*-Methoxy phenyl hydrazine hydrochloride, m.p. 155° was condensed with *p*-methyl cyclohexanone using the procedure of Borsche with slight modification. The resulting hydrazone (IX) was cyclised in presence of 10% aqueous sulphuric acid when the indole (X)

m.p., 110–112° was obtained. This indole (X) was then dehydrogenated with chloranil in sulphur free xylene for 2 hrs. The carbazole obtained was chromatographed over alumina. On sublimation and subsequent crystallization the compound $C_{14}H_{13}NO$ was obtained. It melted at 178–179° alone or when mixed with pure specimen of natural product.

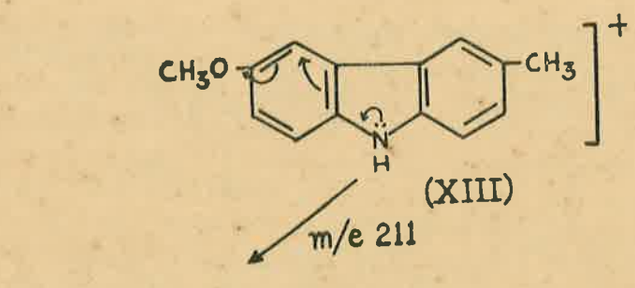
(ii) 2-hydroxymethylene 5-methyl cyclohexanone prepared from 5-methyl cyclohexanone was condensed with 4-methoxy phenyl diazonium chloride by Japp-Klingemann reaction when the 4-methyl cyclohexane 1 : 2 dione 1-*p*-methoxy phenyl hydrazone (XI) $C_{14}H_{18}O_2N_2$, m.p. 197° was obtained ν_{max}^{KBr} 1653 and 3206 cm^{-1}).



(XI) (XII)

Cyclisation of (XI) afforded 6-methoxy-3-methyl 1-oxo-1,2,3,4 tetrahydro carbazol (XII), $C_{14}H_{15}O_2N$, m.p. 212° ν_{max}^{KBr} 1626 and 3205 cm^{-1}; λ max 232, 315 $m\mu$ with log ϵ 4·2280 4·3893 respectively). This on Wolff-Kishner-Huang-Minlon reduction gave 3-methy 1,2,3,4 tetrahydro 6-methoxy carbazole, $C_{14}H_{17}ON$ (X), m.p. 112°. Compound (X) c dehydrogenation with chloranil afforded 3-methyl-6-methoxy carbazole, $C_{14}H_{13}NO$, m.p 177–179°, identical with glycozoline (VIII). The synthetic compound and glycozoline ha superimposable I.R. and UV spectra.

The mass spectral fragmentations as shown below also support the structure of glyco zoline (XIII–XV).

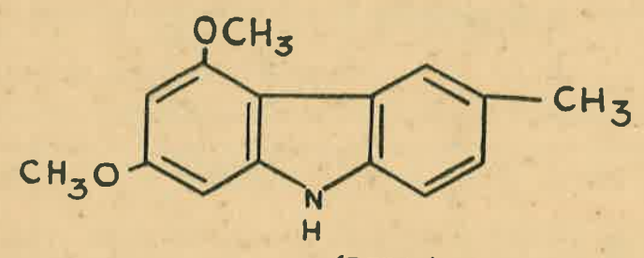


d) *Structure of glycozolidine*

Glycozolidine, the second carbazole derivative obtained from *G. pentaphylla* was shown o be a dimethoxy methyl carbazole, $C_{15}H_{15}NO_2$, m.p. 162°. Further studies on the structure f glycozolidine have been carried out.

Demethylated glycozolidine $C_{14}H_{13}NO_2$, m.p. 240–43°, gave an acetate $C_{16}H_{15}NO_2$, n.p. 194° (XVI). The UV spectrum of the acetate (λ max 237, 256 and 298 $m\mu$ with log ϵ ·55, 4·27 and 4·17) was very similar to that of 2-methoxy carbazole. The phenol acetate howed a molecular ion peak at m/e 269. The peak at (M-43) was very prominent howing the presence of one acetyl group in it. This suggests that one of the methoxyl roups of glyco- zolidine is at position 2 of the carbazole nucleus. Under drastic alkaline ondition glycozolidine furnished a dihydroxy phenol (XVII) $C_{13}H_{12}O_2N$, m.p. 205–208° (λ lax 238, 272, 313 $m\mu$ with log ϵ 4·47, 4·08, 4·15). The phenol afforded a diacetate XVIII) which had UV absorption bands very characteristic for 3-methyl carbazole (λ max 38, 265, 293, 333 $m\mu$ with log ϵ 4·69, 4·33, 4·15, 3·55).

From the similarity of the UV spectrum of (XVIII) with that of 3-methyl carbazole nd the isolation of 3-methyl carbazole by zinc dust distillation of glycozolidine, it is sugges- ve that glycozolidine has a 3-methyl carbazole skeleton with two methoxyl groups on it. rom the previously reported NMR data and the present results it appears that 3-methyl- 7-dimethoxy carbazole formulation (XIX) of glycozolidine is convincing.

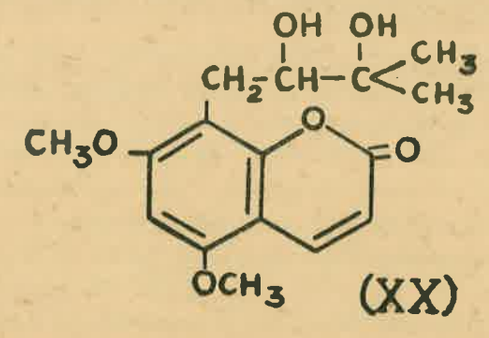


(XIX)

Structure of mexoticin

In connection with chemotaxonomic work, the stem bark of *Murraya exotica* was mined. A new coumarin has been isolated from the stem bark of *Murraya exotica* which been named mexoticin. Mexoticin is a neutral paper chromatographically homogeneous pound which has been assigned the molecular formula, $C_{16}H_{20}O_6$, m.p. 185° (M^+ 308). as two methoxyl groups. The compound reacted with alcoholic alkali like coumarins. UV spectrum of the compound showed bands at 252, 261, 326 $m\mu$ showing the com- nd to be a 7-oxygenated coumarin. The IR spectrum showed bands for hydroxy, δ- ne and C-methyl groups. The NMR data of the compound were much informative. cycnowed the peresence of one highly shielded aromatic proton, two protons assignable to hyd 4 positions of coumarin nucleus. Two hydroxy protons which disappeared on deutera-

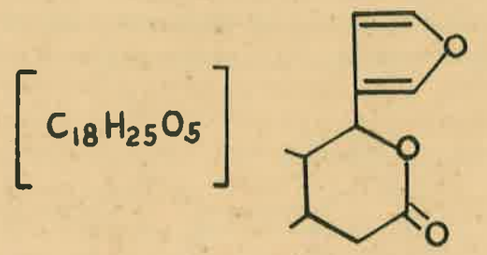
tion could readily be discerned from the NMR spectrum. The NMR spectrum also showed peaks for two aromatic methoxyls, two benzylic protons and one *gem*-dimethyl group. All these data could be accounted for the following structure of mexoticin (XX).



(XX)

(f) *Structure of mahoganin*

In a previous report it was shown that mahoganin, the non-bitter constituent of *Swietenia mahogani*, $C_{27}H_{34}O_8$, m.p. 225–228° could be partially formulated as (XXI)



(XXI)

Further studies on the structure of mahoganin show that the eighth oxygen belongs to epoxide (M-16).

On alkaline hydrolysis mahoganin forms an acid $C_{26}H_{32}O_8$, m.p. 246–247°, $[\alpha]^{2}$ ($CHCl_3$) = −74·68°, which on methylatin with diazomethane furnished a compou $C_{27}H_{34}O_8$, m.p. 242°, identical in all respects with mahoganin. This confirms the prese of a carbomethoxy group in mahoganin.

To sum up the nature of functional groups of mahoganin, it may be stated that lactone function, one carbomethoxy group, one furan oxygen, one hydroxyl account seven oxygen functions of mahoganin. The eighth oxygen function belongs to an epo

The close taxonomic relationship between *S. mahogani* and *S. macrophylla* and similar nature of functional groups suggest the similarity of the structure of mahog and swietenine. The isolation of the biphenyl derivative, λ max at 257·5 $m\mu$, by zinc distillation of the $LiAlH_4$ reduction product of mahoganin shows the relationship bet ring C and ring A in it.

The distinctive features of the NMR spectra of mahoganin, mahoganic acid and swietenine throw further light on the structure of mahoganin. The ethylenic protons of swietenine appears at τ 4·6 to 4·7 as split. In mahoganin and mahoganic acid the ethylenic protons appear as singlets at τ 4·7 and 5·10. The two protons of mahoganin may belong to a terminal methylene group. IR spectra of mahoganin, mahoganic acid and monoacetyl mahoganin show bands at 905, 907, 897 cm^{-1} respectively supporting the presence of a terminal methylene group in mahoganin. This has been substantiated by the isolation of formaldehyde by ozonolysis of mahoganin.

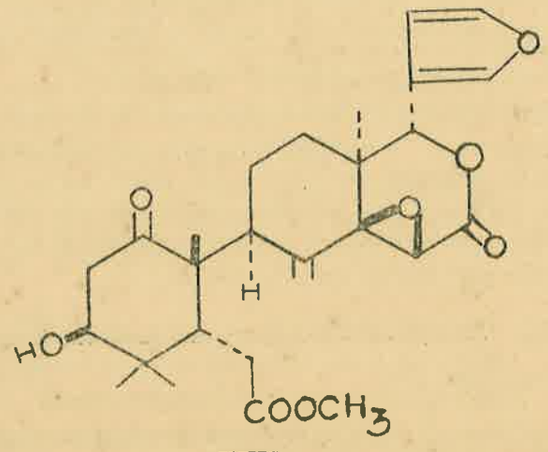
Mahoganin on treatment with hydrochloric acid and acetic acid under controlled condition furnished a *trans*-diene lactone. This observation could only be explained by assuming the presence of the epoxide oxygen conjugated to the lactone carbonyl and the isomerisation of the terminal methylene group between C_8–C_{30}.

The broad multiplets at τ 6·65 to 7·0 of mahoganic acid could be assigned to α-hydrogen of the β-hydroxyl group at position 3 like that in agliol.

On oxidation under Sarret condition mahoganin affords a diketo compound (M 484) which responds to ferric reaction. This clearly establishes the 1 : 3 relationship of the keto and the hydroxyl functions. This is also suggestive of the probable placement of the keto group in position 1 of ring A. The negative cotton effect shown by the ketone chromophore at 288 $m\mu$ also support this.

The IR spectrum of mahoganic acid shows that the ketone is strongly hydrogen bonded. The equatorial hydroxyl is *trans* to the carboxyl group because they do not form lactone in the acid. Stereochemical considerations lead us to assume that the carboxylic group is placed in an axial position in ring A.

Assuming the similarity of the stereochemistry of mahoganin with β-substituted furanolactones isolated from several members of *Meliaceae* and *Rutaceae* and other data obtained, mahoganin could probably be best represented by (XXII).



(XXII)

(g) The studies on the alkaloidal constituents of jute are being carried out in collabor tion with analytical section and Botany Department.

B.2.13. *Synthesis of carbazole derivatives*

(a) Some analogues of glycozoline have been synthesised.

(b) A pyrano carbazole oxygenated at 2 position has been synthesised.

B.2.14. *Biochemical properties of natural products*

(a) *Antifungal action of natural products*

Some constituents of *G. pentaphylla* and some compounds related to it have bee examined for their antifungal action. Some of them are fairly active against *T. rubru* and *M. adouni.*

(b) *Studies on the effect of psoralene in relation to pigment metabolism*

The effect of psoralene on toads under different experimental conditions are bei studied in relation to melanin formation.

B.2.15. *Physical properties of natural products*

(a) Detailed investigations on thin layer chromatography of carbazole and couma derivatives are being made in collaboration with analytical section of this department.

(b) UV spectral studies of carbazole derivatives are being made in detail.

B.2.16.

During the period under report, some synthetic compounds having a structural patte similar to some natural carbazoles were prepared. These natural carbazoles have anti-fun action and the object of the synthetic work was to see if simpler compounds would also ha anti-fungal properties. The compounds are now being tested.

Attempts were also made to isolate new compounds from plant sources and two c pounds have already been isolated. They belong to triterpenoid group.

The synthesis of new types of α-pyrones is in progress.

At present another enzyme metabolically related to the epimerase is being studi UDP glucose dehydrogenase. The dehydrogenase is routinely needed for UDP Gluc epimerase assay but the dehydrogenase itself is very interesting for its involvement in bi gical detoxifications. The enzyme has been purified 120–150 fold. This is a so-ca allosteric enzyme and at present work is being extended to see the effect of various m bolities related to this pathway on the allosteric site.

B.3. Analytical Chemistry and Instrumentation

B.3.1. *Thin-layer-Chromatography*

(i) *Physical and chemical aspect of separation on thin-layer plate.* To determine of the interaction of absorbent, solute and solvent in thin-layer chromatography, the fo

g effects were studied : (a) the effect of dielectric constant of the solvent on the separation solutes, (b) effect of ionisation of the solute on the separation, and (c) effect of substitu- on of functional groups on the solute.

Plates of 0·2 mm thickness were drawn with silica gel-G, alumina-G, and kieselguhr-G Merck). They were dried at room temperature and activated at 110°C for 1 hr. and stored a desiccator containing tell-tale silica gel for cooling. After application of the sample e chromatograms were developed single solvent systems of known dielectric constants. he solvents were methanol, ethanol, *n*-propanol *n*-butanol, *n*-pentanol, benzene, hexane, clohexane. The following groups of dyes were used (a) Water soluble indicator dyes of e sulphonthalein series—Phenol Red, Cresol Red, Thymol blue, Bromophenol blue, Bromo- esol green and Bromocresol purple (b) Fat soluble dyes of the Sudan series—Sudan I, dan II, Sudan yellow Sudan III and Sudan IV.

Results show (a) Indicator dyes separate according to degree of ionisation and the re basic it is the more it is adsorbed on silica gel. The reverse holds true for alumina. The degree of ionisation of the dye is dependent on the polarity of the solvent, hence her the dielectric constant of the solvent better the separation. (c) Addition of functional ups like C-methyl change the pK values of the dyes and hence the R_f. (d) With fat uble dyes in non-polar solvents where ionisation is nil, introduction of C-methyl groups reased the polarity of the solute, and this is concurrent with decrease in its capacity to adsorbed irrespective of the adsorbent used. (e) The adsorbtion capacity of silica gel fat soluble dyes is higher than that of alumina-G, which in turn is a stronger adsorbent n kieselguhr-G, thus thre is a general increase in R_f in the series silica gel, alumina, selguhr.

(ii) *Separation of carbazole derivatives by Thin layer chromatography.*—In order to estigate the possibilities of separating the different derivatives of carbazole on thin layer es, 14 different compounds of this class were used. These were supplied by the plant mistry section of our department. 20×20 cm, plates of 250μ thickness were used. th silica gel-G and alumina-G were used as the solid phase.

For detection, the plates were first observed under ultraviolet light when those spots ch exhibited fluorescence were detected. The plates were then sprayed with an alcoholic tion of picric acid. After a few minutes, brown or red spots of the corresponding ates were observed against a yellow back-ground.

Sixteen different solvent systems were used. Good results were obtained by using roleum ether and di-isopropyl ether (10 : 1) with silica gel-G and petroleum ether and ic acid (100 : 1) with alumina-G.

.2. *Gas chromatography*

(i) *Standardisation of the F and M Model* 700 *Gas chromatograph.*—A versatile modular chromatograph has been purchased from F and M. Instrument Corpn. of U.S.A. The ruments was badly damaged in transit. Some important components including a ial gas-flow control valve were found to be damaged. These were repaired in the institute.

Slight changes had also to be made in the carrier-gas flow system of the instrument to make it suitable for use with nitrogen gas. The instrument was originally meant to be used with argon or helium as the carrier-gas. This instrument has been standardised with a mixture of the methyl esters of even carbon fatty acids (C_8 to C_{20}). Two different columns had been used for this standardisation. These were a 10% 'Apiezon L' column and a 10% polydiethylene glycol succinate column.

(ii) *Investigation of the fatty acid content of some important edible fat by gas-chromatography.*—As a 'part of the above investigation butter samples processed and sold by four different concerns were studied. The samples were saponified and the fatty acids obtained was methylated as indicated in the last report. These mixed methylesters were chromatographed using the "F and M' Model-700" gas-chromatograph. For each brand of butter a typical gas-chromatogram was obtained. In all the four different brands rather high proportion of the following saturated acids, such as, butyric (C_4) Deconic (C_{10}), Lauric (C_{12}), Myristic (C_{14}), Palmitic (C_{16}) and stearic (C_{18}) were detected. Of the unsaturated acids oleic (C_{18} monene) linoleic (C_{18} diene) and linolenic (C_{18} triene) are present in large amounts Indeed the most predominent fatty acid component was found to be oleic acid in all the samples. Other unsarurated acid present in considerable amounts are palmitoleic acid and a mono-unsaturated acid containing 14 carbon atoms. Other minor components such as Pentanoic (C_5), Hexanoic (C_6) and octanoic (C_8) acids are also present along with traces of branched chain saturated fatty acids. One of the brand was found to contain considerable amounts of both saturated and unsaturated fatty acids containing 20 and 22 carbon atoms.

B.3.3. *Studies on Maize seed proteins*

(i) *Extraction of soluble proteins from Maize seeds.*—Previously it was shown that water was very poor solvent for the extraction of proteins from maize seeds. Besides water, extraction was also carried out with 0·1 M phosphate buffer of *p*H-7, and some phosphate buffer solutions with different amounts of NaCl per litre. All these extractions were made separately for 3 hrs., 12 hrs. and 18 hrs. at 3°C and at 25°C. Maximum amount of protein (measured in terms of total nitrogen calculated by micro-kjeldahl method) was obtained at 3°C in the extract with phosphate buffer without NaCl. With this solvent the 12 hrs. and 18 hrs. extracts yielded the same amount of protein whereas the amount of protein in the 3 hr. extract was much lower.

(ii) *Paper Electrophoretic study of the Globulin fraction of Golden sunshine, Golden Beauty and Ganga Hybrid-101 variety of Maize seeds.*—Previously we extracted and studied the paper-electrophoretic nature of the globulin fraction of Giant white, Sugar corn and Golden Bantam Variety of maize seeds. (Vide Annual Report 1962-63, 1963-64, 1964-65). The same kind of work was carried out with other three varieties of maize namely Golden sunshine, Golden Beauty and Ganga Hybrid 101. Soluble protein extraction was made by shaking the powdered seeds in 0·1 M phosphate buffer of *p*H 7 (in the ratio of 1 : 4 w/v) for 12 hrs. at 3°C. Extractions were centrifuged and filtered through filter pulp. The clear filtrate was treated with solid ammonium sulphate and only 70% saturation produced precipitation. The precipitate was dissolved in veronal buffer of *p*H 8·6; $\mu = 0{\cdot}05$ and

electrophoresis was carried out for 6 hrs. along a strip of Whatman No. 1 filter paper of 24 cm. length under an applied potential of 180 volts. In all the three varieties three bands were obtained indicating the presence of three fractions in the globulin.

In order to study the nature of these three fraction total globulin dissolved in the extracting buffer was dialysed against distilled water at 3°C for 72 hrs. with frequent changes. With each variety of maize, a precipitate was found to form in the bag which was removed by centrifugation. Solution of this precipitate in veronal buffer of *p*H 8·6 $\mu = 0{\cdot}05$ was subjected to paper electrophoresis as before. This result was compared with the paper electrophoretic study done prior to dialysis. In the case of the precipitate, the slowest moving band was found to be missing. Electrophoresis of the supernatant portion showed all the predialysis fractions. These results show that one of the fractions of the globulin of these seeds can be partially separated from the other fractions by dialysis.

B.3.4. *Paper electrophoresis of serum proteins*

(i) *Study on paper electrophoresis of serum proteins and its transminase activities in different liver diseases.*—It has been observed that there are some specific changes of electrophoretic patterns of different protein fractions in serum in cases of the liver diseases (vide Annual Report, 1963-64). Along with these electrophoretic changes significant increase in the activities of two transminases namely Serum-Glutamic-Oxalacetic transminase (SGOT) and Serum-Gultamic-Pyruvate transaminase (SGPT) have also been obtained. These increases might have been due to many factors of which the release of the enzymes from the diseased cells could be a possible one. This particular feature has been investigated by simultaneous estimation of the transminase activities in peripheral blood and enzyme content of the liver tissue. This study has been made by taking the liver tissue from normal and pathological conditions but the number of normal cases studied has not been adequate as it was difficult to procure normal material from hospitals. From a study of 300 cases of different liver diseases it has been concluded that the combination of study of paper electrophoresis of serum proteins and its transminase activities may be utilised in clinical medicine to asses the functional capacity of the liver.

(ii) *Study on paper electrophoresis of serum proteins and some serum enzymes in cancer.*—This study is being carried out in different types of cancer. The cases are selected from different hospitals, but this work is not in progress at present due to non-availability of untreated cancer cases. In cancer liver and in maliganancy of the gall bladder some typical changes in the electrophoretic pattern have been observed particularly in globulin fraction. Activities of such serum enzymes as S.G.O.T., S.G.P.T. and serum phosphatase have also been studied in these cases. Apart from these two enzymes the study of lactic dehydrogenase in urine in genito-urinary cancer will be taken up when these cases will be available.

(iii) *Immunoelectrophoresis.*—Immunoelectrophoretic and immunodiffusion techniques have been developed. These methods are being applied to the study of different proteins.

B.3.5. *Studies on the Alkaloid contents in the Bark of Jute plants*

From taxonomic considerations, it was of interest to know whether alkaloids are present in any member of the order Teliales. The genus Corchorus (Jute) belongs to this

order Teliales. Samples of plants of the two available species namely *Corchorus capsularis* and *Corchorus olitorius* belonging to the above genus were examined. The samples were supplied by the Dept. of Botany of our Institute. The barks obtained from each type of samples were subjected to soxhlet extraction with absolute alcohol for 24 hrs. After the removal of the alcohol the residue was refluxed with 5% HCl solution for 6 hrs. The resulting solution was filtered and the solid Na_2CO_3 was added to the filtrate with stirring until the solution was distinctly alkaline. Any alkaloid present in this alkaline solution was then extracted with chloroform. The chloroform extract was washed with water to remove any alkali present and was dried over anhydrous Na_2SO_4. Most of the solvent was evaporated and concentrated solution was obtained. This solution was directly subjected to paper chromatography and thin-layer chromatography. Ascending technique of paper chromatography was used with Whatman No. 1 paper. Three solvent systems were used. The dried chromatogram was sprayed with Dragendorff reagent for the detection of alkaloids. In all the solvent systems two spots were detected in case of *C. capsularis* and one spot in case of *C. olitorius*.

For thin-layer chromatography the samples were applied to 10 cm × 20 cm silica gel-G plates activated at 105°C for 30 minutes. Five different solvent systems were used. The best separation was obtained when a mixture of cyclohexane, chloroform and Diethylamine in proportion of 5 : 4 : 1 was used as the solvent system. Both Dragendorff reagent and chloroplatinate reagent were used as the spraying reagent. Four different spots were detected with *C. capsularis* but three spots were obtained in case of *C. olitorius*. Similar study with extract of Jute seeds of the two species is in progress.

B.4. Biochemistry

B.4.1. *Phosphorylation of sugars and permeation process*

The enzymatic phosphorylation of sugars engaged considerable attention during this period. It is now known that there are two principal ways by which sugar can be phosphorylated in the biological systems :

(i) by a kinase type of reaction where ATP is the phosphate donor as in hexokinase (ii) by a phosphate carrier protein (PCP) dependent phosphotransferase reaction where phosphoenolpyruvate (PEP) acts as phosphate donor.

In order to find out whether the recently reported PCP dependent sugar phosphorylating system (type ii) of microorganisms is anyway related to the biological transport or permeation of sugars by living membranes, the following studies have been made.

A short time incubation experiment revealed that the uptake of 2-deoxyglucose by a number of bacteria such as *Strep. faecalis*, *E. coli* B etc., could be nearly as efficient as with glucose. As 2-deoxyglycose can be phosphorylated but no further metabolized by microorganisms, the transport of this sugar into the cell and its biological phosphorylation could be studied conveniently. It was observed that 2-deoxyglucose disappearing from the cell suspension medium could be fully accounted for as accumulation of 2-deoxyglucose-6-

phosphate inside the cell. The analysis of cell free extracts of *E. coli* B showed the presence of PCP dependent phosphorylating system (type II) and its ability to phosphorylate 2-deoxyglucose. 2-Deoxyglucose could be phosphorylated by glucokinase (type I) present in the soluble fraction of the bacterial extracts according to earlier observations elsewhere. The significance of these two type enzymatic phosphorylation in relation to biological permention process is now being studied.

B.4.2. *Inhibition of nucleic acid synthesis of* 2,4-*bis* (*arylamino*) *pyrimidines*

In previous investigations it was observed that 2,4-bis (arylamino) pyrimidines are extremely potent inhibitors of microbial growth. It is possible to enhance the activity of this class of compounds by increasing the lipid solubility. In fact, if in the synthesis of these compounds the arylamino residue is so chosen as to decrease their water solubility, there is a concomitant enhancement of activity of the pyrimidine derivatives with respect to growth inhibition. For example, 2,4-bis (*p*-chloroanilino) pyrimidine, which has very little water sobulity, is a very potent inhibitor and can completely suppress the growth of *Salmonella typhimurium*, *Staphylococcus aureus* and *Candida albicans* at about 1 μg/ml concentration; whereas 2,4-bis (*p*-hydroxyanilino) pyrimidine, which is much more water soluble, shows growth inhibitory effect only at much higher concentration (100 μg/ml).

The effect of 2,4-bis (arylamino) pyrimidine on bacteria cannot be reversed by natural pyrimidine bases or their nucleosides when the inhibitors are used at slightly higher concentration than is necessary for full inhibition (e.g., 2 μg/ml of 2,4-bis (*p*-chloroanilino) pyrimidine). It is possible that these lipid soluble inhibitors may be acting at the membrane site interfering with the nucleic acid synthesis by some unknown mechanism.

Preliminary experiments revealed that when 2,4-bis (*p*-chloroanilino) pyrimidine is added to a rapidly growing *E. coli*-B culture at 10 μg/ml concentration, the synthesis of RNA is strongly inhibited immediately whereas the synthesis of DNA is relatively unaffected. However, at 15 μg/ml of the inhibitor, RNA and DNA synthesis are both stopped almost fully in *E. coli-B* cells. Inhibitors of nucleic acid synthesis in other microorganisms are now being investigated.

It is expected that the studies of this new class of synthetic nucleic acid inhibitors may throw some light on the actual *in vivo* mechanisms of RNA and DNA biosynthesis of microorganisms.

B.4.3. *Purification and properties of N-acetyl glucosamine* (*N-AcGm*) *kinase of hog spleen*

Considerable advancement in studies of N-acetylglucosamine kinase of spleen has been made. It has been found that the spleen has the highest concentration of the above enzyme when compared to the other tissues of hog. This is probably because the spleen degrades red blood cells when free aminosugars become largely available for re-phosphorylation in that organ.

It has now been possible to purify N-AcGm kinase upto a point where it showed electrophoretic homogeneity. All studies of enzymic properties have been made with this

2000 fold purified electrophoretically homogeneous preparation. The following characteristics of the enzyme have been established :

(a) The enzyme catalyzes the reaction $AcGm+ATP \xrightarrow{Mg^{+}} AcGm\text{-}6\text{-}phosphate+ADP$

(b) Mg^{++} could be replaced by Mn^{++} or Co^{++}, but Ca^{++} or Zn^{++} is inactive.

(c) The enzyme showed absolute specificity for N-AcGm. N-Acetylgalactosamine, glucose or glucosamine are inactive.

(d) CTP and GTP are only slightly active as phosphate donor. UTP is completely inactive. ATP is so much more active than other nucleoside triphosphates in this enzyme reaction that one can safely conclude that ATP is the natural substrate of the enzyme.

(e) Km values for ATP and AcGm respectively are $1{\cdot}8\times10^{-3}$M and $1{\cdot}2\times10^{-3}$M.

(f) A broad optimum *p*H for N-AcGm kinase is obtained between 8·6 and 9·4.

(g) Both pyrophosphate and inorganic phosphate inhibit enzyme activity. The inhibitory effect of pyrophosphate is much stronger than inorganic phosphate. *p*-chloromercuribenzoate is a very strong inhibitor of the enzyme, but its inhibitory activity can be completely reversed by cysteine or 2-mercaptoethanol.

Whether N-AcGm kinase has an allosteric site in addition to active site is now being investigated.

B.4.4.

In connection with the studies of aminosugar metabolism in micro-organisms, it has been found that the cell free extracts of *Staphylococcus aureus* contain two phosphorylating enzymes : one specific for glucose and the other for N-acetyl glucosamine. Zelenick *et al.* in 1965 showed that the crude extracts of *Streptococcus pyogenes* contained an enzyme that catalysed the ATP-dependent phosphorylation of both glucose and acetylglucosamine. They purified the kinase 1500 folds and put forward evidences to show that the same kinase catalyzed the phosphorylation of the two sugars.

In *S. aureus* the ATP-dependent phosphorylations of glucose and acetylglucosamine are not catalyzed by the same enzyme. Instead, two separate enzymes — glucokinase and N-acetylglucosaminkinase are present in this organism. None of the enzymes is active on fructose or mannose.

B.4.5. *Mode of Enzyme action and control of metabolic pathways*

Considerable work has been done in recent years to elucidate the mechanism of enzymatic epimerization involved in the transformation of UDP glucose to UDP galactose. The yeast enzyme with which most of the work has been done differs in some significant respect from the mammalian and *E. coli* enzyme. For instance NAD which is needed for the catalytic activity is bound in one case and free in the other. Again the yeast enzyme is highly fluorescing whereas the mammalian one does not have any fluorescence at all. To understand the implications of these differences the liver epimerase has been purified and some preliminary kinetic analysis made. It seems that UMP inhibits the liver epimerase the same way it does in yeast enzyme but unlike the yeast enzyme no activation of the liver enzyme is observed with glucose at sub-saturation levels.

C. BOTANY

C.1. Plant Physiology

C.1.1. *Study of the action wave propagation in the sub-petiole of Mimosa pudica*

When a stimulus is applied to either end of subpetiole of *Mimosa pudica* the pinnules are found to close in pairs serially from the point of application of stimulus to the other end. An attempt was made to detect whether any electrical variation is produced due to contractile movement of the pinnules along with the propagation of action wave through the sub-petiole.

In the present investigation one end of a subpetiole was connected with an electronic amplifier and the other end was connected with the earth. Local stimulation was applied to the end away from the electrode connected with the amplifier. In the record obtained by the oscillograph it was found that during the closure of each pair of pinnules there was small positive electrical variation, before the action wave finally reached the terminal electrode, as indicated by a large negative action potential there.

It has been proposed to amplify the small deflections and record them photographically for detailed studies. A photographic recording arrangement with fast movement of the recording paper has been proposed to be constructed for the purpose.

In this connection mention may be made of similar study of Sibaoka (1950) with electrometer. He observed small electrical deflections occurring during the closure of the pinnules. But he has not discussed about the sign of such deflections and the cause of their occurrence.

Instanteneous appearance of positive impulse at a distance indicates that these are probably produced by some hydromechanical disturbance due to contraction of the pinnules.

C.1.2. *A device for application of measured electrical stimulus in action wave studies*

From the very beginning of the study of action wave in this laboratory, with electronic apparatus, attempts were made to apply electrical stimulus to produce excitation in the plant. In producing intermittent excitation to the same plant in some experiments, it is essential that the intensity of stimulus must be measured and kept constant throughout. But in the process of application of the stimulus there was violent electrical imbalance in the electronic apparatus. Various attempts have been made to eliminate the electrical imbalance thus produced.

In the previous report a device was described in which the plant had to be enclosed in a metal box with a portion of the leaf protruding through a slit. Arrangement was made to apply electric stimulus to that portion of the leaf, outside the metal box, with the lead wire connections kept inside the chamber. In this way a measured electrical stimulus could be applied without appreciably disturbing the electronic apparatus. But the metal

box had to be discarded afterwards because the plant could not maintain its tonic condition enclosed within it. Recently, a working method has been set up in which the plant is kept in the wire net cage and electrical stimulus applied to any of its parts through an induction coil without much disturbances to the electronic system.

In this method one of the electrodes of the secondary coils is connected to earth and a single induction shock produced by the break current of the primary, is used to stimulate the plant. At the inception of the shock a sharp cross line is produced in the oscillograph which does not distort the propagation of the action wave. The cross line may be taken as the time marker of the application of stimulus.

Some observations in *Mimosa* has been proposed to be performed with this method of stimulation.

C.1.3. *Effect of application of load on the pulsatory movement of the leaflet of Desmodium gyrans*

The purpose of the investigation was to demonstrate how the pulsation is effected when the leaflet has to lift progressively increasing load during its movement towards the abaxial and adaxial directions and how the load-work relation is modified in such condition.

Work done by the leaflet with a particular pull in adaxial direction, is comparatively much less than that with similar pull in abaxial direction. Under abaxial pull the amplitude of pulsation is progressively increased upto 10 mg. load and with greater pull the amplitude gradually decreases. But the leaflet has been found to perform the maximum amount of work with a pull of about 40 mg. load in abaxial direction. Under abaxial pull the leaflet can move and do some work even with a load of 100 mg. Under adaxial pull, on the other hand, the amplitude of pulsation goes on decreasing with increase of load from the start and the capacity of movement is totally lost in the leaflet with a load of 50 mg.

The foregoing observations indicate that the abaxial side of the pulvinule plays the dominent role in the pulsatory activity of the leaflet. The faster movement in abaxial direction is independent of the weight of the leaflet and is probably due to active contraction of the cells in the lower side of the pulvinule. A plausible explanation has also been given, how the amplitude of pulsation can increase with pull of small loads in abaxial direction and how the leaflet can perform more work with loads in that direction.

C.1.4. *Study of diametric changes of different regions of leaflets of Desmodium gyrans concomitant with the pulsatory activity*

In the previous report it was mentioned that the pulvinule undergoes total contraction with the downward movement of the leaflet and expansion with up movement. The diametric change was recorded by photographic arrangement with a magnification of X 12,000. The actual diametric contraction of the pulvinule during the downward movement of the leaflet was found to vary between 2 to 3 μ in different specimens.

As the study of bioelectric potential of the pulvinule and other parts of the leaflet indicated that with the contraction of the pulvinule there is increase of sap pressure elsewhere, arrangement was made to study the diametric changes of other regions of the leaflet for further confirmation of the assumption. No such change was observed in the petiole. It was, therefore, proposed to study the diametric changes of the mid-rib and its correlation with the pulsation of the leaflet. In this experiment the leaf blade being fixed, arrangement was made to record the pulsation in a reverse way from the movement of the petiole which was kept free. By this process recording of uniform pulsation was also possible, but the record of the mid-rib, simultaneously taken, indicated no diametric change with the leaf movement.

It is quite likely that due to structural rigidity, the very low sap pressure is not sufficient to bring about any physical change in the petiole and the mid-rib.

C.1.5. *Electrical variation at different parts of the leaflet of Desmodium gyrans and its correlation with the rhythmic movement*

In the present investigation a comparative study of the patterns of electrical responses of the pulvinule and other parts of the leaflet, concomitantly executed with its rhythmic movement, have given indication that with the downward movement of the leaflet there may be expulsion of sap from the pulvinule through the mid-rib and lamina. The downward movement of the pulvinule indicates a negative potential, whereas, corresponding electrical sign developed in the mid-rib and lamina is a positive one. The electrical sign of the mid-rib and lamina bears similarity with the electrical impulse in *Mimosa* petiole produced by a hydrostatic impact from the contracting pulvinus. The negative potential in the pulvinule of *Desmodium* is produced due to its contraction. The mechanical record of its diametric changes also has shown that it undergoes total contractions during the downward movement of the leaflet. It could, therefore, be reasonably assumed that the electrical positivity at the mid-rib and lamina is produced by hydrostatic impact from the contractile pulvinule.

C.2. Cytogenetics and Plant Breeding

C.2.1. *Effect of ionizing radiations on different organs of the same plant*

During the year under report, investigations on radiosensitivity of different plant species were undertaken. The chief purpose of this study was to provide information on the effects of different ionizing radiations on different organs of the same plant for predicting factors responsible for radiosensitivity.

For the present study, chromosomal aberrations during mitosis and meiosis with special reference to interchange bridges, reunion of broken ends and fragments have been used for measuring the radiosensitivity.

Dry seeds of *Nigella sativa* and *Allium cepa* were treated with ten dosages of X-rays ranging from 10×10^3 to 100×10^3 r and those of *Crotalaria juncea* with five dosages ranging from 10×10^3 to 50×10^3 r, using a Philips C.T. Apparatus operating at 50 Kv. and 2 mA, without filter and a target distance of 2 cm. Seeds of these three species were also treated with 300 and 600 μc initial activity of P^{32} for 24 hours. Roots were treated with four dosages

of X-rays ranging from $2{\cdot}5\times10^3$ to $10{\cdot}0\times10^3$ r and with 280 and 560 μc P^{32}. For treatments with X-rays, dry seeds served as control; while seeds kept immersed in phosphate buffer and distilled water for 24 hours along with dry seeds were considered as control; for treatments with P^{32}. Flower buds while still on the plants were irradiated with four dosages of X-rays.

After irradiation of roots and flower buds, the root tip and pollen mother cells were fixed immediately after treatment and after 24 hours. All the materials for cytological studies were fixed in acetic alcohol and stained in lacto-propiono-orcein.

Except for treatments with P^{32} in *N. sativa* and *A. cepa* when germination decreased with increase in dosages, there was no relationship of germination on dosages of X-rays and P^{32}. In many cases, treatments with X-rays produced increased germination over the respective controls.

The chromosomal abnormalities in root tip cells of *C. juncea* after treatment of seeds with X-rays, increased with increase in dosage. In *N. sativa* and *A. cepa*, however, there was no correlation of chromosomal abnormalities on radiation dosage, particularly with high dosages above 50×10^3 r.

Treatment of seeds of *A. cepa* and C. *juncea* with P^{32} induced increased chromosomal abnormalities in root tip cells with incerease in dosage.

It was observed that chromosomal abnormalities produced in roots fixed immediately after treatments were few compared to those when fixed after 24 hours.

The dosages of X-rays applied on flower buds of *N. sativa* were too high resulting in severe fragmentation of most of the chromosomes and were not suitable for critical cytological analysis.

A considerably larger experiment has been undertaken to confirm the foregoing observations.

C.2.2. *Investigation on Recurring irradiation and Biological Dosimetry*

This is a grant-in-aid scheme financed by the Department of Atomic Energy, Government of India.

Seeds of *Plantago, ovata*, *Lathyrus sativus* and *Hordeum vulgare* (Malda, West Bengal), were treated with 20, 40, 60, 80 and 100 Kr X-rays. The treated and control seeds were sown in tin boxes containing sand and soil mixture which were kept moist by watering and in petri dishes with moist filter paper to observe growth and germination capacity of the seeds. The boxes were kept at 22°C in cold room. The continuous lighting was maintained by 4 flourescent tubes placed 56 cm. above the soil level of the boxes. In *P. ovata* more than 70% of the seeds have germinated in all cases except 100 Kr treatment where the percentage is 50·00. In *L. sativus* percentage of germination decreased with increase in dose excepting 60 Kr, treatment. Again the table shows that in *H. vulgare* there was no relationship of seed germination on X-ray dose (Table 1).

In *P. ovata* the height of the seedlings diminished with increase of doses, but there was no marked difference between the treated seedlings and the control. In *L. sativus* the growth of the seedlings was delayed after treatments with 20 and 40 Kr. In *H. vulgare* the height of the seedlings decreased with increase of doses.

TABLE 1

PERCENTAGE OF GERMINATION OF SEEDS

Treatment (Kr X-rays)	Plant species		
	Plantago ovata	Lathyrus sativus	Hordeum vulgare
0 (Control)	72.20	62.50	88.66
20	85.70	55.00	60.00
40	80.00	32.50	60.00
60	73.60	35.00	66.66
80	70.00	31.20	72.40
100	50.00	19.40	72.40

The study of root-tip squash preparations corroborates the 2*n* chromosome numbers in *P. ovata*, *L. sativus* and *H. vulgare* to be 8, 14 and 14 respectively.

C.2.3. *Radiation mutation in Aus paddy*

Studies on Aus paddy

Three radiation induced mutants viz., S.1 (Black mutant) S.240 (White Dular), S.236 (Awned Marichbati) were put to micro yield trial at the Bose Institute Experimental Stations at Falta and Shyamnagar. Of these, S.1 yielded significantly (at 5% level) more grains than the control in both the locations.

C.2.3.1. *Radiation sensitivity of a mutant*

In this experiment one mutant, namely S.1 (Black Mutant) and its control (Marichbati) were irradiated with 25, 50 and 75 Kr X-rays. The treated seeds were allowded to germinate at 30°C, and the germinated seeds were later put in the field. During harvest time, data on survival, plant height, number of tillers, number and length of panicles were collected both from the treated and control plants.

With equal doses of X-rays applied, the percentage of survival was more in the mutant than the control (Table 2).

TABLE 2

SURVIVAL (%) OF PLANTS AT MATURITY

Treatment (Kr X-rays)	Type	Control (Marichbati)		Mutant (Black Mutant S.1)	
		Absolute	Relative	Absolute	Relative
0 (control)		82.00	100.00	86.50	100.00
25		78.30	95.48	80.25	92.77
50		62.12	75.75	75.70	87.57
75		48.50	59.74	60.50	69.94

From the studies of agronomical characters it was clear that except the comparison of plant height, no tolerance was observed after the treatment of X-rays on Mutant type over control Marichbati variety (Table 3).

TABLE 3

COMPARISON OF AGRONOMICAL CHARACTERS OF THE MARICHBATI VARIETY AND BLACK MUTANT (S.1) AFTER X-RAY TREATMENT

Characters	Plant height (cm)				Number of Panicles				Panicle length			
Treatment	Marich-bati	Percen-tage over control	Black Mutant (S.1)	Percen-tage of control	Marich-bati	Percen-tage over control	Black Mutant (S.1)	Percen-tage of control	Marich-bati	Percen-tage over control	Black Mutant (S.1)	Percen-tage over control
Control	116.03	100.00	113.48	100.00	8.02	100.00	11.96	100.00	20.68	100.00	22.31	100.00
25 Kr	116.32	100.24	110.76	97.60	8.44	105.25	12.78	106.85	20.92	101.45	25.50	114.25
50 Kr	110.73	95.43	112.36	99.01	7.55	94.3	8.58	71.73	21.65	104.63	24.96	111.80
75 Kr	111.39	96.00	109.28	96.29	8.12	101.24	9.06	75.75	20.69	100.00	22.61	101.34

C.2.3.2. *Cytological studies of the induced mutants*

The cytological studies of the induced mutants in Aus and Boro Paddy were made and the results obtained are given below :

Interesting variation in the chromosome number was observed in the Selection S.221 and a very small frequency of anaphase bridge and delayed separation were observed in the Selections S.370 and S.221. (Table 4).

TABLE 4

STUDIES ON THE MEIOTIC DIVISION OF CONTROL AND MUTANTS

Name of Types	Chromosome number (n)	Chromosome Abnormalities (%)			
		Metaphase I	Anaphase I	Metaphase II	Anaphase II
Marichbati (Cont.)	12	0.00	0.00	0.00	0.00
Chinsura Boro II (Cont.)	12	0.00	0.00	0.00	0.00
S. 221	12—13	—	4.25	—	—
S. 370	12	0.00	0.00	0.00	0.70

Lodging analysis of the mutant

Number of lodging resistant (S.401 and S.240) as well as lodging susceptable mutants have been isolated whose agronomical behaviour in different soil conditions have been tested. The observed results will be reported after statistical analysis is completed.

C.2.3.4. *Inheritance studies on Neck leaf mutant*

The neck leaf mutant S.268 was isolated from the X-irradiated population of the variety Marichbati and this character is breeding true. Reciprocal crosses were made using the mutant and the control which is devoid of neck leaf, as the two parents. In F_2 the mutant characteristic segregated in 3 : 1 ratio with neck leaf as the recessive.

C.2.4. *Cytological and Cytotaxonomical studies in some genera of Malvaceae*

The present study is a continuation of the cytological investigations in the family Malvaceae, reported in previous years.

The materials investigated are as follows :

1) Genus—*Malvaviscus* Cav.

Malvaviscus arboreus Cav.

Malvaviscus drummondii Torr., and Gray.

2) Genus—*Hibiscus* Linn.

Hibiscus micranthus Linn.

Hibiscus mutabilis Linn.

(a) White flower variety.

(b) Pink flower variety.

Due to the presence of large number of chromosomes which are extremely small in some cases and variation in the number of chromosomes per cell, extensive observations had to be taken up in the materials. It was also required to modify the prefixation schedule with Aesculin and in the final preparations of root tip squashes with propiono-orcein.

Malvaviscus arboreus Cav.—Plant shrubby : leaves alternate, palmately veined, toothed : flowers convolute in the bud, scarlet, never fully expanding : bactlets erect and narrow.

In this species the chromosome number per cell in the same root tip varied considerably. Skovsted (1935) reported the $2n$ number as ca 84. In the present investigation aneuploid numbers ranging from 24 to 84 have been recorded. However, $2n = 42$ chromosomes occurred in maximum frequency. Critical studies of these numbers and their karyotypes are being made with a view to establish the role of polyploidy in the evalution of this species.

Chromosome length in the $2n$ complement of 42, varies from 5μ to 2μ. Primary constrictions are mainly of median and submedian types. There are three pairs of satellited chromosomes. One pair has the satellites at the end of the short arms of long chromosomes with submedian primary constrictions. The second pair is satellited with big knobs and short stalks attached to the short arms of the long chromosomes also with submedian primary constrictions. The third part having small knobs and long stalks are attached to one of the arms of medium sized chromosomes with median primary constrictions. Root tip cells with 28 chromosome bear only one of each of the second and third pairs of satellites along with the first pair and that in 84 chromosome complements the secondary constrictions were found to be exactly double of those occurring in normal complement. Feulgen-Fast green tests showed six chromosomes attached to a nucleolus at the early prophase stage and also three pairs of small nucleoli at the telophase indicating the probability of their origin from the satellited chromosomes.

In addition to the occurrence of hypo- and hyper-ploid numbers, laggards and bridges at somatic anaphase were also observed as abnormalities.

Malvaviscus drummondii Torr., and Gray.—Tall perennial shrub : leaves round-cordate mostly 3-lobed : flowers red : column of stamens becoming excerted : fruit berry-like, red, finally separable into carpels.

The species has 64 chromosomes in its somatic complement. Lengths of chromosomes vary from 5μ to about 2μ. There are four pairs of satellited chromosomes. One pair having big knobs is attached at the end of the shorter arms of the submedianly constricted long chromosome pair. Second pair with small knobs and long stalks is attached at one of the ends of long chromosome with median primary constrictions, and the third and fourth pairs with small knobs and long stalks occur at the shorter arms of the submediantly constricted

medium sized chromosomes. 4 pairs of small nucleoli at telophase and 8 chromosomes attached to a nucleolus at early prophase were observed.

Laggards and somatic bridges were observed as abnormalities.

Hibiscus micranthus Linn.—Plants shrubby with rod like spreading branches, thinly covered with stellate bristles : leaves oblong, serrate, rough with bristly hairs : petioles short : bracteoles linear, shorter than the calyx : flowers pink (or white) : corolla reflexed : anthers whorled : capsule globose : seeds cottony. (Roxburgh suspects that it may be specifically identical with *H. hirtus*.).

The present investigations with seeds collected from various sources reveal that the normal $2n$ complement consists of 64 chromosomes, which has also been supported by the meiotic studies from the flower buds of the same plants and corroborate the findings of Skovsted (1941). Previously 72 has been reported to be the diploid number of this species, indicating that polyploid forms of this material occur in nature. Length of the chromosomes varies from 4μ to $1{\cdot}4\mu$. Primary constrictions are mainly median and submedian. There are four pairs of satellited chromosomes. One pair at the ends of the short arm of the long chromosomes with submedian constrictions ; the second with stalks and small knobs on the short arm of the submedianly constricted long chromosomes while the third and fourth pairs having stalks and small knobs at the ends of the short arms of submediantly constricted medium sized chromosomes. Eight chromosomes were found to be attached to a nucleolus at early prophase.

Abnormalities like bridges were observed both in mitotic anaphase and anaphase II and occurrence of laggards at the mitotic anaphase only.

Hibiscus mutabilis Linn.—Small tree like shrub : leaves cordate, toothed : bracts shorter than calyx : flowers 7 to 10 cm. in diam : sepals connate below the middle : corolla white or pink on first opening in the morning, deep red by evening : capsules globose : seeds hispid.

Two varieties of this species were studied. At blooming during morning the flowers of one are white, while those of the others are pink in colour. But the flowers of both the varieties turn red by evening. These two varieties have different chromosome numbers.

a) *White flower variety*.—It has a $2n$ complement of 90 small chromosomes having median to submedian primary constrictions. Lengths of the chromosomes vary from $3{\cdot}5\mu$ to $1{\cdot}5\mu$. Six pairs of secondarily constricted chromosomes were observed.

b) *Pink flower variety*.—This variety has 110 chromosomes in its $2n$ complement. Lengths of the chromosomes vary from $3{\cdot}5\mu$ to $1{\cdot}5\mu$. Primary constrictions are generally median but existence of submedian constrictions in a few were also observed. Number of secondarily constricted chromosomes were found to be twelve.

Abnormalities like hyperploid numbers at metaphase and somatic bridges and laggards at anaphase were observed.

C.2.5. *Cytotaxonomical studies on the genus Capsicum*

For cytotaxonomical studies on the genus *Capsicum*, forty five varieties of *Capsicum annum* L., fourteen varieties of *C. frutescens* L., nine varieties of *C. pendulum* Willd., five varieties of *C. pubescens* Ruiz et Pav., and one variety of *C. sinense* Jaques were used.

The species and varieties investigated show a constant diploid number of twenty four chromosomes in the somatic complements. In general, the chromosomes are medium in size ranging from 2·88μ to 6·72μ and possess median to subterminal primary constrictions. The size differences of the chromosomes, in the different varieties vary in *C. annum*, from 6·72μ–3·04μ; 6·08μ–2·88μ in *C. frutescens*; 5·44μ–3·04μ in *C. pendulum*; 5·44μ–3·36μ in *C. pubescens* and 5·44–3·84μ in *C. sinense*. In every species and varieties, a gradual gradation in size from long chromosome to short ones has been noted and three groups of chromosomes —long (5·44μ–6·72μ), medium (4·16μ–5·12μ) and short (2·88μ–4·00μ) can be arbitrarily recognized.

Secondary constrictions are found to be present on two to three pairs of chromosomes. In ten varieties of *C. annuum* and one variety of *C. pendulum*, secondary constrictions are located on three pairs of chromosomes, whereas in the rest of the varieties of *C. annum* and *C. pendulum* and in all varieties of other species studied two pairs of chromosomes bear secondary constrictions. From nucleolar studies at early telophase stage of mitosis, numerical correlation between the number of secondary constrictions and maximum number of nucleoli has been found in each variety of the species.

The chromosomes of the species and varieties studied, do not exhibit much structural variation. However, a detailed analysis of the karyotypes reveals the presence of certain distinct types of chromosomes, different combinations of which are present in different species and varieties. In all, eleven types are classified on the basis of size and position of primary and secondary constrictions.

Table 5 shows the detailed analysis of the chromosome complements of the species of *Capsicum*.

TABLE 5

CHROMOSOME COMPLEMENTS OF DIFFERENT SPECIES OF *Capsicum*

Species (chromosome number 2n=24)	Chromosome size			Average chromosome length in μ	TF%	S%
	Long 5.44 μ — 6.72 μ	Medium 4.16μ — 5.12 μ	Short 2.88 μ — 4.00 μ,			
C. sinense	6.0	16.0	2.0	4.6	45	71
C. pubescens	4.0	17.3	2.7	4.7	45	67.3
C. pendulum	1.6	15.6	6.8	4.3	44.9	68.3
C. frutescens	1.6	12.4	10.0	4.2	44.6	65.6
C. annuum	4.0	13.8	6.2	4.5	43.7	54.4

A striking feature observed during the study of microsporogenesis in *Capsicum* was that some of the varieties belonging to different species showed regular and irregular behaviour of the chromosomes during meiosis at different stages of their floral flushes. Flower buds collected during early flush of flowering exhibited regular meiosis, whereas chromosomal abnormalities like varying proportion of univalents, tripolar spindles, lagging bivalents, micronuclei, more than four microscopes or only two microspores etc., were observed in flower bud of late flush. Associated with irregularities during meiosis there was an increase in the percentage of pollen sterility.

Twin seedlings have been noted in five varieties of *C. annum* viz., Californian Wonder, Chilli-yellow, World Beater, Ruby King and Elephant Trunk. Both the seedlings are entirely separate and one of these was less vigorous in growth and though possessed its own cotyledons were smaller and thinner than the other showing normal growth. Cytological studies revealed that the twin seedlings were of diploid—haploid in nature.

From the karyotypic and meiotic analysis, it has been suggested that structural alteration of chromosomes and gene mutations played important roles in the speciation of the genus. Further, *C. annuum* seems to be the most evolved and *C. sinense* the most primitive among the species studied.

The relationships among these species obtained from crossing experiments (Smith *et al.*, 1957; Hirose *et al.*, 1960) corresponds with the relationship traced from the present cytological studies.

C.3. Anatomy and Taxonomy

C.3.1. *The organization and periodicity of the shoot apex of Mimosa pudica L.*

With a view to have detailed information over the causal aspects of foliar movements in the sensitive plants, a thorough histological and experimental investigation on the primary morphogenetic regions and the leaf pulvinar tissues involved in motility has been undertaken. This approach may be helpful in understanding the effects of chemical, surgical and other physiological experiments on them, and the present report on the shoot apex organization and the leaf histogenesis of *Mimosa pudica* L. is a part of the above project.

The vegetative shoot apex organization of *Mimosa pudica* L. has been studied upon collections in early February and late May, 1966. The apical meristem forms a low mound; the vertical height being about 25μ to 47μ in summer and 20μ to 26μ in winter and the transverse diameter ranged from nearly 75μ to 150μ in summer and 65μ to 90μ in winter.

The seasonal variation and periodicity of growth and development of the plant has been reflected in the apical region which displays in summer a four zoned pattern indicating a 4-layered tunica and a complex corpus of 3 zones of initials, rib- and flank-meristems, which differed in position and cytohistological qualities. In shoot tip collected in winter the zone of corpus initials appeared to be less distinct and the rib-meristem constituting the central core of cells was smaller in extent and lacked in characteristic cellular arrangement.

The leaf primordium was noted to initiate during the maximal area phase of the apical meristem with organizing a leaf growth centre which involved a group of active cells in the inner tunica layers and outer corpus one and was accompanied by the first periclinical division in the third tunica layer. The vegetative bud initial may be traceable in the axial of the third youngest leaf primordium as a bud meristem showing the first periclinal in the third tunica layer.

C.3.2. *A study of leaf histogenesis in Mimosa pudica L.*

In the shoot apex of *M. pudica*, the leaf primordium in the early stages of development forms a lateral protuberance very close to the summit. In the third youngest leaf initial of less than half a mm. height, three regions are distinguishable, e.g., basal petiole, middle with pinna initials and terminal one having vacuolating and inactivating cells. Gradually the pinnules start coming up in an acropetal order on either side of the pinna belonging to the fifth leaf primordium. The first pair of basal pinnules may not grow further and result in the two spine- or scale-like foliar growths at the base of each pinna.

The tiny bristle-like scaly structures as evident at the top of mature leaf and pinna rachises resulted from the vacuolation and inactivation of their primordial apices. The stipules are persistent and initiate very early as lateral paired developments on either side of the second youngest leaf from top and attains quickly almost its full length at the base of the sixth leaf primordium.

The ontogeny of the multicellular linear and bulbous trichomes differs sharply. The smaller linear hairs appear as an outgrowth of the outer epidermal cell wall, but in the initiation of the massive bulbous ones, one entire epidermal cell is involved. Their relative distribution was observed to vary from part to part. The surface of stem, rachis, pinnule and stipule is densely coated with thin linear hairs, but in the pulvinar region and stipular margin, the second type predominates.

The distribution of the axial prickles follows a regular pattern, being three per leaf; the median one appears just below the mid-region of leaf attachment and the lateral two on either side of it, which are shifted down with the elongation of internode.

C.3.3. *Floral anatomy of some members of the sub-family Papilionaceae*

During the year under report, floral anatomical investigations on the following fourteen plant members under five different tribes of the sub-family Papilionaceae have been made critically, in order to justify the taxonomic status of the investigated members in the existing system of taxonomic classifications based on external morphological characters. The study is intended also to trace the probable phylogeny and evolution in the investigated members, tribes and even the sub-family. Cytotaxonomic studies on these members have been made and reported pereviously.

Sub-family—Papilionaceae

Tribe : Genisteae

1. *Crotalaria juncea* Linn.

Tribe : Trifolieae

2. *Melilotus alba* Lamk.

Tribe : Hedysereae

3. *Desmodium gyrans*. D.C.

Tribe : Vicieae

4. *Pisum sativum* Linn.
5. *Lens esculenta* Moench.
6. *Lathyrus odoratus* L.
7. *L. sativus* Linn.
8. *Cicer arietinum* Linn.

Tribe : Phaseoleae

9. *Clitoria ternatea* Linn. (Single flower)
10. *C. ternaetea* Linn. (Double flower)
11. *Phaseolus calcaratus* Roxb.
12. *P. mungo* Linn.
13. *Dolichos lablab* Linn.
14. *Cajanus indicus* Spreng.

External morphology of the flowers of these members and their vascular anatomy have been studied. From the mode of vascular trace departures and their pattern of cohesion and adnation, the investigated members have been divided under the following two main types :—

(A) Two sets of gaps are formed in the stele due to the departures of perianth and stamen traces. This is observed in *Crotalaria*.

(B) One set of gap formed in the stele due to perianth and stamen trace departures.

The latter type has again been sub-divided into two types :—

(1) In which the perianth and stamen traces though arise from a single set of gaps in the stele, the gaps of perianth traces intrude into the departing stamen traces in such a way that the stamen traces appear double in origin. *Desmodium* of Hedysereae, *Melilotus* of Trifolieae, *Lathyrus*, and *Lens* of Vicieae and *Clitoria* of Phaseoleae have been characterized by this feature.

(2) In which one cycle of vascular strands for perianth, stamen and even disc, where present, arises from one set of gaps. This condition has been noted in *Cicer* of Vicieae and in *Phaseolus*, *Dolichos* and *Cajanus* of Phaseoleae. Except *Cicer* all these members possess and additional whorl of organ, the disc.

On the basis of the present study an attempt has been made to find out the phylogenetic status of the investigated tribes, genera and species and the evolutionary trends within the sub-family.

The genus *Crotalaria* appears here to be most primitive in having ten free stamens and two sets of gaps in the stele for perianth and stamen traces.

In the next higher step in evolution, the set of gaps for perianth traces extend upto the gaps of stamen traces and this coalescence makes the two sets of gaps appear as one. Here the evolution seems to have occurred due to shortening of intrernodes between perianth and stamen traces. This phase in the evolution has been found in the members of Vicieae, Trifolieae and Hedysereae. *Pisum* of Vicieae and *Desmodium* of Hedysereae show primitiveness in their vascular behaviour compared to that of the other members of this group.

The highest degree of evolution is attained in the members of Phaseoleae and in *Cicer* of Vicieae. Here, adnation of vascular traces appears in extreme form. The traces for perianth, stamen as well as for disc (where present) on the small radii fusing together form a composite unit which after arising, leaves a single set of gaps in the stele. Among these members *Cicer* appears to be the most evolved genus showing the total absence of disc. The members with disc indicate their origin from a double whorled stamened stock where disc represents the second whorl of vestigeal stamens.

The probable origin of the sub-family is considered here in the light of both monophyletic and diphyletic lines of evolution. Further, some suggestions have been put forward for some amendments regarding the taxonomic position of a few genera investigated.

Anatomical study of the flower of *Clitoria ternatea* somehow does not accord with the taxonomists' view regarding its assignment in the tribe Phaseoleae. Unlike the other members of Phaseoleae, it shows two separate sets of traces for perianth and stamens. The transference of the genus from Phasoleae close to the tibes Vicieae or Trifolieae has, therefore, been suggested. Further, from the floral anatomical studies the difference between Double-flowered variety and Single-flowered variety of the species, two separates species status has been proposed for them.

Taxonomists' view about the position of *Cicer* in Vicieae has again been debated from the present floral anatomical studies. The inclusion of *Cicer* as the highest member in Phaseoleae has been proposed due to extreme form of adnation in the vascular traces and further the absence of disc in it.

Fundamentally, the members of the sub-family follow some basic plan. In most cases, sepals receive three traces each from three gaps whereas the petals generally receive one trace each. Like petals, stamens and disc also receive a single trace each. The nature of gynaecium remains unaltered in all the species studied, where a carpel is supplied by three traces.

C.3.4. *Taxonomical Investigation on the family Boraginaceae*

The taxonomic treatments of the members of Boraginaceae have so far been based mainly on gross morphology. The family classified into 4 sub-families (Bailon, Bentham and Hooker, Englar and Prantl) as follows : 1. Cordioideae, II. Ehretioideae, III. Heliotropioideae, and IV. Boraginoideae. (The fourth sub-family is again divided into several

tribes). Recently Hutchinson (1960) gave a separate status to Cordioideae and Ehretiodeae into a single family Ehretiaceae.

So a detailed work on cytology, anatomy and palynology of several members of this family has been carried out in order to find out their taxonomic position in this family.

Cytology.—The investigated members with their chromosome numbers have been tabulated below :

	Name of Plant	*Somatic Chromosome number* (2*n*)	*No. of chromosome with secondary constriction*
1.	*Cynoglossum amabile* Stepf & Orammond.	24	2
*2.	*C. wallichii* G. Don.	24	2
*3.	*C. lanceolatum* Forsk.	24	3
4.	*Anchusa capensis* Thunberg.	16	2
5.	*Echium plantagineum* Linn. var. blue	16	2
6.	*E. plantagineum* var. pink	16	3
7.	*Onosma hookeri* Clarke.	18	2

Chromosome-numbers for plants with (*) asterisks are reported for the first time.

For the study of somatic chromosomes, the root tips of the plants were treated in paradichlorobenzene for 30 mins. to 2½ hours at a temperature of 8–10°C. followed by fixation in propionic acid and alcohol mixture (1 : 2) and stained in propiono-orcein. Karyotypic study among these species shows that there is a close similarity in the morphology of chromosomes within the same genus but a clear dis-similarity in the morphology of chromosomes among the genera. Short chromosomes are found in the *Cynoglossum* spp., *Echium* spp., medium sized chromosomes in *Onosma* spp., and long chromosomes in *Anchusa capansis*.

Floral anatomy.—In all cases the flowers are pentamerous except in *Coldenia procumbens* and *Cordia sebestina* where the flowers are tetra and 6–8 merous respectively. The central stele in each case is either a ring of 5 and more bundles or a simple siphonostele. In *Ehretia*, *Heliotropium* and *Coldenia*, 9–10 sepal traces depart from the central stele and enter into the calyx tube. The traces, acting as midrib bundle remain undivided, while other traces divide and give rise to one or two laterals. At a higher level, 4–5 petal traces arise alternating the sepal midrib. Simultaneously with the departure of petal traces, 5 stamen traces arise. Both petal and stamen traces enter into the corolla tube and remain in such condition for a long time. At the time of differentiation of filament from the corolla tube, each of the petal traces gives 2 laterals which move rapidly towards the base of the filament. In *Cynoglossum* and *Echium*, the sepal, petal and stamen receive one trace each and the sepal trace divides to give rise to 2 laterals.

After the departure of sepal, petal and stamen traces, the remaining vascular tissue may take the form of a ring or may divide into four masses triangular in shape. In all cases dorsal traces are differentiated before the 4 ventrals are formed. In *C. sebestina* and *E. laevis* 6 ventral traces are distributed equally in two parallel rows. The middle one in each row

remains inactive. Four ovarian chambers having a single ovule each, are developed. Each ovule gets a supply from each of the ventrals. In case of *Cordia sebestina* the basic plan of vascular distribution is different. A number of traces from the axial stele along with some accessory bundles arising from the cortex of the pedicel pass into the calyx tube. A number of small bundles of the axial stele separate out after giving rise to petal, stamen, disc and dorsal traces. Ultimately these bundles of the axial stele give rise to ventral traces. After the departure of sepal traces, petal and stamen traces arise and enter into the corolla tube. Before the insertion of filaments, each of the petal traces divides several times. After the departure of petal and stamen traces the remaining vascular tissue is divided into a number of small bundles which run towards the circumference of ovary to supply the disc. So a well supplied disc like structure is formed around the ovary. Two dorsal traces are differentiated out from the central ring of vascular elements before the departure of petal and stamen traces. The two dorsals remain undifferentiated in the bundles of disc for a considerable period. But when locules are formed, they become prominent and extend towards the end of style.

Palynology. A detailed study of pollen grains was made in *Cordia sebestina, C. aublutii, Ehretia laevis, Coldenia procumbens* and *Heliotropium indicum.*

Cordia sebestina.—Pollen grains spheroidal (diameter varies from 52·9μ to 39·1μ), tricolpate. Colpa syncolpate lolongate, (average length and breadth about 37·5μ to 25·3μ and 2·5μ). Mean inter colpian distance 14·8μ. Exine 3μ to 3·6μ thick, inhectate, psilate. Sexine about 2·7μ to 4μ thick, reticulate. Reticulum homobrochate (lumina slightly varying in size). Muri 1–1·5μ wide, simplibaculate. Bacula about 2·7μ to 3μ in length, broader and united at the apex. Lumina penta or hexa-angular, length and breadth about 2μ×1·3μ). Nexine very thin about 0·6μ thick.

Cordia aubletii.—Pollen grain spheroidal, (diameter about 39·2μ to 40μ), triporate. Pore more or less circular (diameter about 5·1μ). Exine about 3·9μ thick, intectate. Sexine about 2·8μ thick reticulate. Reticulum homobrochate. Muri about 1 to 1·8μ wide, retipilate. Bacula about 2·5μ to 2·8μ long, slightly broad at the apex, simpybaculate. Lumina more or less circular, about 3·2μ wide. Nexine very thin, about 0·6μ thick.

Ehretia laevis.—Pollen grain prolate to spheroidal (length and breadth about 18·8μ ×1·6μ), Tricolporate, colpa syncolpate (length and breadth about 12·5μ×1·6μ). Amb hexagonal due to the thinness of sexine in central part than in the marginal part of the mesocolpia. Pore more or less circular (diameter about 1·3μ). Mean intercolpian distance about 3·8μ to 4μ). Exine about 1·25μ thick, tectate, psilate. Sexine about ·75μ thick, gradually thinner towards the pore, punctitegillate (puncta inconspicuous). Bacula uniformly spread, length about ·7μ. Nexine nearly as thin as sexine about ·5μ thick.

Coldenia procumbens.—Pollen grain prolate (length and breadth about 21μ×15·9μ). Triporate, Pore lalongate (diameter about 45μ×2·7μ). Mean interporian distance 4·6μ. Exine about 4·5μ thick, tectate, punctitegillate (puncta inconspicuous). Sexine about 1·8μ thick, more thicker towards the poral region. Bacula uniformly scattered. Nexine nearly half of the sexine, about ·5μ to ·6μ thick.

Heliotropium indicum.—Pollen grain prolate (about 30·65μ×25·2μ). Tricolporate. Colpa syncolpate (about 21·8μ×3·6μ), lalongate. Pore almost lolongate (about 4·6μ×2μ). Mean intercolpian distance 8·2μ. Amb hexagonal due to preasence of pseudocolpia. Exine about 1·5μ thick, tectate, psilate, Sexine and Nexine not distinguishable, punctitegillate (puncta very much inconspicuous).

C.3.5. *Taxonomic Position of the Genus Nyctanthes*

The Genus *Nyctanthes* Linn. with only one species of *N. arbortristis* Linn. was originally included in the family Oleaceae mainly on account of the structure of the flower which is somewhat like that of *Jasminum.* A second species *N. aculeata* Craib was later described from Siam (Craib, 1939). Till recently the status of the genus *Nyctanthes* as belonging to the family Oleaceae was maintained by all workers on Taxonomy.

Recently Shaw (1952) included the genus in a new sub-family Nyctanthoideae under the family Verbenaceae mainly on the basis of some morphological characters.

A detailed comparative study of *Nyctanthes* along the with some members of Oleaceae, Loganiaceae and Verbenaceae on anatomy including floral anatomy, cytology, palynology etc., has been made in order to evaluate the correct taxonomic position of the genus.

Except such characters as lobate-dentate leaves and quadrangular stem, the general morphological characters of *Nyctanthes* particularly of the flower show much resemblance to those of the members of Oleaceae.

Although Stant (1952) supported the inclusion of *Nyctanthes* under Verbenaceae, it appears from the detailed anatomical evidences of trichomes, petiole structure, stomata, nodal structure, structure of the secondary wood, sclerosed cells etc., that the genus should be excluded from Verbenaceae.

The cytological study on the other hand, show that *Nyctanthes arbor-tristis* Linn., like some other genera of the Oleaceae (Darlington and Wylie, 1955) possesses 46 somatic chromosomes in its diploid cell.

From palynological point of view *Nyctanthes* appears to be related to *Jasminum* of Oleaceae rather than any members of Verbenaceae in the surface pattern of pollen grains.

Lastly, the alkaloidal, constituents of *Nyctanthes* which appear to be indoleaceous provide evidences in support of its affinity to the members of Loganiaceae (sens. Lat),

Hutchinson (1926), in his system of classification of Dicotyledons included Oleaceae and Loganiaceae under the same order Loganiales. Again the members of these two families in comparison to those of Verbenaceae have more characters related to those of *Nyctanthes.*

The family Loganiaceae has been split into six separate families (Hutchinson, 1959, 1964) where Strychnaceae comes as the last family prior to Oleaceae in the order Loganiales. From the evidences obtained it seems to be justified in shifting the genus *Nyctanthes* from Oleaceae and assigning it to a separate family which may be named as Nyctanthaceae. The proper phylogenetic position of Nyctanthaceae will be between Strychnaceae and Oleaceae under the order Loganiales of Hutchinson.

C.4. Palynology

C.4.1. *Study of pollen morphology with special reference to Taxonomy*

Convolvulaceae.—This family consists of about 50 genera and 1200 species in its world wide distribution but in India it is represented by 20 genera only. For the pollen morphological studies 9 genera have so far been investigated, namely *Erycibe*, *Argyreia*, *Jacquemontia*, *Ipomoea*, *Convolvulus*, *Rivea*, *Lettsomia*, *Hewittia* and *Evolvulus*.

The pollen grains were acetolysed in the classical method of Erdtman which was later slightly modified with an adaptation to the tropical climate. This experiment gave a good result for the preservation of micro-slides. For sealing paraffin of higher than usual melting point has been used (60°–62°C) which provided resistance to the hot summer climate of India.

The morphological pattern and character of the pollen grains of this family vary to a considerable extent. Four species from *Erycibe* have been investigated, i.e., *E. expansa*, *E. laevigata*, *E. princei* and *E. paniculata*. Pollen grains are prolate ($28\mu \times 38\mu$), psilate, tricolpate, punctitegillate. In some cases colpa is surrounded by a thick margo, but in others margo is absent (*E. princei*, *E. paniculata*).

Three species from *Argyreia* (*A. cuneata*, *A. speciosa*, *A. tiliaefolia*) pollen morphologically differ from *Erycibe* by having spherical grains which are also considerably larger in size (diam. 71μ–117μ), echinate, panotoporate (number of pores 22 to 85), pores circular.

Rivea and *Lettsomia* show a close similarity with *Argyreia* in pollen morphology suggesting closer affinity. The pollen grains of two species from *Convolvulus* (*C. arvensis* and *C. pluricaulis*) are spheroidal, tricolpate in *C. pluricaulis* and pantocolpate in *C. arvensis*, both are punctitegillate.

Jacquemontia, *Hewittia* and *Evolvulus* together form a similar pollen morphological unit. *J. violacea* has spheroidal, pantocolpate pollen grains, colpa connected with each other by their ends and thus forming a continuous channel which are situated in grooves.

Ipomoea is the most interesting genus in this family. Species investigated are *I. bona-nox*, *I. hederifolia*, *I. batatas*, *I. obscura*, *I. biloba*, *I. pulchella*, *I. reptans*, *I. carnea*, *I. versicolor* (*Mina lobata*). Pollen grains in general are spheroidal, echinate, pantoporate. They exhibit a wide range of variation in exine stratification. The form and structure of spines are useful characters in making specific distinction, they are mush-room sphaed in *I. bona-nox*; blunt and more or less without neck in *I. versicolor* and *I. hederifolia*: broad at base and gradually tapering towards apex in *I. reptans*, *I. biloba* and *I. pulchella*; bulbous and broad at base but gradually tapering towards apex with a blunt tip in *I. obscura*, *I. batatas* and *I. carnea*; form a concave tuft of bacula at the spine-base in *I. pulchella* and *I. obscura*.

Solanaceae.—Three genera from this family have so far been studied—*Solanum*, *Cestrum* and *Datura*. Pollen grains are spheroidal to subspheroidal, triocolporate, colpa lolongate with a colpoid streak (in *Solanum trilobatum* colpoid streak is absent) faintly striate (*Cestrum diurnum*) but in *S. trilobatum* there is no striation. Pollen grains of *Datura* sp. have striato-reticulate pattern.

Casuarinaceae.—This is taxonomically a highly interesting mono-generic family. Only 12 species, *Casuarina humilis*, *C. chamaecyparis*, *C. frasariana*, *C. leuhmanni*, *C. suberosa*, *C. montana*, *C. cunninghamia*, *C. deplantheana*, *C. muricata*, *C. distyla*, *C. glauca* and *C. stricta* so far have been studied pollen morphologically.

Pollen grains generally are triporate, tectate, exine with faintly delimitied arcii. There is, a striking similarity between the pollen grains of *Casuarina* and those of Betulaceae, particularly *Betula*, also Myricaceae and certain pollen types of Juglandaceae.

C.4.2. *Pollen analysis, Geochronology and Palaeoecology of the peat bog deposits of Belgachia, Birati and Howrah*

Lack of data prevents the progress of pollen analytical work in West Bengal. No systematic approach has yet been done since there is no reference pollen herbarium. About one thousand microslides for the pollen herbarium in the Palynological unit have been made which give the possibility of the initial study of the microfossiliferous deposits of the Bengal Basin.

In the early quaternary time Calcutta, it is assumed, was a part of Sundarbans. Wood fragments in subfossil state have been found in Belgachia deposit. The anatomical study of the wood fragments are being done in collaboration with the Wood Anatomy Section of the Forest Research Institute, Dehradun. A pollen type (grains spheroidal, tricolporate, psilate, punctitegillate) of Belgachia deposit has got a very close resemblance with the pollen of *Bruguiera*, a constituent of the present mangrove vegetation of the Sundarbans. If the anatomy suggests the wood to be a part of mangrove vegetation, then it could be conclusively proved that mangrove vegetation once thrived in the Calcutta region.

C.4.3. *Geochemistry of peat stratigraphy and palaeolimnology of the quaternary deposits in relation to the pollen concentration*

The study will trace the residual organic substances in the sediments to explore its generalised relationship with the tropical climatic history of the body of water.

There is every chance of degradation of the chlorinoid pigments of the plants growing in lakes.

Small scale biochemical changes in lake sediments, if found, will be helpful for correlation with the fluctuations of the concentration in pollen spectra in the same strata.

Such biogeochemical investigation in the Palynological Section in collaboration with the Chemistry Department has been undertaken. At present quantitative estimation of free amino acid and protein is being made. The water soluble protein extracted from the peat samples, collected from Birati, amounted at first step to be 48μ gm per c.c., which after dyalisis came down to 32μ gm per c.c. keeping the volume constant. This suggests the possibility of having free amino acid from the same sample. In the second step extraction of water soluble protein by homogenising was found to have increased in volume to 132μ gm per c.c. Qualitative analysis is yet to be made.

C.5. Tissue Culture

C.5.1. *Normal and Crown Gall tissue culture*

It was reported in the previous report that for 14 successive fourteen-day transfers there was no cumulative 'poison' effect on tissue growth when iron was supplied in the form of iron chelate (Fe-EDTA complex). The experiment was continued and it was confirmed that Fe-EDTA complex could be used safely and continuously in culture media. Of the iron sources studied for the tissues, ferric tartrate was found to be most satisfactory for both normal and gall tissues, followed by Fe-EDTA, ferric sulphate, ferric chloride, ferric citrate, and ferrous ammonium sulphate.

C.5.2. *Single Cell Suspension Culture*

The method used for obtaining cell suspension culture of plant tissues was reported previously. However, the percentage of freely floating cells was comparatively low. In order to study the totipotency of plant cells it is necessary to increase this percentage. This can be done only by increasing the friable character of the tissues and using suitable method of agitation which provides aeration to suspended cells and physically aiding in the separation of cells from each other. Increase in speed beyond a certain limit is not desirable as this has deleterious effect upon the cultured cells. This was observed in experiments with high speed reciprocating shaker. However, the percentage of free cells was more. It was, therefore, thought worthwhile to see whether with low speed and vessels agitated end-on-end in a horizontal shaker result in good suspension. This can be done only when air-tight stoppered vessels are used. It is the usual practice, unlike animal culture, to close the culture flasks with cotton plugs. It was, therefore, necessary to examine the effect on growth of tissues grown in a closed atmosphere. For this purpose glass stoppered conical flasks (110 ml) were used and the tissues grown for 12 days. The result revealed a 41% decrease in tissue growth, suggesting that air-tight stoppered vessels for suspension culture studies are not satisfactory.

Increase in the speed from 1 r.p.m. to 4 r.p.m. of the revolving shaker resulted in a better suspension culture. Rotary shaker which was not used so far in this laboratory was tried and preliminary result indicates that it may give sufficiently thick suspension without much cell damage.

For increasing the friability of the tissues, twelve different media used in tissue culture studies are being tried. Along with this, effect of casein hydrolysate, calcium pantothenate, nicotinic acid, yeast extract, kinetin, 2-4-D, NAA, EDTA, sucrose, nucleic acid bases and calcium on tissue friability and growth in being investigated. Increase in friability was observed with 6% sucrose, EDTA, Calcium pantothanate and nicotinic acid when added individually to the medium. Decrease in friability as well as growth rate of gall tissue resulted when kinetin, 2-4-D and NAA was added to the medium. While for normal tissue, though there was increase in growth rate the friable character remained unaffected. Addition of casein hydrolysate and yeast extract, at the concentrations used so far, had no effect friable nature of the tissue.

Work on suspension culture of normal tissue cells was carried out using the same method as used for gall tissue. Here also the percentage of freely floating cells was poor. So far, only in a very small number of cases 'embryo-like' structures were noticed; but further growth was not observed on transfering these cells to solid substratum.

C.5.3. *Cytological Studies on gall tissue*

Recently cytological studies on tissue cultures have been started. Methods for preparing cell suspension, hydrolysis and staining have been standardised. However, the degree of stainability of gall tissue cell was not satisfactory. Considerable difficulty was encountered in preparing suspensions of fixed material for want of pectinase. A modified method, though not satisfactory, using hydrochloric acid and chromic acid mixture is being followed. The study is being carried out to determine the time of cell division, frequency of cell division and division cycle after tissue transfer.

C.5.4. *Isolation of new cultures*

It was observed, in hollyhock suspension culture studies, that friability of tissues is the most important limiting factor in studies on totipotency of cells. From the literature it is evident that the nature of friability depends not only on the medium constitution but also on the inherent character of the tissue source. Considerable difficulty in the cytological studies was observed because of the high chromosome number and poor degree of stainability of chromosomes of the plants belonging to the family Malvaceae. With successful report on cultivation of large amount of tissue in a short period the attention of tissue culture workers has been drawn towards the application of the technique in production of plant constituents of medicinal and of economic importance. Considering these points, attempts were made to isolate and establish new tissue cultures. Success has been achieved in establishing cultures from embryo and endosperm tissues of *Abelmoschus esculentus*; stem tissue of *Cestrum nocturnum*, *Nigella sativa* and *Murraya koenigii*; rhizome tissue of *Alocassia indica* and *Rhoeo discolor*; and petiole tissue of *Cycas revoluta*. Tissues could not be established from rhizome of *Pachyrhizus angulatus*, *Zingiber officinalis* and *Podophyllum hexandrum*; bulb of *Allium cepa*; stem of *Plantago ovata* and fruit of *Lycopersicum esculentum*.

C.6. Plant Biochemistry

C.6.1. *Phytin, other phosphoinositols and their role in Phosphate transfer in germinating seeds*

In continuation of the last year's work on phytin and synthesis of nucleic acids, the changes of different inositol phosphates with the different period of soaking in relation to germination of seeds has been carried out with mung beans. A good correlation has been achieved so far as the decrease of inositol hexaphosphate (phytin) and corresponding increase of penta, tetra, tri and other inositol phosphates as the period of soaking is increased. Concomitantly a novel phosphoinositol phosphotransferase system has been detected in the germinating mung beans which can transfer phosphate from phytin to ribonucleoside diphosphates producing the corresponding ribonucleoside triphosphates. When crude extract

was incubated with 4 ribonucleoside diphosphates separately in the presence of P^{32}–phytate and the corresponding nucleoside triphosphates isolated, all four were found to be labelled, indicating probably the presence of this enzyme system. Further purification of this crude extract showed a preference for GDP and approximately 200 fold purification of this activity has been achieved after passing through DEAE and hydroxylapatite columns. It is observed that without the addition of Mg^{++} there is no transfer of phosphate. Mn^{++} is twice as effective as Mg^{++} and 5μ mole/ml is the optimal concentration. Neither deoxyguanosine diphosphate nor GMP could accept the phosphate from phytate. With high concentration of GTP the transfer is inhibited. The presence of ATP does not influence the reaction. The *p*H of this reaction has been found to be around 7. The reaction is linear upto 20 min. and after that period the rate of reaction decreases. There is a linear increase in the reaction with protein concentration upto 2 mg/ml. That the product is (γ P^{32}) GTP has been confirmed by the fact that after hydrolysis with 1 N HCl at 100°C for 7 min. more than 85% of the radioactivity is liberated as inorganic phosphate. The reaction is not analogous to that of transphosphorylase since the presence of ATP does not stimulate the reaction. Inositol penta or tetraphosphates can also act as a phosphorus donor in this reaction but to lesser extent than hexaphoaphate. That the reaction is reversible has been proved by the fact that when (γ P^{32}) GTP is incubated in presence of inositol pentaphosphate there is a transfer of P_i^{32} to the latter, producing hexaphosphate; the rate of reaction being 1/10th of the forward reaction. That the transfer of P_i^{32} is mediated through a phosphorylated enzyme product has been established by a series of short term experiments and finally by isolating phosphohistidine, a component of phosphoprotein from the reaction mixture of those experiments. In presence of AMP there is an increase of phosphorus transfer to GDP indicating most probably an allosteric transition of the protein. The over all reaction may be written as follows :

$$\text{Inositol hexaphosphate} + \text{GDP} \xrightleftharpoons[Mn^{++}]{\text{Phosphoinositol phosphotransferase}} \text{Inositol pentaphosphate} + \text{GTP}$$

In germinating seedlings this type of phosphotransferase system may play a vital role in the over all economy of energy balance. Other systems which can transfer phosphorus to ADP, CDP and UDP are now being investigated.

C.6.2. *Enzymatic synthesis of polyribonucleotides by chloroplast extract and amino acid incorporation into protein*

In continuation of the last year's work polyguanylic acid attached at the end of the primer RNA (poly G) has been isolated and tested as a messenger RNA for different amino-acid incorporation. Incorporation of glycine, and to a less extent of tryptophane, into protein has been found to be stimulated by the poly G containing product. A somewhat

similar promotion in the incorporation of aspartic acid was also observed. The stimulation of glycine incorporation was further confirmed by an experiment in which the GMP incorporating enzyme was added to the incubation mixture containing preincubated RNP, *p*H 5 fraction and other components. A control was kept in which an equivalent quantity of enzyme inactivated by heating at 90°C for 5 min. was added. The stimulation of glycine incorporation occurs only in the presence of active enzyme. Furthermore, this stimulation was similar to that obtained with the poly G containing product synthesized by the enzyme. About 50 out of 64 triplets in RNA have already been decoded from the stimulation in the corporation of amino acids by poly U, poly A, poly C and various copolymers. The coding properties of poly G have remained obscure. Attention has recently been focussed on the possibility that the end of a protein chain in a polycistronic messenger RNA may be indicated by a special nucleotide configuration containing secondary structure as a result of intra-molecular hydrogen bonding between guanine residues. The stimulation in the incorporation of glycine and to a lesser extent of tryptophane by the polynucleotide extract observed in this study indicates that the GGG may represent another codon for glycine and perhaps for tryptophane as well. This conclusion is supported by the earlier code assignments of GUG, GAG and GCG for glycine; on the basis of a doublet sharing code, GGG would also be expected to code for glycine. One of the advantages of the polynucleotide tested here is its association with the primer RNA which is likely to lessen secondary structure formation in the chain. When poly G residue, after stripping off the primer RNA by pancreatic RNase was used, no stimulation of glycine incorporation was observed. This could result either from failure to recover the oligopeptides or from secondary interaction in the poly G itself which may inactivate the messenger function. It has been shown that the number of guanylate residues added by the enzyme to the polynucleotide chain does not exceed 20, which could code only for 6–7 glycine residues on the basis of a triplet code.

C.6.3. *Interaction of proteins with nucleic acids and its role in RNA synthesis*

Nucleic acids can interact with histones or other basic proteins to form insoluble complexes and the extent of interaction can be measured turbidometrically. Turbidity increases steadily until a plateau is obtained when the reactants are present in equivalent amount. DNA can react with polylysine (a synthetic basic protein) and maximum turbidity as measured from Optical Density (O.D.) at 460 $m\mu$ was obtained when the ratio of polylysine : DNA is 1 : 2 This is in conformity with the equivalent weights of DNA and polylysine. With the concentration of 40 μg polylysine and 80 μg DNA per mililiter, the optimal NaCl concentration was found to be 0·1 M. In the case of phosvitin (a phosphoprotein extracted from hen's egg yolk) and polylysine the optimal O.D. at 460 $m\mu$ was obtained at a concentration of 2.5 μg phosvitin and 40 μg polylysine and this interaction was found to be very sensitive to salt concentration below 0·1 M. From compositions the equivalent weights of phosvitin and polylysine have been calculated to be 120 and 147 respectively and hence in the complex formation the optimal condition is expected at a ratio of polylysine : phosvitin of 1 : 1, instead a ratio of 16 : 1 was obtained experimentally. Furthermore, when DNA-polylysine complex was prepared as mentioned earlier and then phosvitin

was added for knocking off DNA from the complex competitively, 4–5 μg of phosvitin was found to be optimal concentration whether phosvitin was added instantaneously or after a lapse of minimum period for the binding of DNA and polylysine first. For the binding of DNA and polylysine sufficient time was necessary for complete precipitation as an increase in O.D. at 460 $m\mu$ was recorded upto 40 min. after which a plateau was obtained. Though the equivalent weight of DNA, RNA, lysozyme and polylysine was obtained approximately correctly with the procedure applied here yet that of phosvitin has been found to be different in each case so far tried. Thus the equivalent weight of phosvitin varies from 8–280 against the theoretical value of 120. In phosvitin, most of the phosphate is linked with serine moiety but phosphoserine when used in place of phosvitin neither turbidity with polylysine nor any competition with DNA polylysine complex was obtained. When the basic proteins are added along with the primer DNA the synthesis of RNA is inhibited. With each fraction of histones isolated from goat liver nuclei and also with polylysine, RNA polymerase (from Azotobacter vinelandii) activity was inhibited to a considerable extent (70–90%). The significant observation in this that phosvitin when added with polylysine in RNA-polymerase assay, 60% of inhibition was restored. Phosphoserine was not effective at all. This observation is also in conformity with the binding capacity of those substances with polylysine. It has not yet been tested with homologous system. Further investigation is now in progress to find out whether derepression of RNA-polymerase activity with other phosphoproteins is operative *in vivo*.

C.6.4. *Informational macromolecules in growth and development*

The fundamental problem of chemistry of growth and development is to understand why tissue cells do not all express, all the time, all the potentialities inherent in their genome. A cell is not a cell unless it can regulate its activity. An enzyme may be extracted from the cell and purified thousand fold and *in vitro* unlimited product catalyzed by the particular enzyme can be obtained. However, the cell containing this enzyme and the requisite substrate is not capable of synthesizing unlimited product. Which factors determine that rate of production is the vital question and is none the less easy to solve. The pattern of growth of a particular cell cannot be computed in terms of input (chemical substance) and output of the cell growth but each and every point in the circuit of the cell will have to be checked so that one can detect the primary impulse or primary effect of growth. In the seeds, all the physiological activities are highly suppressed. As soon as the seeds imbibe water enzymatic activities start. Since the current concept visualizes the histones as gene regulators it is expected that the pattern of histone biogenesis at any given stage of imbibition or germination will determine the nature of flow of message from the genome. In other words the transcription of different segment of DNA for the synthesis of *m* RNA will be dependent on the availability of those regions to RNA-plymerase. It has also been demonstrated that all the histones are not equally effective in inhibiting *in vitro* transcription of DNA. The data so far obtained indicate that an active biosynthesis of basic proteins including histones immediately follows the imbibation of water by seeds in a very short period. This is also applicable in the case of RNAs (separated through MAK column,

So far as the biosynthesis of basic proteins is concerned there is an indication that the rate of synthesis of fraction F_1 and F_{2A} (after fractionation of CM cellulose column) is the highest in early stages of imbibition. The nature of RNA biosynthesis in different periods has also been found to be different indicating most probably that as soon as imbibition starts, even without any visual sign of germination different segments of DNA are being transcribed due to derepression of suppressing effect of basic protein (histones). The essentiality of RNA and protein synthesis associated with growth has now been established. When growth hormone such as IAA has been applied to a tissue section the change in the pattern of RNA synthesis has been observed. Within 4 hr. of imbibition of seeds, RNA associated with DNA peak (in methylated albumin kiesselguhr column) exhibits high radioactivity (P^{32}) whereas with increased time this radioactivity gradually declines and a peak after ribosomal RNA appears indicating most probably the presence of messenger RNA. In the last year's report it was stated that RNA content of the ribosomal pellet increased by 12–13% in the case of IAA treated tissues. In fact, an increase of 4–5% associated with *m* RNA peak has been observed indicating most probably an altered nature of ribosomes in constitution. This is now being followed up to see whether there is any change in translation mechanism of those ribosomes.

C.6.5. *Physicochemical studies on gram-staining*

This work is being carried out in collaboration with Dr. M. K. Pal, now in Department of Chemistry, Kalyani University. The aim of the project is to study from different view points the mechanism of gram stanining. In contrast to other's finding it has been observed in this laboratory that uptake of dye (crystal violet) by gram positive bacteria (*Staphylococcus aureus*) is much higher than that by gram negative bacteria (*E. Coli*). In presence of different alcohols the uptake of dye is reduced in both the groups. This decrease in uptake seems to be directly proportional to the increase of carbon chain in the alcohol. But in each case the difference in uptake of crystal violet is maintained in both the groups. Gram staining involves the formation of a complex between the crystal violet and iodine and the gram positivity of the cell is due to this complex being insoluble and undissociable in the differentiating medium e.g., 95% alcohol, generally used. Gram positive bacteria after staining washed with sodium thiosulphate or dimethylaniline-ethanol (95%), nitrobenzene-ethanol, aniline-ethanol, xylol-ethanol are rendered colorless suggesting that permeability barrier is not the only factor for this. So far, only gram positive becteria may be converted to gram negative ones by variety of physical or chemical treatments. Gram negative bacteria (*E. Coli*) after treatment with cetylperidinium chloride (a detergent) have been found to retain some dye and can partially behave as gram positive bacteria. It has been observed that cetylpyridinium chloride can extract from *E. Coli* some lipid, protein and RNA. The lipid component which is present in much higher quantity in *E. Coli* (—) than that in *S. aureus* (+) is the lipid. Most probably the lipid fraction removed by cetylpyridinium chloride treatment render *E. Coli* cells to retain the conjugated dye molecule partially which otherwise is washed off with differentiating solvent. The mechanism of developing gram positivity in *E. Coli* is now being studied in detail.

C.6.6. *Mechanism of movement of Desmodium leaves*

A high phosphatase activity has been detected in the leaves of *Desmodium* sp. and purified 20 fold. In presence of light this phosphatase activity is decreased where as in dark it is increased. Tannic acid activates the phosphatase activity suggesting a correlation of movement of leaflets e.g., in dark or in presence of tannic acid the movement of leaflets is inhibited. Phosphatase is not strictly specific for ATP. It can hydrolyze other phosphate containing compound such as F-D-P, G-6-P or AMP, ADP and other nucleotides to a limited extent. The *p*H optimum has been found to be around 5·5–6·0.

D. MICROBIOLOGY

D.1. Antibiotic production

D.1.1. *Biosynthesis of polyene antibiotic* Ac_2 435 by *Streptomyces* sp.

The medium used for fermentation in Roux bottles was C-D synthetic medium replacing $NaNO_3$ by $(NH_4)_2SO_4$ at concentration 1 : 5 gm/litre. The experiments were carried out at 28°C and observations were made every 48 hours.

During fermentation the *p*H, growth, antibiotic titre and consumption of sugar and requirement of nitrogen sources were determined. The changes occurring in the fermentation medium were thus followed and the results are given in Table 1 which tended to show an acid condition of the medium. The antibiotic titre showed a peak on the 8th day while growth continued unaltered until the 12th day of incubation.

TABLE 1

CHANGES IN THE CULTURE MEDIUM DURING THE FERMENTATION OF STREPTOMYCES STRAIN Ac_2 435

Days of incubation	pH	Antibiotic titre-diameter of inhibition zone in mm. against C.lunata	Growth :—dry wt. of mycelium in mg/20 ml. medium	Residual glucose in mg/ml. of medium	Residual nitrogen (NH_3) in mg/ml of medium
0	7.2	—	—	21.0	0.333
2nd	6.7	11.6	7.0	17.85	0.322
4th	5.8	15.0	12.6	15.75	0.317
6th	5.4	21.6	14.6	14.7	0.286
8th	4.9	35.0	23.0	13.65	0.286
10th	4.8	28.6	30.6	12.6	0.275
12th	4.8	27.0	32.0	9.45	0.264

D.1.2. *UV-induced mutation in* Ac_2 435 *for increased production of the polyene antibiotic*

The spores of the *Streptomyces* strain Ac_2 435 suspended in normal saline solution were exposed to UV rays and several different doses suitable for Streptomyces were administered. The irradiated spore suspension was plated out as usual, probable mutants were isolated and antibiotic titre was tested. The test organisms *Candida albicans* was used for assay. Only a few mutants showing higher antibiotic titre were isolated.

The UV dosages at 60, 80 and 100 seconds were found most suitable for this experiment as higher mutagenic activity was observed at this dose level. It will be seen from Table 2 that of the surviving spores only 10·8 per cent showed increased antibiotic titre while significant decrease in the above activity was recorded in 31·4 per cent cases. In 56·7 per cent cases there was no change in the titre while in 0·38 per cent cases the activity was completely lost.

TABLE 2

ALTERATIONS IN ANTIBIOTIC PRODUCTION OF MUTANTS INDUCED BY UV IRRADIATION OF STREPTOMYCES STRAIN Ac_2 435

Time of irradiation in secs.	Total Number of isolates tested	Antibiotic activity							
		Increase		Decrease		Lost		No change	
		Number of isolates obtained	Number of isolates in %	Number of isolates obtained	Number of isolates in %	Number of isolates obtained	Number of isolates in %	Number of isolates obtained	Number of isolates in %
60	297	45	15.1	85	28.6	—	—	167	56.2
80	492	43	8.7	151	30.6	3	0.6	295	59.8
100	500	51	10.2	169	33.8	2	0.4	278	55.6
Total	1289	139	10.8	405	31.4	5	0.38	740	56.7

—indicates no isolates scored.

D.1.3. *Studies on the taxonomy of antibiotic producing Streptomyces strain* Ac_5 658

The *Streptomyces strain* Ac_5 658 has been deposited in the culture collection of the National Institute of Microbiology, New Jersey, New Brunswick, U.S.A. A report has been received about the identity of the strain which suggests its position among the substrains of *Str. griseus* producing antibiotics other than streptomycin. The studies on the biochemical properties of the strain under investigation were compared and it could be said that this could possibly be a new aggregate substrain of the well-known parent *Str. griseus* as there is already indications that the antibiotic substance isolated from the culture has not been reported before.

D.1.3.1. *Purification of the antibiotic from Streptomyces strain* Ac_5 658

Fermentation. The fermentation of the antibiotic was done in media containing cotton seed meal now extensively used in the U.S.A. as a substitute for corn steep liquor In the reciprocating shakers, the time for complete growth and attainment of highest titre of antibiotic was 72 hours.

Extraction and purification. The fermented broth is shaken vigorously with *n*-butanol several times (broth : solvent ratio 2 : 1) and the extract is concentrated in a film evaporater under reduced pressure. The residue is dissolved in methanol in which the inactive fraction separates out. The methanol fraction is concentrated and treated with distilled acetone. There appears a flocculent ppt. which is inactive. The mixture is filtered, centrifuged and concentrated by evaporation. On thin layer chromatographic analysis it is found to contain 3 fractions of which the major one is active. The active fraction was tested and is found to be positive against ninhydrin. The detailed chemical analysis is in progress to establish the identity of the antibiotic.

D.1.4. *Studies on the screening of anticancer antibiotics from Streptomyces* spp.

The screening of anticancer substances has been a difficult and lengthy process ever since antibiotics were appearing in the field of chemotherapy. Dr. G. F. Gause (Prof. and Scientific Director of the Institute of Antibiotics of the Academy of Medical Sciences, Moscow) and coworkers in Russia discovered novel method of assay in which mutant strains of *Staphylococcus aureus*, yeasts and protozoa were employed. The test organisms were found equivalent to cancer cells. They have been characterised by their impaired respiratory system. On analysis of the coenzyme contents of the bacterial mutants and their parent strains it was observed that the absorption spectrum of the former is different from the latter. The following important characters of the bacterial mutants are considered.

(i) The catalase content of the mutants were lower than that of their parent.

(ii) They take up lesser quantity of oxygen as compared to the wild parents.

(iii) They showed remarkable resistance to well-known antibiotics while showing sensitiveness towards probable anticancer antibiotics showing a different antibiotic spectrum.

The authors provided further evidence in support of their claim by making a comprehensive study of the mutants of *Staph. aureus* (e.g., UV_1 and UV_2). Comparative assay of anticancer antibiotics was made with cancer cell lines Sarcoma 108 (Foley) and Hela cells (Human cervix carcinoma) both of which are grown in suitable media. The method is presumptive and requires further studies on toxicity etc., for confirmation of potentiality as a chemotherapeutic agent. Another well-known method was adopted for presumptive tests of anticancer antibiotics. In this test *E. coli* phage was allowed to infect and multiply within the cells of the host. The metabolic filtrate of the Streptomyces isolates were assayed by the dilution method with bacteriophage in *E. coli* cultures.

The inhibition of phage activity is indicated by the turbidity of the bacterial suspension. Above methods were employed for screening antibiotics with anticancer potentiality. The results are obtained quickly in this case and an idea of the activity of the metabolic filtrates of Streptomyces isolates is obtained for further studies.

After screening more than 900 cultures of *Streptomyces* only 12 isolates stood the positive test in the present investigation and the results of comparative assay are given in Table 3. It has been found from our experiment that the metabolic filtrate, which is active against UV_2 and UV_3, is either inactive or less active against the parent and other G (+) and G (—) organisms.

Moreover, the metabolic filtrates having selective action against *Staph. aureus* mutants (NCIB 9309, NCIB 9310) can prevent phage multiplication. Some of these active metabolic filtrates possess degenerating action on tumour cells (Sarcoma S 180 and Hela 229) *in vitro*.

TABLE 3

SCREENING TEST OF ANTICANCER COMPOUND FROM STREPTOMYCES ISOLATES

Streptomyces isolate	pH at the 7th day	Diameter of the zone of inhibition (mm) Staph. aureus .209 NCIB 9308	UV_2 NCIB 9300	UV_3 NCIB 9319	Degenarating action on* Sarcoma S. 180 (mouse)	HeLa 229 (Epitheloid Carcinoma, Cerveix, human)	Antiphage activity**
Ac/W_9	6.8	—	25	25	++	++	+++
Ac/W_5	8.0	—	30	25	++	++	+++
Ac/C_1	8.0	15	30	35	+	—	++
Ac/Z_1	7.0	—	20	18	++	+	++
Ac_4	8.0	10	10	30	—	—	—
Ac/D_4	7.4	15	30	28	—	—	+
Ac/Z_2	6.6	12	25	25	—	—	—
Ac/D_5	7.5	10	20	23	—	—	—
Ac/Y_1	7.5	10	20	16	—	—	—
Ac/Y_2	8.2	13	20	20	—	—	—
Ac/W_{12}	6.3	—	25	28	++	+	++
Ac/B_2	7.4	10	32	25	+	+	+
Control	7.2	—	—	—	—	—	—

*Degenarating effect shown by slide cultures.

**Antiphage activity is shown against *E.coli* phage by turbidimetric method.

D.1.5. *Multiple resistance to antibiotic*

Development of antibiotic resistance among patients kept on prolonged treatment with *Streptomycin* and other antitubercular drugs has been observed in a T.B. hospital in West Bengal. The histories of the cases were reported by the hospital authorities as follows :

(1) The first patient (pt. 11) was female aged 27 years. She was suffering from pulmonary tuberculosis for 3 years. She had been treated with 150 gms. of streptomycin, 600 gms. para amino salicylate (PAS) and 147 gms. of isonicotinamide hydrazide (INA).

(2) The second patient (pt. 2), a male aged 14 years was supposed to have been suffering from pulmonary tuberculosis for one year. He had been treated with requisite quantity of streptomycin, INA and PAS.

(3) The third patient (pt. 3) aged 52 years was also suffering from pulmonary tuberculosis for more than one year. He was subjected to the usual treatment with streptomycin INH and PAS.

The interesting feature of this investigation was that, all of them had their sputum free from *Mycobacterium tuberculosis* since their admission into the hospital.

Recently the sputum of the three patients was supplied to us for microbiological analysis. The sputum was diluted and plated into Nutrient agar Sabourad's agar for the isolation of bacteria and pathogenic fungi respectively. The isolates were treated with different dilutions of streptomycin, penicillin, chloramphenicol and tetracycline antibiotics. Some of them were found to be strongly resistant to streptomycin and penicillin as well. The results obtained are shown in Table 4, where the maximum concentration of the antibiotics which the bacteria can stand are shown. Eight bacterial cultures were isolated. Of them, the sputum of the second patient (pt. 2) contained a strain of *Staphylococcus aureus* highly resistant to both streptomycin and penicillin in plate culture. It is also evident that, the bacterial cells, resistant to streptomycin and penicillin, are sensitive to chloramphenicol at concentrations 2–10μ gm/ml.

TABLE 4

MAXIMUM CONCENTRATION OF ANTIBIOTICS THAT THE BACTERIAL CELLS CAN WITHSTAND

Patient Number	Streptomycin µgm/ml	Penicillin u/ml	Chloramphenicol µgm/ml	Erythromycin µgm/ml	Aureomycin µgm/ml	Terramycin µgm/ml	Gram character. And morphology
Pt. 1	2000	160	2	100	75	80	G+ Round cells
Pt. 2A	2000	1600	5	80	100	100	G- Rods.
Pt. 2B	2000	1600	10	100	75	75	G+ Round Cells.
Pt. 3	2000	1600	10	50	50	75	G+ Round Cells.

D.2. Induced mutation in microorganisms

D.2.1. *Modifying effects of chemicals on UV irradiation*

There are several factors which could modify the effect of UV irradiation in microorganisms. Experiments were performed to determine the effect of chemicals on ultraviolet irradiation in *A. chevalieri* strain (73070). The following chemicals were used (a) nucleic acid, (b) casein hydrolysate, and (c) vitamins. In each experiment, spore suspension was made from seven day old cultures, which was suspended in sterilized 'Calsolene' solution (1 : 10,000) and Ringer's solution. To show the modified effect of UV radiation three sets of experiments were done with 3 chemicals in each case.

For treatments of spores of *A. chevalieri* with nucleic acid hydrolysate (concentration 1 ml. stock DNA+3 ml stock RNA per 100 ml of MM media) was used.

The treatments were given in three different steps.

(i) *Treatment during irradiation.*—In this treatment spore suspension of *A. chevalieri* were centrifuged twice followed by the addition of nucleic acid hydrolysate in desired concentration. Irradiation was given for 2, 4, 6 and 8 minutes. After irradiation the fluid of nucleic acid hydrolysate was rejected immediately by centrifugation and the spores were resuspended in sterile Ringer's solution and plated out in CM media.

(ii) *Post irradiation treatment.*—The spore suspension in this case was subjected to UV irradiation for required time periods. The suspension was centrifuged and the supernatant was rejected. The nucleic acid hydrolysate was added to it in required concentration. After the treatment was over the spores were centrifuged and washed with water and diluted for plating.

(iii) *Pre-irradiation treatment.*—In this experiment, nucleic acid hydrolysate solution was irradiated alone for 2, 4, 6 and 8 minutes. To this centrifuged spores of the fungus irradiated nucleic acid was added immediately after irradiation and the mixture was treated as before for required period of time. During this treatment all the flasks were kept on a shaker and were shaken throughout the treatment. At the end of the treatment the spores were washed twice and plated out in CM media after different dilution.

Experiments with casein hydrolysate and vitamins were performed with 3 sets of treatments. The concentration of amino acid was measured on the basis of 5 ml casein hydrolysate and 3 ml stock vitamin solution in 100 ml MM medium. A set of control culture was run for each experiment. Auxanographic tests were performed to isolate the biochemical mutants and morphological mutants were however, isolated from CM plates. The results obtained are given in Table 5 and 6.

TABLE 5

FREQUENCY OF SURVIVAL AND MUTATION

Dose—100 ergs/mm²/sec.

Treatment	Time of irradiation	Spore concentration 32.2×10^6		
		Survival %	Biochem. %	Morpho. %
UV irradiation	0	100.0	—	—
"	2	11.5	2.7	3.0
"	4	4.5	3.5	7.1
"	6	0.68	5.3	8.0
"	8	0.13	2.6	5.3

It will be seen that treatment of spores of *A. chevalieri* with nucleic acid hydrolysate, amino acid, and vitamin solution during irradiation per cent killing was significantly higher than that of UV alone. Post irradiation treatment with the above chemicals modified the injurious effect of UV and pre-irradiation treatments showed reduced rate of killing of spores. Both biochemical and morphological mutations showed increased frequency in the case of

TABLE 6

MODIFYING EFFECT OF DIFFERENT TREATMENTS ON UV-IRRADIATION

Dose 100 ergs/mm²/sec.

Treatment	Time of irradiation	Spore concentration 24.8×10^6				32.2×10^6				28.2×10^6			
		Nucleic acid hydrolysate				Casein hydrolysate				Vitamin			
		Survival %	Biochem. %	Morpho. %		Survival %	Biochem. %	Morpho. %		Survival %	Biochem. %	Morpho. %	
During irradition	0	100.0	—	—		100.0	—	—		100.0	—	—	
	2	9.9	2.7	4.1		9.7	2.3	4.6		3.0	1.9	2.5	
	4	3.9	3.4	4.8		0.21	3.8	6.2		2.3	3.5	5.3	
	6	0.13	5.5	9.7		0.11	6.2	9.3		1.3	6.2	10.7	
	8	0.05	4.1	8.3		0.09	3.4	6.9		1.0	2.5	4.4	
Post-irradiation	0	100.0	—	—		100.0	—	—		100.00	—	—	
	2	10.2	2.2	2.7		28.5	2.6	5.3		8.8	2.0	4.1	
	4	1.4	4.1	6.2		4.7	5.3	7.0		1.7	2.7	4.8	
	6	1.2	5.8	8.3		0.34	8.0	12.5		1.2	6.2	10.7	
	8	1.1	2.0	7.6		0.25	4.0	10.7		0.93	1.3	3.4	
Pre-irradiation	0	100.0	—	—		100.0	—	—		100.0	—	—	
	2	80.2	—	—		77.6	—	—		70.9	—	—	
	4	64.5	—	—		75.1	—	0.8		65.9	—	—	
	6	34.1	2.1	1.5		69.5	0.4	2.6		62.4	2.0	1.5	
	8	28.7	0.6	1.0		62.7	0.7	1.7		57.4	0.3	1.0	

—indicates no iselates scored

post-irradiation treatment. Number of mutations declined in the case of pre-irradiated treatment.

D.2.2. *Segregation of heterokaryotic colonies produced by the recombination of different auxotrophs of the Streptomyces strain* Ac_{21} 460

Heterokaryons were produced by mixing two kinds of auxotrophs in the limiting medium, incubated at 28°C for 24 hours and then plated on minimal medium. Colonies appearing on minimal media were presumed to be recombinant of the two strains. By several successive transfers stability was determined and stable heterokaryons were characterised.

Twelve heterokaryotic colonies from the recombination group $B_{66/14}$, eight from $B_{27/34}$, six from $B_{34/23}$, five from $B_{23/14}$ and five from $B_{66/90}$ were taken for studying the segregation pattern (Table 7a–7c). Heavy spore suspension of the heterokaryotic strains were plated out in complete agar medium and they were transferred to MM agar medium by the total

TABLE 7(a)

SEGREGATION PATTERN FROM DIFFERENT HETEROKARYONS

Component strains	Heterokaryon studied	Number of colonies tested	Segregation type (%)				
			I	II	III	IV	V
66—w pig_+-hist- × 14—y pig_+ribo- (B66/14)	H—1	224	100	Nil	8.9	72.2	100
	H—2	144	100	,,	Nil	41.6	100
	H—5	220	100	,,	12.2	77.3	100
	H—8	192	100	,,	Nil	78.1	100
	H—9	160	100	,,	8.7	75.0	28.2
	H—14	156	100	,,	Nil	28.8	Nil
	H—15	140	100	,,	3.5	82.1	26.0
	H—18	182	100	,,	9.8	16.4	18.6
	H—21	200	100	,,	7.5	77.5	100
	H—22	240	100	,,	11.7	70.8	100
	H—25	316	100	,,	12.6	77.9	100

isolation method. Some colonies were seen to grow in MM media while majority did not. Colonies not growing in MM media were tested by auxanographic technique in the basal media supplemented with growth factors to determine the ratio by which the heterokaryons were segregated. Colonies thus growing on supplemented media were taken as segregants and from the different sets of recombination the behaviour of each segregant was studied. As far as their segregation pattern is concerned the following types were recognized.

TABLE 7(b)

SEGREGATION PATTERN FROM DIFFERENT HETEROKARYONS

Component strains	Heterokaryon studied	Number of colonies tested	Segregation type (%)				
			I	II	III	IV	V
27—O Pr-/val- × 34—br Orn- (B27/34)	H—28	180	Nil	100	Nil	Nil	100
	H—35	144	,,	100	4.1	,,	100
	H—41	212	,,	100	8.4	,,	100
	H—45	192	,,	100	Nil	,,	Nil
	H—46	140	,,	100	8.5	,,	100
	H—48	192	,,	100	6.2	,,	100
	H—50	160	,,	100	Nil	,,	31.5
	H—56	200	,,	100	,,	,,	Nil
34—br Orn- × 23—w gly- (B34/23)	H—60	112	83.9	16.9	Nil	Nil	41.2
	H—61	140	77.1	22.9	5.7	,,	100
	H—63	112	75.8	24.2	2.6	,,	88.2
	H—65	160	81.8	18.2	7.5	,,	41.5
	H—68	140	87.1	12.9	Nil	,,	100
	H—72	192	83.3	16.7	3.6	,,	100

TABLE 7(c)

SEGREGATION PATTERN FROM DIFFERENT HETEROKARYONS

Component strains	Heterokaryon studied	Number of colonies tested	Segregation type (%)				
			I	II	III	IV	V
23—w gly- × 14—y pig+ribo- (B23/14)	H—78	176	100	Nil	Nil	89.7	100
	H—82	112	100	Nil	,,	42.8	100
	H—86	200	100	,,	,,	45.5	100
	H—89	130	100	,,	,,	78.4	100
	H—91	160	100	,,	,,	77.5	98.2
66—w pig-hist- × 90—bu lys ant- (B66/90)	H—116	128	89.1	10.9	1.5	Nil	Nil
	H—123	140	96.4	3.6	3.5	,,	78.6
	H—125	128	90.6	9.4	Nil	,,	5.6
	H—130	144	91.6	8.4	0.7	,,	Nil
	H—131	112	87.5	12.5	Nil	,,	,,

Type I—*Colonies not growing on MM media.* Resemble one member of the auxotroph pair with regard to superficial colour and colony appearance; segregations occur in varying proportions.

Type II—*Colonies not growing on MM media.* No resemblance with any member of the auxotroph pair; segregation occurred in varying proportions.

Type III—*Colonies growing on MM media.* No further segregation into any of the parental components.

Type IV—*Colonies growing on MM or supplemented MM.* Produce diffusible pigment into the culture medium.

Type V—*Colonies growing on MM or supplemented MM.* Possess antibiotic activity. Further work is in progress.

D.2.3. *UV induced mutation in pigment forming Streptomyces strain* Ac_1 805

To study the genetics of *Streptomyces* spp., strains are generally selected on the basis of stable genetical characters e.g., pigment production in presence of specific carbohydrate source and antibiotic synthesis.

In the present study screening of fresh isolates of *Streptomyces* spp. was made of which six strains were finally isolated and characterised. All the selected strains could produce antibiotics as well as pigments of various grades at *p*H 7·2 and required incubation at 28–30°C for 8 days. The medium had glucose as the sole source of carbon. The active filtrates of the cultures were tested for thermostability (Table 8). It will be seen that the antibiotic activity was afterwords lost at 60°C in most strains except one (Ac_2 806) in which the activity is retained even when heated at 100°C for 10 minutes.

Of the strains, Ac_1 805 and Ac_2 806 were selected for mutation by exposure to ultraviolet irradiation. The present report deals with results obtained with strain Ac_1 805 which has coloured aerial mycelium and spore. The reverse colour of colonies is orange and diffuses a light yellow pigment into the culture media. The spores were irradiated with UV rays (at 2537Å of 100 ergs/mm²/sec) for 20, 40, 60 and 80 seconds respectively. The percentage of spore survivability decreased with the increase of the time period of exposure and the nature of the curve was exponential.

The isolation of the mutants was done by filtration technique. The irradiated spore suspension was allowed to grow in 20 ml. minimal liquid medium. After 24 hours of germination at 28°C the spore suspension was filtered through the sterilized sintered glass filter (No. 4) and the filtrate was plated in complete agar medium. Total isolation method of Fries—was then followed and the morphological and biochemical mutants were isolated. The characterization of biochemical mutants was made by the usual method of auxonography. Out of 680 colonies tested 105 strains were scored as morphological mutants and 6 as biochemical ones. When these 6 strains were characterised further, it was observed that 5 strains were deficient in nucleic acid and one in amino acid synthesis. Vitamin deficient mutants were yet to be isolated. Further work with these mutants is in progress.

TABLE 8

THERMOSTABILITY OF ANTIBIOTIC PRODUCED BY THE DIFFERENT STRAINS OF STREPTOMYCES

Strain Index	Pigment	Antibiotic activity was different temperatures (°C)				
		30	40	60	80	100
$Ac_1$805	(yellow)	+	+	+	—	—
$Ac_2$805	(greyish black)	+	+	+	—	—
$Ac_2$806	(white)	+	+	+	+	+
$Ac_4$790	(grey)	+	+	+	—	—
$Ac_4$789	,,	+	+	—	—	—
$Ac_1$797	(state colour)	+	+	—	—	—

+ and — indicate presence and absence of activity respectively.

D.2.4. *Modifying effects of chemicals on UV-irradiation*

D.2.4.1. *Effects of thiourea on UV-irradation*

The experiment was performed to show whether there is any protecting power of thiourea against ultraviolet radiation. Five species of *Aspergillus* were selected for this experiment. Those used were *Aspergillus niger* (V_{35}), *Aspergillus aureus* (50567), *Aspergillus oryzae* (17299), *Aspergillus avanaceous* (16140), and *Aspergillus terreus* (16043), the first two spp. belonging to group *A. niger* and the remaining three to the groups *A. flavus*, *A. wentii* and *A. terreus* respectively. The strains of the fungi selected were first purified by single conidium micro-manipulation. Spore suspension was prepared from 8 day old cultures in sterile calsolene-water (1 : 10,000) solution and the Ringer's fluid.

Ultraviolet irradiation was given from a 4-watt General Electric Germicidal lamp. The lamp was fitted above a shaker to have equal distribution of energy during radiation. The distance of the lamp from the shaker, was adjusted so that the dose was 100 ergs/mm²/sec.

To demonstrate the modifying effects of thiourea on ultraviolet irradiation in fungi, the spores of the selected strains were treated with 0·1 Molar solution of thiourea before, during and after irradiation.

(i) *Pre-irradiation treatment.* The solution of thiourea 0·1 Molar was added to the spores and kept shaking for 2 hours at 28°C. After 2 hrs. the spores were washed three times by centrifugation and then irradiated for the required time period, resuspending in sterile distilled water. The spore suspension was finally diluted and plated out in complete medium and incubated at 22°C for 3 days.

(ii) *Treatment during irradiation.* The spore suspension was centrifuged and 0·1 Molar thiourea was added to the spores before irradiation was given for the required time period. The treatment fluid was rejected by centrifugation. The spores were washed twice and after proper dilution, plated out in CM and incubated as usual.

(iii) *Post irradiation treatment.* The spore suspension in this case was subjected to UV-irradiation for the required time period and was treated in the same manner as in (ii).

Besides these treatments two sets of control were set up—(1) without UV irradiation and thiourea treatment, and (2) with irradiation but without thiourea treatment. The results are given in Table 9.

TABLE 9

MODIFYING EFFECT OF THIOUREA ON UV IRRADIATION

Incubation temperature —28°C
Dose—100 ergs/MM²/sec.

Organism	*A. niger*	*A. aureus*	*A. oryzae*	*A. avanaceous*	*A. terreus*
Time of irradiation in mins.	02	4	3	2	1.5
	Percent survival				
(1) Without treatment (UV control)	2.0	2.08	2.6	2.4	3.0
(2) Two hrs. pre-irradiation treatment	1.8	2.33	3.2	2.6	3.7
(3) During irradiation treatment	100	100	100	100	100
(4) Two hrs. post-irradiation treatment	2.5	2.25	2.7	3.1	3.2

From the table it will be seen that complete protection against UV-irradiation was found when the spores were irradiated in the presence of 0·1 M thiourea.

From the study on the effect of modifying factors it was found (Table 9) that pre- and post-treatments with thiourea had effects in protecting the spores of fungi against the injurious action of ultraviolet rays. It is interesting to note that use of the same concentration of thiourea during irradiation resulted in completely eleminating the killing effect of UV radiation in all the spp. of *Aspergillus*. It is possible that the presence of the chemical in the substratum must have influenced the indirect action of non-ionizing ultraviolet radiation. Another interesting phenomenon was observed in which the survival percentage decreased in *Aspergillus* niger with 2 hrs. of pre-irradiation treatment. This is a striking example in which the effect of treatment augumented the magnitude of the killing in the given dose. Further work on the survival kinetics with other modifying factors is in progress.

D.3. Microbial Genetics

D.3.1. *Studies on UV induced mutation in Penicillium vermiculatum* 77·62.

Mutation experiments with UV radiation have been continued with more suitable range of doses. The technique followed is the same as described in the earlier report. In the previous experiments 2 mins. treatment gave a maximum killing effect on conidiospores. As such fresh experiments were done with treatments of 30, 60, 90, 120, 150, 180, 240, 360,

380 and 600 sec. At 30 secs., the percentage of survival was found to be 61·8 and at 60 secs. 16·76. Beyond 60 sec. there is a gradual fall in the survival percentage upto 180 sec. At 240 sec., however, a resistance is observed. At higher treatments survival is very poor.

A large number of transfer of colonies were done on minimal medium. From each treatment 1000 colonies were transferred to minimal plates following the usual pattern of 16 colonies in each, wherever possible. Table 10 shows the percentage of survivals, total number of colonies transferred from complete to minimal medium, total number of biochemical and morphological mutants scored, and percentage of both biochemical and morphological mutants obtained. Detailed biochemical assay of mutants was done by auxanographic test. Mutants having deficiencies in nucleic acids, vitamins, amino acids and various combinations of these as also sulphate non-reducing and nitrate non-reducing mutants have been isolated. Table 11 shows the number of biochemical mutants having various requirements under different categories.

A mutant requiring two nutrients *viz.*, nucleic acid and amino acid was obtained from preliminary assay. In the final assay however this particular mutant did not respond to any individual nucleic acid or amino acid added to the minimal plates.

TABLE 10

SHOWING DURATION OF TREATMENT, SURVIVAL OF CONIDIOSPORES, NUMBER OF MUTANTS ISOLATED, TOTAL NUMBER OF COLONIES TESTED ON MINIMAL MEDIUM IN IRRADIATION EXPT. WITH UV WITH *Penicillium vermiculatum*

Time of irradiation in seconds	% Survival of Conidiospores	Colonies transferred to minimal	Total number Biochemical mutants	morphological mutants	% of Biochemical mutants	% of Morphological mutants
0	100	1052	—	—	—	—
30	61.8	864	14	13	1.62	1.50
60	16.77	1008	20	34	1.98	3.37
90	2.6	768	25	25	3.25	3.25
120	0.84	896	20	19	2.23	2.12
150	0.28	1146	19	26	1.66	2.27
180	0.046	704	9	18	1.28	2.55
240	0.01	669	13	13	1.94	1.94
360	0.005	974	14	16	1.43	1.64
480	0.0028	772	6	9	0.78	1.2
600	0.0024	592	3	6	0.5	1.0

Legend—indicates no isolates scored.

TABLE 11

SHOWING THE NUMBER OF DIFFERENT BIOCHEMICAL MUTANTS OBTAINED

1. Mutants requring one or more than one vitamin	Riboflavin 4	Ca-pantethanate/ Riboflavin 1	Thiamine 2	*p*-amino benzoic acid/ Thiamine/ Inositol 1	Inositol 1
2. Mutants requiring one or more than one amino acid	Asparagine 1	Aspartic acid/ valine/ Threonine/ Ornithine 1	Glycene/ Methionine/ Leucine/ Cysteine 1		
3. Mutants requiring one or more than one nuclic acid	Adenine/ Guanine 1	Xanthine/ Guanine 6	Adenine/ Hyposanthine 2	Adenine/ Hypoxanthine/ Guanine 1	Guanine/ Xanthine/ Adenine 1
4. Sulphate non-reducing mutants	3				
5. Nitrate non-reducing	2				

Quantitative cultural studies of the mutants with different nutritional requirements is in progress.

D.3.2. *Studies on the variation of antibiotic activity of mutants*

The antibiotic activity of *Penicillium vermiculatum* cultured in Czapek-Dox medium with 1% cotton seed meal was tested with *Staphylococcus aureus* and *Escherichia coli* by cup assay method. The filtrate was found to be active against *S. aureus* only showing an inhibiting zone of 24 mm. diam. Antibiotic activities of some of the biochemical and morphological mutations have also been tested against a few pathogenic fungi and bacteria. The mutants were grown in complete medium without cotton seed meal and zones of inhibition were observed on the 4th, 5th and 6th day. The following tables (Tables 12a–12c) show the activity against different test organisms :

It will be seen from the above tables that *Penicillium vermiculatum* in C.D. (Bhattacharya) plus cotton seed meal produces an antibiotic which is active against *S. aureus* but in complete medium none of the mutants nor the parent strain could inhibit *S. aureus*. From the above data it is evident that mutation can influence antibiotic production in as much as different mutants show activities against different organisms. Though the zones of inhibition as shown in the Table 12a–c are not much pronounced yet enough to give an indication of differential behaviour of the mutants with regard to different test organisms. It seems that the antibiotic activity is affected by the composition of the medium. A search to find out the suitable medium for the studies in antibiotics with *P. vermiculatum* is in progress.

TABLE 12A

SHOWING OBSERVATION ON ANTIBIOTIC ACTIVITY OF THE MUTANTS OF *Penicillium vermiculatum* AGAINST DIFFERENT TEST ORGANISMS

Observation on the 4th day of incubation	Aspergillus niger	Curvularia lunata	Fusarium sporum oxy-	Alternaria solani	Candida albicans	Staph. aureus	Salmonela typhosa	Vibrio choleri	Escherichia coli	Unknown bacteria
Control	—	—	—	—	—	—	—	—	18	—
$V_2$8/23M	—	—	—	—	—	—	—	—	24	—
$V_6$120S10	—	—	—	—	—	—	—	—	24	—
$V_7$240S7	—	12	12	—	—	—	—	—	20	—
$V_7$150S6	—	—	—	—	—	—	—	—	26	—
V_2M2/14	—	—	12	—	—	—	—	—	—	—
$V_5$150S4	—	9	12	—	—	—	—	—	—	—
$V_6$120SM_{31}	—	—	—	—	—	—	—	—	—	—
$B_7$150S5	—	11	—	—	—	—	—	—	—	—
$V_5$120S1	—	11	10	—	—	—	—	—	—	—

TABLE 12B

SHOWING OBSERVATION ON ANTIBIOTIC ACTIVITY OF THE MUTANTS OF *Penicillium vermiculatum* AGAINST DIFFERENT TEST ORGANISMS

Observation on the 5th day of incubation	Aspergillus niger	Curvularia lunata	Fusarium oxysporum	Alternaria solani	Candida albicans	Staph. aureus	Salmonela typhosa	Vibrio choleri	Escherichia coli	Unknown bacteria
Control	—	—	—	—	—	—	8	—	—	—
$V_2$8/23M	—	—	—	—	—	—	—	—	—	—
$V_6$120S10	—	—	—	—	—	—	—	—	—	—
$V_7$240S7	—	15	12	—	—	—	—	—	—	—
$V_7$150S6	—	—	—	—	—	—	—	—	—	—
V_2M2/14	—	14	—	20	—	—	—	—	—	—
$V_5$150S4	—	13	12	18	—	—	—	—	—	—
$V_6$120SM_{31}	—	—	—	—	—	—	—	—	—	—
$V_7$150S5	—	—	—	—	—	—	—	—	—	—
$V_5$120S1	—	—	—	13	—	—	—	—	—	—

TABLE 12c

SHOWING OBSERVATION ON ANTIBIOTIC ACTIVITY OF THE MUTANTS OF *Penicillium vermiculatum* AGAINST DIFFERENT TEST ORGANISMS

Observation on the 6th day of incubation	Aspergillus niger	Curvularia lunata	Fusarium oxysporum	Alternaria solani	Candida albicans	Staph. aureus	Salmonela typhosa	Vibrio choleri	Escherichia coli	Unknown bacteria
Control	—	—	10	—	—	—	—	—	—	—
$V_2$8/23M	—	—	—	—	—	—	—	—	—	—
$V_6$120S10	—	—	—	—	—	10	—	—	—	—
$V_7$240S7	—	11	—	—	—	—	—	—	—	—
$V_7$150S6	—	—	—	—	—	—	—	—	—	—
V_2M2/14	—	14	14	—	—	—	—	—	—	—
$V_5$150S4	—	—	—	—	—	—	—	—	—	—
$V_6$120SM	—	—	—	—	—	—	—	—	—	—
$V_7$150S5	—	—	—	—	—	—	—	—	—	—
$V_5$120S1	—	11	14	—	—	—	—	—	—	—

D.3.3. *Studies on the sporulation of P. vermiculatum*

Irregularities in the conidiospore forming habit had been observed in the strain of *P. vermiculatum*. Investigations on the conditions favouring sporulation were, therefore, taken up. To start with, cultures were grown in the following media :—

(i) Czapek—Dox agar
(ii) ,, ,, ,, Modified (Bhattacharya I.J.M.A.R.I.)
(iii) ,, ,, ,, plus KH_2PO_4
(iv) Penicillium sporulating medium (Northern Regional Research Laboratory, Canada)
(v) Potato glucose agar (Mycologia Vol. 52, p. 47)
(vi) A_1 (Chaudhuri)
(vii) Minimal medium (Pontecorvo)
(viii) ,, ,, (Westergaard)
(ix) ,, ,, (Francis J. Ryan)
(x) ,, ,, (Horowitz)

It was observed that conidia were produced only in media No. vii and ix. However C.D. (Bhattacharya) with 20% glucose encourages sporulation favourably and this medium had so far been used as the sporulating medium.

D.3.3.1. *Effect of carbon sources*

Carbon sources *viz.*, glucose, sucrose, dextrin and glycerine were also added to the basal medium (C.D. Bhattacharya) separately at 4% level. Out of these carbon sources only glucose responded favourably.

Attempts were made to formulate a medium without high sugar content which might prove useful. Various growth promoting substances, such as carrot extract, glutamic acid, corn meal agar, vitamins, casamino acid, asparagine, sodium acetate, ammonium tartrate and also trace elements were added to the basal medium separately. It was found that sodium acetate, trace elements and ammonium tartrate individually helps sporulation to some extent in presence of 4% glucose.

As repeated experiments with the more suitable media did not give uniform results, it was obvious that some other factor or factors, apart from nutrition, influence sporulation. As such, further experiments were done with variation in the environmental factors, such as *p*H of the medium, types of glassware, temperature, humidity and light.

D.3.3.2. *Effect of pH of the medium*

Initial *p*H of the basal medium was varied from 4·0 to 8·0 at a difference of 0·5. It was found that *p*H of the medium does not have any marked effect on sporulation within this range.

C.D. (Bhattacharya) is a buffered medium with a *p*H 6·5. It was thought that the buffered condition of the medium might interfere with conidia production. So, while setting up the experiment the buffered action was disturbed by adding strong acid or alkali (2N). *p*H of one medium was adjusted at 5·4 and the other at 8·0 and the original buffered medium was kept as the control. It was observed that the former two media produced more conidia than the latter. But this also was unsatisfactory as sporulation was not at all perfect. Further attempts were, therefore, made with temperature, humidity and light.

D.3.3.3. *Effect of temperature*

Temperature has no effect on sporulation; only vegetative growth is more at a higher temperature of 28°±1°C.

D.3.3.4. *Effect of humidity*

Humidity has an important role on conidia formation as it had been found in the initial experiments that lesser humid condition is better suited to sporulation. Humidity was controlled inside the desicators used with different concentrations of sodium chloride in water. Culture plates were kept within the desiccators at 25°C and allowed to grow for 7 days. After incubation 5 discs were taken out at random and shaken well in 2 ml of water for 10 minutes so that the spores get separated from the mycelium to make a suspension. Haemocytometer counts are then taken to determine the strength of spores in the suspension. Mean of ten haemocytometer readings were taken. The spore concentration of the suspension is given below :

Humidity control with different concentrations of NaCl			*Number of spore per ml* $10\times^{6}$
Absolutely dry control (fused $CaCl_2$)			9·95
40% NaCl	...	...	10·4
30%	...	...	2·0
20%	...	...	1·6
10%	...	...	1·5

D.3.3.5. *Effect of light*

Light has been found to be the most vital factor amongst all those experimented with. In absence of light no sporulation ever occurred. When plates were exposed to continuous light (fluorescent tube) intense sporulation was observed. Detailed studies on the effect of light on sporulation is being continued.

D.3.4. *Studies on UV induced mutation with Penicillium purpurogenum Stoll Strain* P_{14}

A number of strains of *Penicillium* spp. were isolated from platings of soil collected from various parts of West Bengal and Bihar. Several strains were selected taking into consideration characters like sporulating habit, colour of spore, formation of perithecia, pigmentation, production of antibiotic substances etc., with a view to study their genetic behaviour.

Penicillium purpurogenum Stoll Strain P_{14} was selected for study.

Description of strain P_{14}

Colonies superficially appearing velvetty, usually heavily sporulating in central and subcentral areas, sometimes definitely zonate or azonate; massed conidial heads arising from aerial hyphae or directly from the substratum; odour indistinct; reverse deep red to dark reddish purple, surrounding agar being similarly coloured in somewhat lighter shades; conidiophores arising from the substratum and measuring upto 100 to 150 μ; penicilli typically biverticillate and symmetrical, compact, usually consisting of a single vertical of 4–5 or sometimes more metulae, each terminating in a compact cluster of 4–6 parallel sterigmata bearing short conidial chains; metulae 10–14μ by 2·5–3·0μ sterigmata mostly 10–12μ by 2·0–2·5μ, lanceolate in form, characteristically tapered; conidia elliptical to subglobose, sometimes more or less apiculate, mostly 3·0–3·5μ by 2·5–3·0μ with walls typically heavy and somewhat roughened; selerotia or perithecia absent.

Cultural studies with the selected strain were done with different media such as Czapek-Dox, Fries minimal, P-complete and various modifications. In all these media sporulation was profuse in as much as, the spores proved to be disadvantageous to the handling of colonies during transfers in mutational assay.

Quantities of organic and also inorganic constituents in the P-complete medium were differently varied to see whether these ingredients had any effect on the sporulation of the strain. Lessening of the production of conidiospores was observ ed in the medium with 4% NaCl. But this medium proved disadvantageous because of some retardation in germination. P-complete with sorbose was found to be very suitable for both restriction of colonies and suppression of sporulation.

Initial difficulties with spore harvest were encountered because of adherence of conidiospores in chains and also because they float on the surface of water. To make the spore suspension free from chains and clumps of spores sintered glass filteres No. G2 and G3 were used. But this technique did not prove quite satisfactory as the spore suspension passed through with the chains of spores. Various wetting agents such as calsolene (I.C.I.), sodium tauroglycocholate, polyoxy ethylene sorbitan mono oleate commonly known as "Tween 80" were tried. The last mentioned was found to be the most satisfactory.

Suspension of spores were made in 0·01% sterile "Tween 80" solution in 250 ml Erlenmeyer flask. 100 ml. of spore suspension was shaken vigorously in a shaker for 2 mins. with 20 g. washed and sterilized sand and filtered through cotton plug. The clear spore suspension thus obtained was used for the experiments.

Conidiospores from 5–6 days old cultures were subjected to UV radiation for 30, 60, 90, 120, 150, 180, 240, 300 and 360 sec. The usual technique of plating irradiated spores in complete medium and subsequently transferring to min. agar for the isolation of biochemical and morphological mutants were followed. The maximum number of biochemical and morphological mutants were obtained at 90 and 150 sec. respectively. The following table (Table 13) shows the frequency of mutants at different treatments.

TABLE 13

SHOWING DURATION OF TREATMENT, NUMBER OF MUTANTS ISOLATED, TOTAL NUMBER OF COLONIES TESTED ON MINIMAL MEDIUM IN IRRADIATION EXPERIMENT WITH UV WITH *Penicillium Purpurogenum* STOLL

Time of irradiation in sec.	Number of colonies transferred to minimal plate	Number of biochemical mutants	Number of morphological mutants	Percentage of biochemical mutants	Percentage of morphological mutants
0	960	nil	nil	nil	nil
30	1232	1	3	0.08	0.24
60	1264	4	6	0.13	0.47
90	1088	8	7	0.73	0.64
120	576	1	2	0.17	0.35
150	416	3	7	0.72	1.68
180	976	7	11	0.71	1.12
240	2048	11	14	0.53	0.68
300	1456	2	5	0.14	0.34
360	1434	1	6	0.06	0.41

Of the biochemical mutants, the more important ones are deficient in amino acids, vitamins, and nucleic acids. Detailed assay of the nutritional mutants is in progress.

D.3.5. *Experiments on induced mutations of Sordaria bosensis*

X-ray mutants.—Detailed assay of primary selections of mutants, from colonies cultured on agar plates after treatment of ascospores with X-rays, were done by following the auxanographic technique. On rigid testing most of the initial selections were left out and detailed assay with individual mutrients was done with only seven biochemical mutants. These mutants responded to (i) Biotin/Choline/PABA—mutant No. 1, (ii) Biotin-mutant Nos. 2, 3 and 6, (iii) Choline/Riboflavin/Pyridoxine/Biotin—mutant No. 4 and (vi) Choline/Biotin—mutant No. 5 and (v) Choline—mutant No. 7.

UV mutants.— large number of UV mutants were selected initially. 94 of these mutants were obtained from the 60 min. UV treated population and 97 from 90 min. treated population. On repeated transfers in the most suitable modification of the A/1 minimal medium such as A_1 with 2 g. NH_4NO_3, and A_1 with 2 g. NH_4NO_3 plus succinic acid. Many of the mutants reverted to the wild type. Those mutants including the slow-growing ones were subjected to a very critical test by growing them individually in the liquid minimal medium in 100 ml flasks. Thirty three biochemical mutants have been characterized so far; the remaining isolations reverted to the wild type during assay experiments. The mutants responded variously to vitamins, amino acids and nucleic acids. The technique adopted for the characterisation of biochemical mutants is a very elaborate one requiring a large number of glasswares and repeated observations in different supplemented media through several weeks.

A few morphological mutants have also been isolated. The mutants are characterised by (a) crumpled colony formation, (b) compact growth, (c) flesh coloured mycelium, (d) petit colony formation, (e) nonsporulating behaviour, and (f) accumulation of dark brown pigment in the medium.

D.3.6. *Experiments with Neurospora crassa*

Studies in photoreactivation on treatment of conidiospores of *Neurospora crassa* 489 !a (methionineless) with acridine orange were done. The experiment yielded positive results and it is being planned to take up the work on an elaborate scale.

D.4. Rhizobium bacteriophage

D.4.1. *Field trial experiments on legume inoculation with phage-resistant (R) and non-resistant (NR) Rhizobium spp.*

In the field trial experiment the design and layout were based on 8 legume crops belonging to different cross-inoculation groups. The *Rhizobium* strains both non-resistant (NR) and resistant (R) to bacteriophage were previously tested for their efficiency to form nodules, under laboratory conditions. The present investigation deals with the growth and nodulation of legumes as a result of seed inoculation with non-resistant (NR) and resistant (R) *Rhizobium* strains. There were control plants in each set which received no inoculation of bacteria.

The method of inoculation of legume seeds before sowing is briefly described below. Every care was taken to maintain aseptic condition until the seeds were actually sown in soil. Watering on a moderate scale was done after sowing to keep the layer of inoculated bacteria on the surface of seeds in variable condition until the seeds fully germinated. As rhizobia are known to be killed on prolonged exposure to direct sunlight, precautions were taken to perform the treatment experiment in a shady place. Application of chemical fertilisers were avoided as they have been known to affect the multiplication of nodule bacteria on inoculated seeds. A list of legumes grown for the experiment is given below :

List of legumes

	Plant Genera and species	*Plant group*	*Bacterial strain (R)*
1.	*Trigonella faenum graecum*	... Lucern	... Rh. meliloti (1)
2.	(i) *Lathyrus sativus*	... Pea	... Rh. leguminosarum (2)
	(ii) *Lens esculanta*	... ,,	... ,,
3.	*Phaseolus radiatus*	... Bean	... Rh. phaseoli (3)
4.	(i) *Arachis hypogaea*	... Cowpea	... Strain specific
5.	*Cicer arietunum*	... Specific	... –do–

Among the 6 legumes grown, *Arachis hypogaea* is a summer crop. The rest were cultivated in the climatic condition of the winter season of West Bengal. In the experimental plots the design was that there were 3 rows of plots each 4′ ft. apart. The crops received irrigation during season and care was taken to keep the plots free of weeds. The crop remained in the field for ten weeks and records were taken in the following manner.

The first set of data were collected on the fourteenth day after sowing and thence at intervals of 7 days which continued upto the fortyninth day. The average of the number of nodules, height of the shoots and dry wts. of shoots and roots were recorded on each scheduled date, and figures in the tables show an average of 20 plants collected at random from 2 plots of each treatment group.

It has been shown that nodulation in legumes starts at about the stage when the first true leaves emerge out. The commencement of nodulation varies from plant to plant as well as is conditioned by the climatic and edaphic factors. As the record of the results of the present experiment started after 14 days of sowing, most of the plants in the field plots must have grown sufficiently and nodulation, if not disturbed, must have occurred at an earlier date. The longevity of the nodules bears a correlation with the growth habits of

(1), (2) and (3) indicate numbers of strain.

the host legumes and in the case of ineffective nitrogen-fixers the degeneration of the nodules takes place after 7–8 days growth in most herbaceous plants. The effective nodules in the majority of cases may remain intact on the roots for 5–10 weeks from the date of formation. With the commencement of fruiting or under extreme conditions of temperature and moisture the carbon and nitrogen ratio of the host metabolism is disturbed and they induce a change in the rate of nitrogen fixation followed by a degenerative effect on nodules.

In *Trigonella faenum graecum* (Table 14) the peak of the nodulation was observed on the fortyninth day of growing of NR and R sets of plants while in control plants the nodulation was appreciably different from those of treated ones. The nodules were absent from the roots of control plants on the twentyeighth day. This indicated that the appropriate *Rhizobium* spp. were probably scarce in the soil and that there were ineffective strains in the experimental field soil.

In *Lathyrus sativus* and *Lens esculanta* (Table 15a and 15b) both belonging to the pea group had shown a very distinctive picture concerning nodulation of the control plants, as the nodules were completely absent from their roots. The highest figure in the number of nodules in the inoculated plants was observed on the fortyninth day. The height of shoots of *Lathyrus sativus* differed from those of the control from the twentyeighth day leading to an appreciable difference on the fortyninth day. However, in *Lens esculanta* the heights of the treated sets of plants showed difference from those of the control since the beginning of growth but to a lesser degree.

The field trial experiments on *Phaseolus radiatus* (Bean group) showed (Table 16) an altogether different picture of nodulation as well as height of plants and dry wt. of shoots and roots. The nodulation in the control in this case was almost equal to the number recorded in the treatment set of plants. Little difference in the height of plants among the control and treated sets was seen and dry wt. of shoots and roots of plants showed no appreciable difference.

The results obtained in the field trial experimental of *Arachis hypogaea* (Table 17) are remarkably different from the other four legumes described above. Here the number of nodules increased until the fortyninth day and figures for the same in control plants were significantly different from those inoculated with non-resistant bacteria. Furthermore, a wide difference between two inoculated sets, (NR) and (R), was most significant in this case.

In *Cicer arietinum* the nodules were absent in the plants of uninoculated C plots (Table 18) and there was sharp difference in the growth between the plants in the uninoculated and inoculated plots. The difference in the growth and nodulation was also noticeable in the plants belonging to NR and R plots. The nodulation was maximum on the fortysecond day in the inoculated ones and the number of nodules per plant as recorded here in fact, represented complex structures which were quite different from single nodules of other plants. The dry wts. of the shoot and root were greater in the inoculated than those of the uninoculated ones and the increase was nearly two-fold.

TABLE 14

GROWTH OF *Trigonella faenum graecia* IN FIELD PLOTS

Growth in days

	28			35			42			49		
	C	NR	R	C	NR	R	C	NR	R	C	NR	R
No. of Nodules	—	5.9	6.2	—	9.4	10.0	—	9.8	10.8	—	10.6	11.8
Ht. of shoots (cm)	5.9	6.2	6.3	6.6	7.1	7.4	8.1	8.5	8.7	9.8	9.8	10.8
Dry wt. of shoots (gm)	0.026	0.03	0.03	0.059	0.06	0.06	0.078	0.08	0.08	0.088	0.089	0.09
Dry wt. of roots (gm)	0.008	0.008	0.008	0.01	0.01	0.01	0.017	0.017	0.018	0.024	0.024	0.024

TABLE 15A

GROWTH OF *Lathyrus sativus* IN FIELD PLOTS

Growth in days

	28			35			42			49		
	C	NR	R	C	NR	R	C	NR	R	C	NR	R
No. of nodules	—	5.5	6.4	—	7.6	8.3	—	9.4	10.2	—	10.6	11.9
Ht. of shoots (cm)	9.2	11.4	11.7	9.6	11.9	12.3	10.2	14.3	15.4	11.8	17.5	17.5
Dry wt. of shoots (gm)	0.07	0.25	0.26	0.11	0.36	0.36	0.13	0.46	0.47	0.17	0.61	0.70
Dry wt. of roots (gm)	0.013	0.03	0.03	0.013	0.034	0.034	0.014	0.036	0.035	0.015	0.042	0.036

TABLE 15B

GROWTH IN *Lens esculenta* IN FIELD PLOTS

Growth in days

	28			35			42			49		
	C	NR	R	C	NR	R	C	NR	R	C	NR	R
No. of Nodules	—	7.0	7.0	—	8.2	8.3	—	9.0	9.0	—	10.03	10.05
Ht. of shoots (cm)	7.9	9.0	9.0	10.1	11.1	11.2	11.4	12.0	11.8	12.7	14.8	14.7
Dry wt. of shoots (gm)	0.04	0.06	0.059	0.05	0.07	0.07	0.06	0.08	0.086	0.014	0.21	0.21
Dry wt. of roots (gm)	0.006	0.007	0.007	0.007	0.008	0.009	0.08	0.011	0.01	0.011	0.013	0.013

—indicates absence of nodules

TABLE 16

GROWTH OF *Phaseolus radiatus* IN FIELD PLOTS

	Growth in days											
	28			35			42			49		
	C	NR	R	C	NR	R	C	NR	R	C	NR	R
No. of Nodules	3.4	3.5	4.6	1.9	3.5	3.9	1.75	1.88	1.7	1.0	0.75	1.2
Ht. of shoots shoots (cm)	8.25	8.8	8.9	9.5	9.6	9.8	10.2	10.4	10.8	10.75	11.2	11.6
Dry wt. of shoots (gm)	0.1	0.11	0.12	0.14	0.15	0.16	0.176	0.18	0.18	0.21	0.24	0.25
Dry wt. of roots (gm)	0.013	0.013	0.014	0.015	0.02	0.015	0.019	0.026	0.026	0.024	0.029	0.03

TABLE 17

GROWTH OF *Arachis hypogea* IN FIELD PLOTS

	Growth in days											
	28			35			42			49		
	C	NR	R	C	NR	R	C	NR	R	C	NR	R
No. of Nodules	19.6	16.1	64.0	32.0	62.0	79.1	41.2	75.0	89.0	64.0	98.0	129.0
Ht. of shoots (cm)	13.6	16.6	23.0	15.1	22.2	28.8	21.2	35.5	42.1	30.6	40.9	42.1
Dry wt. of shoots (gm)	1.2	1.4	1.8	2.8	3.6	4.0	6.1	8.5	10.2	8.2	11.2	11.8
Dry wt. of roots (gm)	0.14	0.15	0.28	0.28	0.31	0.45	0.51	0.68	0.82	0.67	0.91	0.92

TABLE 18

GROWTH OF *Cajanus indicus* IN FIELD PLOTS

	Growth in days											
	28			35			42			49		
	C	NR	R	C	NR	R	C	NR	R	C	NR	C
No. of Nodules	5.2	6.5	7.7	5.5	7.8	8.5	10.5	12.5	13.2	3.7	8.5	8.2
Ht. of shoots (cm)	28.6	34.1	35.0	39.1	42.8	44.0	51.2	55.0	55.9	59.8	62.8	63.2
Dry wt. of shoots (gm)	0.41	0.48	0.49	0.58	0.65	0.66	0.9	1.2	1.3	1.21	1.9	1.91
Dry. wt. of roots (gm)	0.03	0.04	0.04	0.07	0.08	0.08	0.12	0.18	0.19	0.2	0.28	0.29

D.5. Ecology of air micro-organisms

D.5.1. *Survey of Cladosporium, an allergenic fungi*

A survey of the fungal units occurring in air has been carried out for three years in Calcutta, Shyamnagar and Falta, the results of which have been reported in the previous report.

Among the various fungal genera, *Cladosporium*, a common dominant air-borne fungus, showed a sharp seasonal variation. They are totally absent during the summer and rainy seasons, but as soon as the temperature goes down, they appear on the exposed plates. The increase in number starts at the beginning of November and persists till the end of February. With the rise of temperature of air the count gradually disappear.

The results are given in Table 19.

TABLE 19

OCCURRENCE OF *Cladosporium* SP. IN AIR

Localities	Nov. 62	Dec. 62	Jan. 63	Feb. 63	March 63	Apr. 63	Nov. 63	Dec. 63	Jan. 64	Feb. 64	Oct. 64	Nov. 64	Dec. 64	Jan. 65	Feb. 65
	Percentage														
Calcutta	25	23	53	39	—	—	3	33	31	16	5	4	12	28	27
Shyamnagar	24	33	56	58	8	28	14	33	42	52	4	24	50	60	41
Falta	2	41	52	49	5	0.5	8	19	70	33	—	22	35	48	40

— indicates absence of *Cladosporium*.

D.5.2. *Cellulose-decomposing ability of microorganisms isolated from air samples*

The following fungi—*Ceratocystis paradoxa, Sordaria hypocoproides* and *Stachybotrys* sp. were tested for their ability to grow on cellulose made from filter paper as the shole source of carbon in pure cultures.

A number of conical flasks (Cap. 250 ml.) were taken and washed thoroughly, first with chromic acid and then with distilled water. Filter papers (9 cm. diameter) were dried at 60°C for 24 hours, cooled, weighed and their respective weights recorded. Two such filter papers were moistened in each of the flasks with 50 ml. of Czapek-Dox mineral solution.

The sterilized media were inoculated with 1 ml. spore suspension of each of the above fungi. In all the experiments, five replicate flasks were inoculated with each fungal isolate and five were left uninoculated as controls. The flasks were then incubated for 30 days at 25°C. The relative humidity was maintained at 66%. After the growth period, the filter papers were taken out rinsed thoroughly in distilled water and dried at 60°C for 24 hours. The mycelial weights of the different flasks were determined. Results obtained are given in the following table (Table 20).

TABLE 20

LOSS IN DRY WEIGHT OF FILTER PAPERS INOCULATED WITH THREE FUNGAL ISOLATES AND THE RESULTING MYCELIAL WEIGHTS

Test fungus	Percentage loss in dry weight of filter paper. (mean of 5 replicates)	Mycelial weight mg.of the resulting fungal cultures. (mean of 5 replicates).
Ceratocystis paradoxa	1.5	24.3
Sordaria hypocoproides	5.8	46.6
Stachybotrys sp.	15.2	90.2

D.5.3. *Extraction of antigenic principles from Cladosporium sp.*

The test fungus was grown in malt extract 1·5% broth in Roux bottles, at 25°C. After seven days of incubation, the mycelial mats were collected squeezed off and weighed.

Half of the mats were then ground with sufficient quantity of aluminium oxide in a mortar with frequent addition of 10% sodium chloride solution. The grinding process, required more than two hours after which the mycelial mats form a pastse. Then the semi-liquid mass was dissolved in sufficient quantity of the said sodium chloride solution and thoroughly mixed in paring blender. The mixture was then centrifuged at 4000 r.p.m. for 30 minutes and the supernatant was collected. It was passed through a No. 5 Sintered glass filter to remove bacteria.

Another set of extraction was carried out with the remaining half of the mycelial mats, by grinding them with aluminium oxide in which 10% sodium desoxychlolate solution was added little by little (about 25 cc.). The grinding process required more than two hours and sodium chloride solution (10%) was added in quantity sufficient to form a paste. The mixture was blended throughly and centrifuged at 4000 r.p.m. for 30 minutes and the supernatant fluid was taken and passed through a No. 5 Sintered glass filter.

The filtered samples were used separately for the determination of protein contents. On analysis the first sample was found to contain 0·5 mg. and the second, 0·7 mg. of protein per ml. of the samples. The filtrates are stored at 10°C. Further studies are being carried out for the determination of their antigenic properties.

D.6. Industrial fermentation by isolated *A. niger* strains

D.6.1. *Selection of strains of A. niger producing gluconic acid.*

A group of 35 strains of *A. niger* was freshly isolated after screening 209 samples and their gluconic acid productive capacity was determined. Of the isolated 30 selected, strains for fermentation the results of 14 strains were compared with the type strain *A. niger* 67 along with the isolates screened in the previous year (1964-65). The medium used for testing the selected strains was modified and sterile air was forced into the culture bottles under pressure. The conversion was rapid and higher titre was attained within 54 hours of growth.

The following table (Table 21) shows the acid titre and quantity of mycelium produced within 72 hours of fermentation. Substitution of glucose with other hexose sugars did not increase the titre and the conversion rate was poor except with glucose. The best sugar medium was commercial corn syrup which contains 85% glucose and a few other hexose sugars in traces.

TABLE 21

GLUCONIC ACID PRODUCTION IN STATIONARY CULTURE

Strain Index (A. niger strain)	Glucose consumed (Conc. 30 gm/ 100 ml.)	Gluconic acid produced** (gms)
Type strain 67	25.6	22.2*
40K	13.2	11.0
112	15.0	12.4*
106	16.7	14.0*
502	13.0	10.5
763	12.2	9.8
134	22.0	18.0*
154	18.7	16.4*
176	14.9	12.2*
182	14.0	11.0
192	12.7	10.6
206	11.9	10.0
207	11.0	8.8
208	10.3	8.5
209	10.5	8.5

* The corresponding strains are selected for testing at the next step in tall gas-washing bottles.
** Yield calculated on the basis of calcium gluconate produced.

D.6.2. *Glass fermenter for gluconic acid fermentation*

Gas-washing jars made of pyrex glass with bottoms replaced by sintered glass plates are used for gluconic acid fermentation. The jars are filled 2/3rd with culture media and kept closed in a small metal chamber. Filtered air from a air-compressing system was introduced into the chamber and the medium was kept agitated by air bubbles. The conversion of glucose into gluconic acid starts after 4 hours of incubation and calcium carbonate was added to the fermentation bottles after 10 hours of incubation for neutralizing the acid produced. The rate of production of gluconic acid is given in Table 22.

TABLE 22

GLUCONIC ACID PRODUCTION IN GLASS FERMENTERS

Glucose in medium 20 gm/100 ml.

Strain Index (A. niger strain)	Experiment No.*	Air pressure lb/sq. in.	Gluconic acid** in 5 hours (per 100 ml.)	Gluconic acid** in 10 hours (per 100 ml.)
	1 (type strain 67)	15.0	2.0	4.5
	2 ,,	15.0	2.2	4.6
	3 ,,	16.0	2.6	4.9
	4 (Type strain 112)	17.0	1.0	2.1
	5 ,,	16.0	1.1	2.5
	6 ,,	16.0	1.6	2.9
	7 (Type strain 106)	15.0	1.6	3.2
	8 ,,	15.0	1.7	3.6
	9 ,,	15.0	1.7	3.6
	10 (Type strain 134)	17.0	2.1	4.0
	11 ,,	17.0	2.0	4.1
	12 ,,	17.0	2.0	4.2
	13 (Type strain 154)	16.0	1.9	3.9
	14 ,,	16.0	1.9	3.7
	15 ,,	17.0	2.0	4.0
	16 (Type strain 176)	15.0	1.0	2.1
	17 ,,	16.0	1.0	2.4
	18 ,,	16.0	1.0	2.2

* In each experiment 500 ml. gas-washing jars are used and the data presented are average of five replicates.

** Yield calculated on the basis of calcium gluconate produced.

D.7. Transformation in microorganisms

D.7.1. *Transformation in Aspergillus nigar* (V_{35})

In transformation the following genetic markers were chosen for the strains donor or recipient strains.

(a) Conidial colour
(b) Size and shape of the colony
(c) Pigment production
(d) Nutritional specificity

It is expected that DNA of the donor parents could cause changes in the characteristics of the recipient ones and the progeny of the transformants assumes a stable genetic constitution after a few generations. In many cases the changes caused are intermediate between the donor and the recipient.

In the present study the transforming DNA was isolated from *A. niger* strain V_{35} (parent) which grows moderately on agar medium and produces black conidia. The recipient was a strain morphologically similar to the wild parent V_{35} but nutritionally deficient in

methionine. As a result of DNA treatment the biochemical mutant strain (Leucineless) of the above parent V_{35} was found to develop transformants amongst which about 10 different intermediate transformants were isolated and they were tested repeatedly in plate cultures. Their growth pattern and colony characteristics are given in Table 22. The intermediate forms are mostly stable for 12 succeeding generations and segregations are not recorded.

The occurrence of intermediate characters amongst transformed recipient strains was recorded by Ephrussi-Taylor (1956) in *Klebsiella pneumoniae* indicating abnormality in the acquisition of hereditary characters.

TABLE 23

GROWTH CHARACTERS OF TRANSFORMANTS OF A. NIGER (V_{35})

Type	Colony colour	Colony diameter (CM)	Sporulation	Other character of the colony
I	Black	7.6—8.0	Heavy	White margin Reverse wrinkled.
II	Black	8.5	Sparsed	Colony with radiating furrow from centre; white margin.
III	Black with a small white patch in the centre.	8.0	Thickly sporulated in the centre.	Irregular sporulation all over except centre.
IV	Black	7.5	Alternate thick and thin sporulation.	Sporulation uneven occurring in patches; margin ridged.
V	Black	8.5	Centre heavily sporulated	Margin with ridge and furrow.
VI	Black	7.0	Uneven sporulation	Formation of three sectors with thin sporulation.
VII	Black	9.0	Centre heavily sporulated.	Yellow pigment on the reverse sectors as in VI.
VIII	Black	7.0	Medium with dusty spores in the centre.	Diffused yellow pigment on the reverse; radiating sector; yellow pigment on the reverse.
IX	Cinnamon	9.5	Heavy sporulation in the centre.	Margin ridged and furrowed, divided into two sectors; unequal growth.
X	Black	8.5	Uneven sporulation	Very outer margin.
XI	Black	8.5	Conidiophores with small conidia.	Secondary mycelium with wrinkled reverse.
XII	Black	7.5	Heavy	Lower surface wrinkled with white margin.

D.8. Physiology of microorganisms

D.8.1. *Solubilization of insoluble phosphates by "Rhizosphere" fungi*

A detailed survey for phosphate solubilizing fungi was made. Isolations of fungi from "Rhizoplane", "Rhizosphere" and "Non-rhizosphere" soils were done after the methods of Harley and Wild (1955) and Peterson (1958) on Czapek-Dox agar with streptomycin 50 μg/ml. for controlling bacterial growth. Phosphate solubilizing capacity was tested according to the method of Katznelson and Bose (1959) and also by estimation of phosphorus by photoelectric colorimeter (Fiske and Suba Rao, 1925). Legume rhizosphere (*Arachis*, *Desmodium*, *Lens*, *Pisum*, *Crotolaria* etc.) in general contained higher percentage of phosphate solubilizing fungi than non-rhizosphere soil. Among the phosphate solubilizers variations have been noted with respect to the capacity to solubilize insoluble phosphates as also some sort of preferential behaviour has been noted for the solubilization of various sources of insoluble phosphates. Growth of solubilizing fungi in presence of insoluble phosphates was not affected at all or only slightly affected. Incubation of soil with insoluble calcium and aluminium phosphates markedly stimulated the general fungal and actinomycetal population as also the percentage of solubilizers. Concomittent with this there was also an increase in the available phosphorus content of soil after such enriched incubation. Investigation with Lucern, Berseem, Pea, Gram, Lens are being conducted to determine precisely the role played by rhizoplane and rhizosphere fungi in breaking up the insoluble phosphates, factors affecting the breakdown, tracing the fate of solubilized phosphorus and the mechanism of solubilization.

D.8.2. *Microbiological investigations on a Pellagra-like disease caused by infected rice*

Recently there was an out-break of pellagra-like disease of unknown origin in the district Nadia, West Bengal. The disease was characterized by the blackening and hyperkeratosis of feet and hands and the appearance of "Malar flush". Though many features resemble pellagra, the affliction differed in the association of jaundice, hepatomegaly or hepatospleomegaly, and the occurrence of haemorphagic manifestations in some of them. On detailed survey of the affected regions complaints regarding the blackish discolouration of rice which after boiling became bitter to taste and malodorous were heard of. It was felt that the disease might have been cuaused by some thermostable toxin which was not destroyed during the normal cooking process. Paddy seeds, rice grains, and soil samples from the affected areas were received for microbiological investigation. The surface scraping of rice grains revealed the presence of the fungal structures like spores and mycelial bits which gave rise to well-formed colonies during moist chamber studies. These preliminary observations led to the thinking that the thermostable fungal toxin, might be the causative factor for the disease and the following investigations were made.

D.8.3. *Isolation of and studies on microorganisms of the paddy seeds*

Isolations were made from the surface sterilized whole seeds, husks and rice grains on glucose-peptone agar medium with streptomycin 50 μg/ml. Seed samples were collected

from three locations in West Bengal where pellagra-like disease was recorded. *Heterosporium* sp. and *Aspergillus* sp. were found to occur in all the samples. *Chaetomium* sp. was quite frequently recorded from two samples. Other species recorded were *Helminthosporium*, *Mucor*, *Penicillium* and *Masoniella*. It was interesting to note that 40% of the total fungal population had quite good antibacterial activity notably among the species of *Aspergillus* and *Chaetomium*. The possible effects on paddy and also on public health remain to be worked out.

D.8.3.1. *Role of Heterosporium sp. in causing pellagra-like disease*

While carrying out the total isolation it was interesting to locate one organisms, which, though resembled *Helminthosporium* was sharply differentiated by the presnence of echinulations on the spore wall. Further, isolations from whole paddy seeds, seed husks, rice grains and soil samples consistently gave rise to colonies with spores of the above fungus. This organism elaborated deep brown pigment on glucose-peptone medium which had a characteristic smell. In order to find out the relationship with paddy seeds the fungus was inocula ted onto the surface of sterilized paddy seeds and incubated. After 6–8 days of incubation the fungus was found to have completely colonized the seeds and the grains were transformed into sooty black fungal spore mass, the husk, however, remained unaltered.

During the normal cooking process of rice all the organisms present are killed and hence it was thought that the termostable fungal toxin might be the cause of malady. In order to find out if the fungus elaborated any toxic substance, it was grown on glucose-peptone medium in a rotary shaker. After 7 days of incubation the culture medium was filtered through sintered glass filter no. 5 and evaporated under vacuum at 50°C. The concentrated cultures filtrate was tested for its effect on experimental animals.

From morphological and cultural studies it is concluded that the fungus belongs to the genus *Heterosporium* and appears to be a species not recorded so far. Further studies on exact taxonomic position, survival under natural conditions and possible relationship with paddy seeds along with toxicological properties are under progress.

E. ANIMAL PHYSIOLOGY

E.1. Physiological studies on the liver, thyroid and pituitary of tadpoles (Bufo melanostictus and Rana tigrina)

E.1.1. *Histochemical studies on liver of tadpoles.*

E.1.1.1. *Neutral lipid, tyrosine and melanin contents in liver of tadpoles at various stages of development.*

Histochemical studies on the content of neutral lipid, tyrosine and melanin in liver of tadpoles at different stages of development were performed for an attempt to correlate the changes with the advancement of stages.

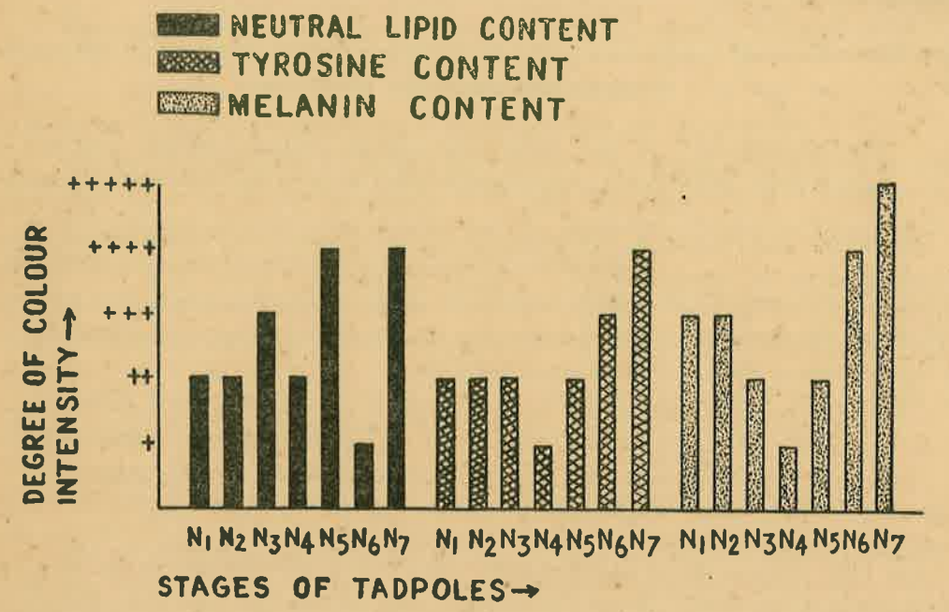
The tadpoles of *Bufo melanostictus* and *Rana tigrina* at different stages of development were selected. The seven different stages of tadpoles as used in the present as well as in the following experiments and their corresponding stages described by Taylor and Kollros are given in Table 1.

TABLE 1

DIFFERENT STAGES OF TADPOLES USED AND THEIR CORRESPONDING STAGES (AFTER TAYLOR AND KOLLROS)

Stages designated		Stages in larval development of *Rana pipiens* (after Taylor and Kollros).	
No.	Corresponding stage number according to the classification of Taylor and Kollros	General designation	Stage number
N_1	V	Limb bud stage	I–V
N_2	Between the stages IX and X	Paddle stage	VI–X
N_3	Between the stages XII and XIII (with small hind limbs).	Foot stages (Pre-metamorphic stages).	XI–XVII
N_4	XVII (with large hind limbs).		
N_5	XX (with four limbs).	Metamorphic stages.	XVIII–XXV
N_6	Between the stages XXII and XXIII (with four limbs and partially retracted tail).		
N_7	XXV (completely metamorphosed tadpoles; froglet).		

The result of the content of neutral lipid, tyrosine and melanin in liver of tadpoles at different stages of development during their larval life are shown in Graph 1. Similar nature of distribution of neutral lipid, tyrosine and melanin was observed in both the species of tadpoles under study. The individual tadpole of each species at a particular stage exhibited similar results.



It is clear from Graph 1, the content of neutral lipid in liver remained more or less at the same level of distribution upto the end of the foot stages excepting at the beginning of foot stages (stage N_3) when a slight enhancement in the level was observed. It increased markedly at the time of appearance of front legs (stage N_5) which was followed by a sharp fall during the period of absorption of tail (stage N_6) but again by a sharp rise when the tadpoles exhibited complete metamorphosis (stage N_7). Maximum content of neutral lipid in liver was observed when the tadpoles showed just emergence of fore limbs as well as completion of metamorphosis.

Tyrosine in liver was at the same level from the later period of limb bud stages upto the earlier period of foot stages (stages N_1 to N_3). A sharp fall of tyrosine was found at the end of the foot stages (stage N_4) when the hind limbs appeared to be fully grown. It then started to increase gradually from the beginning of the metamorphic stages and became maximum when the tadpoles showed completion of metamorphosis (Graph 1). Tyrosine level was found to be maximum at the end of the metamorphic (stage N_7).

Melanin was observed to be at the same level of distribution at limb bud stages (stage N_1) and also at paddle stages (stage N_2). This pigment then started to decline at the beginning

of pre-metamorphic stages (stage N_3) and reached the lowest level at the end of these stages *i.e.*, at stage N_4. As in case of tyrosine, the emergence of fore limbs (stage N_5) was associated with a rise of melanin content. But the increase of melanin was more marked than that of tyrosine during the absorption of tail (stage N_6) and also at stage XXV (stage N_7). The heaviest distribution of this pigment granules occurred at stage N_7, that is, when the tadpoles were completely transformed into froglets.

E.2. Histochemical studies on liver of tadpoles under thyroxine, vitamin B_{12}, thiourea and penicillin treatments

E.2.1. *Histochemical studies on the alkaline and acid phosphatases, glycogen, neutral lipid, protein, RNA, DNA, tyrosine and melanin under thyroxine and vitamin B_{12} treatments.*

Thyroxine and vitamin B_{12} increased the activity of both the enzymes, alkaline and acid phosphatases, in liver of tadpoles. This increase was more prominent in case of thyroxine treatment. These two enzymes underwent similar pattern of fluctuations according to the stages to which the tadpoles had reached as observed in case of normal tadpoles, but the variations were always at an enhanced level.

When the tadpoles were induced to pass through the different developmental stages by the application of thyroxine, the glycogen and the neutral lipid were stored rapidly in liver at a more increasing order upto the time of emergence of fore limbs (stage XX) in comparison to the corresponding stages of controls. As in untreated controls, the storage of glycogen and lipid reached the same highest level during the appearance of fore limbs and also when metamorphosis was completed. But during the retraction of tail there was a profound decrease in the amounts of glycogen and neutral lipid, and they reached the same level as that of the controls. The pattern of changes of the level of these substances in liver at different stages of development of thyroxine—treated tadpoles was the same as found in control tadpoles.

The level of hepatic glycogen and neutral lipid of tadpoles was apparently unaffected by vitamin B_{12} treatment. As vitamin B_{12} stimulated the advancement of stages of development of tadpoles, the time of stage to stage variations of these substances was slightly accelerated than those of normals.

Protein contents and its variations in liver of thyroxine-treated tadpoless and their controls were similar upto the end of foot stages. The level of protein in metamorphic stages was higher in treated tadpoles than that in controls, even during the regression of tail. Thyroxine was not found to cause any change on the contents of RNA and DNA in liver of tadpoles. As usual, these substances varied in amounts in accordance with the stages of development.

In liver of vitamin B_{12} treated tadpoles, the contents of protein and RNA were enhanced to some extent. But DNA remained unchanged. The form of variations of them at different stages was similar as found in normals. But protein and RNA varied at an enhanced level.

The tadpoles under the treatment of thyroxine exhibited a reduced level of tyrosine and melanin contents in liver. Vitamin B_{12} produced no effect on the level of distribution of tyrosine and melanin. But the nature of variation was the same as in normal tadpoles.

E.2.2. *Histochemical studies on the alkaline and acid phosphatases, glycogen, neutral lipid, protein, RNA, DNA, tyrosine and melanin under thiourea and penicillin treatments.*

Thiourea and penicillin decreased the activities of both alkaline and acid phosphatases. The form of variations was dependent on the stages of development of tadpoles of the treated series like that of normal tadpoles but continued all along at a reduced level. The effect of thiourea on both the enzymes was more marked than that of penicillin.

The level of glycogen and neutral lipid in liver of tadpoles was found to be reduced to a great extent by thiourea treatment, while they were unaffected by penicillin. But in both the cases, the pattern of changes of both the substances was always stage-specific, as noticed at the corresponding stages of normal tadpoles.

Thiourea produced no alterations of protein, RNA, DNA, tyrosine and melanin in liver of tadpoles. Penicillin caused a slight fall in the amounts of protein and RNA but showed no effect on the contents of DNA, tyrosine and melanin. The variations of them in liver upto the final stages of development of tadpoles were more or less the same as in normal ones.

E.3.1. *Histometric studies on liver of tadpoles at various stages of development.*

The cell area and the nuclear area of liver of tadpoles at various stages of development were measured. The exact area in μ^2 of each cell and nucleus was determined. The area of the cells and their nuclei of liver of normal tadpoles was progressively increased with their gradual growth and their advancement through the different stages of development. But in case of nuclear area there was no appreciable difference (1·7% in case of *Bufo melanostictus* and 3·0% in *Rana tigrina* tadpoles) between the end of foot stages (stage XVIII) and just after the appearance of fore limbs (stage XX). In individual tadpoles at a particular stage of development both the cell area and nuclear area were found to be more or less the same, only negligible differences were observed in a few cases. The rate of increase of cell area of liver was high during the growth of the hind limbs and gradually decreased after the emergence of fore limbs (stage XX) and the completion of metamorphosis (stage XXV). In the case of nuclear area, the rate of increase was highest at the end of foot stages (stage XVII). The rate of increase of nuclear area was minimum at the time of emergence of fore limbs, (stage XX) but the increase was again found to be phenomenal when the tadpoles developed into a tiny froglets (stage XXV).

A definite relationship between the cell area and the nuclear area of liver was noticed during the larval life of both the species of tadpoles. Percentage of nuclear area to total cell area of liver of *Bufo melanostictus* tadpoles varied from 8·9% to 12·6% and that of *Rana tigrina* tadpoles from 7·2% to 10·3%. So the percentage of nuclear area to total cell area varied with the stages of development.

E.3.2. *Histometric studies on liver of tadpoles under thyroxine, thiourea, penicillin and vitamin B_{12} treatments.*

The exogenous thyroxine caused an increase of cell area and nuclear area of liver of tadpoles. Both the cell area and the nuclear area also showed a gradual rise with the induced development of stages. Thiourea remarkably slowed down the growth of the hepatic cells and their nuclei. But the increment of cell area and nuclear area of liver of the retarded larvae was associated with their stages of development. Penicillin and vitamin B_{12} had apparently no effect on the growth of the hepatic cells and their nuclei. As usual, the cell area and the nuclear area increased in accordance with the stages of development. The only difference from the controls was that the period of metamorphosis of tadpoles was delayed by penicillin and slightly shortened by vitamin B_{12}, the interval between the stages was proportionately lengthened and shortened respectively which led to the corresponding delay or acceleration of the usual rise of the area of the hepatic cells and nuclei.

E.4. Histological studies on the activity of thyroid and pituitary glands of tadpoles

It has been reported previously that the activity thyroid glands of tadpoles gradually increases with the gradual progress of the tadpoles in their stages of metamorphosis. Penicillin treatment depresses the activity of thyroid glands and vitamin B_{12} stimulates the activity of these glands. Thyroid glands of tadpoles became atrophied by thiourea treatment and consequently the metamorphosis of tadpoles was inhibited. Considering the inhibitory action of thiourea and the metamorphosis inducing action of vitamin B_{12}, studies have been undertaken on the activity of these glands in tadpoles under various doses of thiourea, thiourea plus vitamin B_{12} treatments and also adding thyroxine on the retarded larvae by thiourea treatment. The work is in progress.

F. WORKSHOP

(*a*) The workshop consists of mechanical, electrical, refrigeration, electronic, glass-blowing and carpentary sections. Besides general maintenance and repair, Workshops manufacture equipments/apparatus/installations in the laboratories and field stations, all of which are covered by about 700 departmental requisitions, the workshop undertook the following major works during the year under review.

1. Mechanical arrangement for low level liquid Scintillation coincidence counting including device for dark transfer of Scintillators.
2. Apparatus for uniform sensivity Photon counter.
3. Thin layer Chromatography apparatus of modified design and its accessories.
4. Modification of Gas phase chromatography apparatus.
5. Construction of X-ray tube with three windows for physical investigations.
6. 50 Cm. Bent crystal cauchoies type X-ray Spectrometer.
7. Neutron Generator Target assembly with liquid air cooling system.
8. Vacuum valve.
9. Ion source probe of Aluminium for Neutron Generator.
10. Electrostatic fluxmeter of short response time for use in studies of Transient field charge.
11. Fabrication of Oscilloscope housing and fitting it with a cossor camera.
12. Driving mechanism of a camera by a fraction H. P. motor with reduction gear, to give a film drive of 4 inch per/min. fitted with a time switch relay.
13. Lead shield with attachment for β -counting system.
14. Fabrication of spares for Refrigeration and Air conditioning units of the Institute.
15. Repair and maintenance of instruments such as Centrifuge, Shaker, Auto-clave, Ovens, recorders, microscopes, vacuum pumps, fraction collectors etc. of the Institute.
16. Fabrication of some instruments made by Sir J. C. Bose viz. Microwave apparatus, Potometer.

In addition to the maintenance of Refrigerators Air Conditioners and other refrigerated equipments, the refrigeration section completed installations of two more O° cold rooms and assisted in servicing and maintenance of 'Spinco' Ultra centrifuge M.S.E. centrifuge of the Chemistry Dept.

The Electronic section is also maintaining the electronic instruments including the Spinco ultra centrifuge and DK-2 Spectrophotometer.

(*b*) The General maintenance, repairs and remodelling constructions of the laboratories at the Institute and outstations were done under the direct supervision of this department.

G. LIBRARY

The library contains 8006 volumes (3756 books and 4250 bound periodicals). During the year under report 663 volumes (335 books and 328 bound periodicals) were added to the stock as against 824 volumes during the previous year. The total number of periodicals received was 283 out of which 166 were subscribed and the remaining 117 were received either in exchange of Institute publications or as gifts. The library also received a fairly good number of technical pamphlets, circulars, reports, etc. from various institutions and governments, both Indian and foreign.

The library is kept open for 63 hours a week from 9 A.M. to 8 P.M. on weekdays and from 9 A.M. to 5 P.M. on Saturdays.

The library is primarily meant for use of its research staff. But provision has been made to admit, on the basis of reader's card, bonafide non-Institute research workers and scientists for use of the Reading Room only.

The library grant received during the year under report was Rs. 49,000.00 (Rs. 30,000.00 as recurring and Rs. 19,000.00 as non-recurring). To meet the mounting cost of periodical subscriptions, specially of American origin, the recurring grant was ear-marked for subscriptions to periodicals and binding of books and periodicals. The non-recurring grant was spent on purchasing of books and important reference works.

Need is being felt for providing additional floor space both for the display of current periodicals as well as for additional stack rooms. Plans have been included in the Fourth Five Year Plan programme for raising another storey on the library buildings for this purpose.

PUBLICATIONS

During the year under report Vol. 26, Nos. 3 and 4 (September and December 1963 issues) and Vol. 27, Nos. 1 and 2 (March and June 1964 issues) of the Transactions of the Bose Research Institute were published.

Several important books and monographs of Jagadis Chandra Bose are out of print for some time. Following a plan of reprinting them, the popular accounts written by Jagadis Chandra of his investigations "Plant Autographs and their Revelations" and "Response in the Living and Non-Living" have already been published. A collection of ten lectures delivered under the auspices of Jagadis Chandra Bose Memorial Lectures, Series I (1959-61) and Series II (1962-63) have also been published.

Other publication during the year was "Report of the Bose Institute for 1964-65."

APPENDIX A

I. Governing Body

Dr. Debendra Mohan Bose
Dr. Sunando Bose
*Shri Kedar Nath Chatterjee
Shri Saibal Kumar Gupta, I.C.S. (*Retd.*)
Prof. Satis Ranjan Khastgir
Shri Sudhir Kumar Mandal
Shri Amiya Nath Mitra
Dr. Sivotosh Mukherji
†Shri S. C. Mukherjee, I.C.S. (*Retd.*)
Dr. Basanti Dulal Nagchowdhuri
Prof. Bhupati Mohan Sen, I.E.S. (*Retd.*)
Dr. Jyotish Chandra Sengupta

II. The Council

*Shri K. N. Chatterjee
Shri S. K. Mandal
Dr. S. Mukherji
Dr. B. D. Nagchowdhuri
Prof. B. M. Sen
Dr. J. C. Sengupta
} Nominees of the Governing Body

Dr. D. M. Bose, Director, Bose Institute (*Ex-officio*)
Secretary to the Government of India in the Ministry of Education or his nominee.
Director-General, Scientific and Industrial Research or his nominee.
Financial Adviser to the Ministry of Education.
Shri P. R. Ramakrishna, M.P. — Representative of Lok Sabha.
Prof. P. Parija, Vice-Chancellor, Utkal University.
Prof. G. N. Ramachandran, Madras University.
} Scientists from outside West Bengal, nominated by Govt. of India.

Dr. P. C. Mukherji, Director of Public Instruction, — Representative of the Govt. of West Bengal.
Accountant-General, West Bengal.
Shri Nandadulal Sreemany (Representative of the Calcutta Corporation)
Registrar, *Secretary*.

III. Finance Committee

Prof. B. M. Sen, Chairman (Representative of the Council)
Accountant General, West Bengal.
Financial Adviser to the Ministry of Education.
Dr. B. D. Nagchowdhuri (Representative of the Governing Body).
Dr. D. M. Bose, Director (*Ex-officio*).
Registrar, *Secretary*.

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* Died on 16 May 1965 † Died on 1 May 1965

APPENDIX B

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

Department of Physics

Head of the Dept.: Prof. S. R. Khastgir, Ph.D., D.Sc., F.N.I.
Reader : Shri A. M. Ghose, M.Sc.
Research Fellow : Dr. T. C. Bhadra, M.Sc., D.Phil.
Research Scholar : Shri A. K. Chatterjee, M.Sc.
Shri Satyanarayan Chongdar, M.Sc.

Mary and Richard Keating Scholar : Shri Amitava Ghosh, M.Sc.
C.S.I.R. Pool Officer: Dr. (Mrs.) Purnima Sinha M.Sc., D.Phil.

Department of Chemistry

Head of the Dept.: Dr. A. Sen, Ph.D. (Leeds)
Reader : Dr. A. K. Barua, M.Sc., D.Phil.
Dr. Sudhamoy Ghosh, M.Sc., D.Phil.

Research Fellow : Shri J. Dutta, M.Sc.
Dr. D. P. Chakraborty, M.Sc., D.Phil.
Research Assistant : Shri H. C. Chakraborty, B.Sc.
Research Scholar : Md. Monimal Haque, M.Sc.
Shri Shib Prasad Datta, M.Sc.
Sm. Roma Chatterjee, M.Sc.
Shri Asoke Ghose, M.Sc.
Shri Swapan Kr. Ghose, M.Sc.

Govt of India : Shri Basudev Das, M.Sc.
Training Scholar : Shri Asoke Ghose, M.Sc. (upto 9-3-66)
Shri Asis Dutta, M.Sc.
Shri Sunil Kr. Paul, M.Sc.
Sir. J. C. Bose Fellow: Dr. S. R. Ghoshal, M.B.B.S., D.C.H., (Cal. University).
C.S.I.R. Pool Officer: Dr- (Mrs.) Ashoka Ray M.Sc., D. Phil.
do Dr. N. K. Sinha, M.Sc., D. Phil.
do Dr. Sankar Mitra M.Sc., Ph.D.
do Dr. A. N. Bhaduri M.Sc., D. Phil.

Guest Workers : Prof. S. N. De, M.B., D.T.M., Ph.D. (Lond) D.Sc. (Lond)
Dr. P. N. Das. M.B.B.S.
Shri Sudhir Kumar Ghosh M,Sc.
Sm. Anima Dutta M.Sc.

Department of Botany

Head of the Dept.: Dr. Sunando Bose, M.Sc. (Cal.) Fil-Doct. (Lund)
Reader : Dr. B. B. Biswas, M.Sc., D.Phil.
Research Fellow : Dr. R. K. Basu, D.Sc. (Cal.)
Dr. V. N. Gadgil, M.Sc., D.Phil.
Shri Balaram Mazumder, M.Sc.
Statistician : Shri A. K. Roychowdhury, M.Sc.
Research Assistant : Shri B. K. Dutta
Shri A. T. Guhathakurta
Shri Amiya Kumer Adhikary, M.Sc.
Research Scholar : Shri Sukumar Gupta, M.Sc.
Sm. Sukla Sengupta, M.Sc.
Govt of India Training Scholar : Sm. Pratima Roychowdhury, M.Sc.
Shri Pritisadhan Basu, M.Sc.
Shri Kali Krishna Ghoshal, M.Sc.
C.S.I.R. Pool Officer: Dr. Biswambar Saha M.Sc., D. Phil.
do Dr. Sunirmal Chanda, M.Sc., D.Sc.

Department of Microbiology

Head of the Dept.: Dr. P. N. Nandi, Ph.D. (Lond), D.I.C.
Research Fellow : Shri K. L. Chowdhuri, M.Sc.
Dr. (Mrs.) Ashalata Chandra, M.Sc., D.Phil.
Dr. A. K. Mishra, M.Sc., D.Phil. (on leave)
Dr. S. L. Chakraborty, M.Sc., D.Phil.
Research Assistant : Shri Arun Kr. Chatterjee, M.Sc.
Research Scholar : Sm. Jharna Mitra, M.Sc.
Sm. Roma Barat, M.Sc.
Sm. Kalyani Bhattacharya, M.Sc.
Govt of India Training Scholar : Sm. Ujjaini Chowdhuri, M.Sc.
Shri Panchu Gopal Roy, M.Sc.
Shri Sisir Kr. Chattapadhyaya, M.Sc.
C.S.I.R. Pool Officer: Dr. S. B. Ghosh, M.Sc., D. Phil.

Physiology

Research Fellow : Dr. A. K. Medda, M.Sc., D.Phil.
Research Scholar : Sm. Rama Ghose, M.Sc.
Attached Worker : Shri G. C. Bhattacherji

Workshop

Superintendent : *General* Shri M. L. Chatterjee
–do– : *Electrical and Refrigeration* Shri S. R. Ghosh.

Library

Librarian : Shri P. K. Chakraborty
Asstt. Librarian : Sm. Ila Biswas, B.A.
Library Assistant : Sm. Protima Chakraborty, B.A.

STAFF APPOINTED UNDER GRANT-IN-AID SCHEMES

A. Department of Atomic Energy, Government of India

Scheme I : Nuclear Physics investigations with Neutron Generator
Investigators-in-charge : Shri A. M. Ghose, M.Sc.
Shri B. Mitra, M.Sc.
Junior Scientific Officer : Dr. Amalendu Nath, M.Sc., D.Phil.

Scheme No. II : "Investigation on recurring irradiation and biological dosimetry".
Investigator-in-charge : Dr. Sunando Bose.
Junior Scientific Officer : Shri Asoke Kumar Das, M.Sc.

Scheme No. III : "Biosynthesis of histones and its implications on the metabolism of nucleic acids".
Investigator-in-charge : Dr. B. B. Biswas, M.Sc., D.Phil.
Junior Scientific Assistant : Shri Nitai Chandra Mandal. M.Sc.

B. Indian Council of Agricultural Research

1. Studies on the occurrence of Rhizobium bacteriophage and its effect on host legumes grown in Indian soils.
Investigator-in-charge : Dr. P. N. Nandi, Ph.D. (Lond.)
Junior Research Assistant : Sm. Itu Sanyal, M.Sc.

C. Council of Scientific and Industrial Research

1. Application of Gas chromatography to (1) the analysis of fatty acid.....mixture".
Investigator-in-charge : Shri J. Dutta, M.Sc.
Junior Research Fellow : Sm. Anita Ghosh, M.Sc.

2. Physical chemical investigation on milk proteins etc.
Investigator-in-charge : Dr. A. Sen, M.Sc., Ph.D. (Leeds)
Senior Research Fellow : Dr. Sheela Chowdhury, M.Sc., D.Phil.

3. Chemical Examination of Indian Medicinal Plants belonging to the families etc."
Investigator-in-charge : Dr. D. P. Chakraborty, M.Sc., D.Phil.
Senior Research Fellow : Dr. Kalachand Das, M.Sc., D.Phil.

4. Study of the Frequency of Power Spectrum of Lightning discharges.
Investigator-in-charge : Prof. S. R. Khastgir, Ph.D., D.Sc., F.N.I.
Junior Research Assistants : Shri Deepak Niyogi, M.Sc.
Shri Janakinandan Maity, M.Sc.

5. Studies in Ionospheric Absorption.
Investigator-in-charge : Prof. S. R. Khastgir, Ph.D., D.Sc., F.N.I.
Senior Research Assistants : Shri Suman Ganguly, M.Sc.
Junior Research Assistants : Shri Sisutosh Samanta, M.Sc.
Shri Debi Prasad Mitra, M.Sc.

6. Metabolism of aminosugars in mammalian and microbial system.
Investigator-in-charge : Dr. Sudhamoy Ghose, D.Phil.
Junior Research Fellow : Sm. Manju Dutta, M.Sc.
Shri Santimoy Banerjee, M.Sc.

7. Phytin, synthesis of nucleic acids etc.
Investigator-in-charge : Dr. B. B. Biswas, D.Phil.
Senior Research Fellow : Dr. Susweta Biswas, D.Phil.

8. Regulation of RNA and protein Synthesis etc.
Investigator-in-charge : Dr. B. B. Biswas, D.Phil.
Junior Research Fellow : Sm. Anjali Mukherjee, M.Sc.

9. Studies on the chemical constitution of some antibiotics isolated from Streptomyces.
Investigator-in-charge : Dr. A. K. Barua, D.Phil.
Senior Research Fellow : Dr. Deb Kumar Mukherjee, D.Phil.

10. Studies on chemical Taxonomy in relation to the family *Rutaceae*.
Investigator-in-charge : Dr. D. P. Chakraborty, D.Phil.
Junior Research Fellows : Shri Bejoy Kirhsna Chowdhuri, M.Sc.
Shri Saktipada Basak. M.Sc.

11. Chemical Investigation of some Plants of the family Verbenaceae.
Investigator-in-charge : Dr. A. K. Barua, D.Phil.
Junior Research Fellow : Shri Pranab Kr. Sanyal, M.Sc.

12. Studies in semi large scale production of antibiotics.

Investigator-in-charge : Dr. P. N. Nandi, Ph.D.

Senior Research Fellow : Shri S. K. Mukherjee, M.Sc. (upto 30-7-65).

13. Effect of ultraviolet light on DNA transformation etc.

Investigator-in-charge : Dr. P. N. Nandi, Ph.D.

Junior Research Fellow : Sm. Meena Guha, M.Sc.

14. Emeritus scientist. Dr. P. K. Bose, D.Sc., F.N.I.

Junior Research Fellow : Shri Budhadeb Paul, M.Sc.

D. PL-480 project on "VLF—Propagation"

Investigator-in-charge : Dr. S. R. Khastgir, Ph.D., D.Sc., F.N.I.

Senior Research Assistants : Shri P. V. Manoranjan Rao, M.Sc.
Dr. Hamansu Bhattacharya, M.Sc., D.Phil.

Junior Research Assistant : Shri Salil Kumar Banerji, M.Sc.

APPENDIX C

LIST OF PUBLICATIONS (1965-66)

	Title of papers	Authors	Where published	Reference : Vol. page and year
1.	The constitution of spergulagenic acid.	P. Chakrabarti, D. K. Mukherjee A. K. Barua	*Tetrahedron*	*22*,1431, 1966.
2.	The constitution of entagenic acid—a new triterpenoid sapogenin from Entada phaseoloides Merrill.	A. K. Barua	*Tetrahedron*	In Press
3.	The constitution of entagenic acid.	A. K. Barua, P. Chakrabarti B. C. Das	*Tetrahedron*	In Press
4.	Studies on the constitution of spergulagenic acid.	P. Chakrabarti, D. K. Mukherjee, R. Chatterjee A. K. Barua	*J. Ind. Chem. Soc.*	*43*, 41, 1966.
5.	The ultraviolet absorption spectra of simple carbazole derivatives.	D. P. Chakraborty, Amitabha Ghosh J. Datta	*Sci. & Cult.*	*31*, 529, 1965.
6.	Structure of mahanimbine.	D. P. Chakraborty, K. C. Das, P. K. Bose	*Sci. & Cult.*	*32*, 83, 1966
7.	Glycozoline, a simple carbazole drivative from *G. pentahylla* (Retz.) DC.	D. P. Chakraborty	*Tetrahedron* Letters	661, 1966
8.	Glycozolidine, a new carbazole drivative from *G. pentaphylla*.	D. P. Chakraborty and B. P. Das	*Sci. & Cult.*	*32*, 181, 1966
9.	Synthesis of glycozoline.	D. P. Chakraborty. K. C. Das B. K. Chowdhury	*Sci. & Cult.*	*32*, 245, 1966

	Title of papers	Authors	Where published	Reference : Vol. page and year
10.	Paper chromatographic separation of some carbozole derivatives.	D. P. Chakraborty, K. C. Das B. P. Das	*Indian J. Chemistry*	*4*, 416, 1966
11.	Influence of pituitory on the melanin formation on psoralene treated toads.	N. M. Biswas, D. P. Chakraborty C. Deb	*Naturwiss*	*52*, 622, 1963
12.	Some biochemical and chemotherapeutic aspects of leucoderma.	D. P. Chakraborty	*Indian J. Dermatol.*	*11*, 69, 1966
13.	Structural studies on the constituents of *Swietenia mahagoni*—some functional groups of mahoganin.	D. P. Chakraborty, K. C. Das C. F. Hammer B. Das	*Proc. International Symp. on Terpenes*	In Press
14.	Haemoglobin polymorphin in Indian Zebu Cattle and the Indian buffalo.	A. Sen, Debdutta Roy, S. Bhattacharya N. C. Deb.	*J. Animal Since*	*25*, 445, 1966
15.	The Physics of the Return-stroke ascent and the time-varition of its current in a lightning discharge.	Manoranjan Rao S. R. Khastgir	Trans. Bose Res. Institute	In Press
16.	Geometrical methods of finding Refractive Index, Absorption coefficient and polarization parameters of radio waves in the Ionosphere.	S. K. Banerjee S. R. Khastgir	Proceedings of the Nat. Inst. Sciences (India)	*31*, No. 5, 455, 1965
17.	Effect of Ionospheric reflections on the nature of radio-atmospherics.	H. Bhattacharya Manoranjan Rao	Radio Science (USA)	*1*, No. 3, 309, 1966 (New Series)
18.	Lateral Corona Currents the return-stroke and the slow field-change after the return-stroke in a lightning discharge.	Manoranjan Rao H. Bhattacharyya	Journal of Geophysical Res.	*71*, No. 11,

	Title of papers	Authors	Where published	Reference : Vol. page and year
19.	Experimental Investigations on Polarization characteristics, Electron number density and Electron collisional frequency of Down-coming Radio-waves at Oblique indidence.	S. R. Khastgir Y. S. N. Murty	Indlan Journal of Physics'	*49*, No. 7, 373, 1966
20.	The Corona currents after the Return-stroke and the Emission of ELF-waves.	Manoranjan Rao.	Radio Science (USA)	In Press
21.	Shock-Induced Freezing of Supercooled water.	T. C. Bhadra	Jour. of Applied Meteorology	*4*, 156, 1965
22.	Induction of Freezing of Supercooled water by Physical Methods.	T. C. Bhadra	Ibid	In Press
23.	On the Acoustics of shock generated by the Rupture of Diaphragms in a shock tnne.	T. C. Bhadra	Ibid	in Press
24.	Effect of high intensity Ultarsonic waves on Ice.	T. C. Bhadra	Ibid	in Press
25.	Sphere Transmission method for the Measurement of Atomic Photo-electric Cross sections.	A. M. Ghose	Nucl. Instr. and Meth.	*35*, 45 (1965)
26.	Photoelectric cross sections of Gamma Rays for Heavy Elements.	A. M. Ghose	Nucl. Phys.	*75*, 539, 1965
27.	Photoelectric cross section of Mn^{54} photons in Pb.	A. Dasgupta, A. Nath A. M. Ghose	Proc. Nucl. Phys Symp. Bombay.	265, 1966
28.	An empirical formula for the coherent scattering cross sections of Gamma Rays.	A. Nath A. M. Ghose	Ibid	260, 1966

	Title of papers	Authors	Where published	Reference : Vol. page and year
29.	Estimation of the incoherent component in the measurement of coherent scattering of gamma rays at small angles.	A Nath	Trans. Bose Res. Inst.	*26*, 33 1964
30.	(n, p) cross sections of some elements for 14.8 Mev neutrons.	B. Mitra A. M. Ghose	Nucl. Phys.	83, 157, 1966
31.	Absolute (n, $2n$) cross section for 14.8 Mev neutrons.	B. Mitra, Arun Chatterjee A. M. Ghose	Proc. Nue. Phys. Symp. Bombay	66, 1966
32.	Response of Plastic Senitillators towards fast neutrons.	Arun Chatterjee A. M. Ghose	Ibid	339, 1966
33.	Measurement of Non elastic cross sections of Nuclei for fast neutron.	Arun Chatterjee A. M. Ghose	Ibid	69, 1966
34.	A New Method for the measurement of self-diffusion coefficient in liquids.	A. Nath	Ibid Part II,	157, 1966
35.	Alkaline and Acid Phosphatase Activity in Livers of Tadpoles at Different stages of Development.	Rama Ghosh, A, K. Medda, C. Deb, G. C. Bhattacharya	*Sci. & Cult.*	*31*, P 153-155, 1965
36.	Ptotein, RNA and DNA contents in Liver of Tadpoles at Different stages of Larval Life.	Rama Ghosh, A. K. Medda C. Deb, G. C. Bhattacharya	*Sci. & Cult.*	*31*. 255-257. 1965.
37.	Level of Glycogen in Liver of Tadpoles at various stages of Development.	Rama Ghosh, A. K. Medda, C. Deb, G. C. Bhattacharya	*Sci. & Cult.*	*31*, 538-539, 1965
38.	Histones as gene repressors.	B. B. Biswas	*Sci. & Cult.*	*31*, 396, 1965
39.	Polyguanylic acid and its effect on amino-acid incorporation.	A. K. Chakravorty B. B. Biswas	*Indian J. Biochem*	*2*, 275, 1965

	Title of papers	Authors	Where published	Reference : Vol. page and year
43.	Enzymatic synthesis of guanosine triphosphate from phytin and guanosine diphosphate.	Susweta Biswas B. B. Biswas	*Biochim. Biophys, 108*, 710, 1965 *Acta*	
41.	A novel enzyme system : Phytin phosphotransferase.	Susweta Biswas, B. B. Biswas	*Sci. & Cult.*	*31*, 634, 1965
42.	The effect of UV on protein synthesis by chloroplast ribosomes.	Susweta Biswas B. B. Biswas	*Expt. Cell Res.*	*41*, 682, 1966
43.	Nonterminal incorporation of adenosine monophosphate from adenosine triphosphate by chloroplast extract.	Susweta Biswas B. B. Biswas	*Arch. Biochem. Biophys*	*115* 1966
44.	Characterization of Ribonucleoprotein particles and protein synthesis in chloroplasts.	Susweta Biswas B. B. Biswas	*Indian J. Biochem*	*3*, 1966
45.	Informational macromolecules in regulation of growth.	B. B. Biswas	*Indian J. Plant Physiol.*	In Press
46.	Interaction of phosvitin with basic proteins and its implication on the activity of RNA-polymerase.	B. B. Biswas, A. Roychoudhury M. K. Pal	*Information Exchange Group No.* 7	310, 1966
47.	Mutagenic response of a thiamineless strain of *Neurospora* crassa to the action of 5-bromouracil.	K. L. Chaudhuri	*Sci. & Cult.*	*32*, 44-45, 1966
48.	Ultraviolet induced mutation in *Penicillium vermiculatum*	Jharna Mitra K. L. Chaudhuri	*Trans. Bose Res. Inst.*	*28*, 1965
49.	Effect of 2-2-dimethyl Ethylene immine on conidiospores of *Neurospora crassa* S4894a	K. L. Chaudhuri	*Sci. & Cult.*	*32*, 419-421

	Title of papers	Authors	Where published	Reference : Vol. page and year
			Ibid	*32*, 1966
50.	Studies on the pigment production of in UV induced mutants of *Penicillium purpurogenum.*	K. L. Chaudhuri S. K. Chattopadhaya		
51.	Ultraviolet induced biochemical mutation in *Aspergillus chevalieri.*	Geeta Nanda P. Nandi	*Sci. & Cult.*	*32*, 257, 1966

APPENDIX D

(Presented at the 49th Aniversary meeting)

DIRECTOR'S REPORT

We are celebrating this evening the fortyeighth anniversary of the foundation of the Bose Institute in 1917. At that time the first World War was being fought in Europe and in the adjoining regions of Asia and Africa. Jagadis Chandra in his inauguration address warned us of the danger in following too closely the methods used in the West in building up the material riches of the country and the resulting feverish rush which has invaded even the field of science for exploiting the application of knowledges not so much for enrichment of human worth as for destruction; civilization in the absence of some power of restraint is trembling in an unstable poise on the brink of ruin.

In the years following this inauguration address of Jagadis Chandra, an uneasy piece prevailed in the western world; the war affected countries of Europe helped by funds from the USA were able successfully to obliterate the destruction caused by War. Through the effort of scientific technology new heights of material prosperity began to spread over these countries. Within twenty years, peace was again threatened by the rise of totalitarian forms of government in several countries of Europe.

A year after the passing away of Jagadis Chandra (1937) the second world war commenced. Rabindranath appalled at this resurgence of scientific devilry and at the continuous drainage of resources of life, appealed in his Jagadis Chandra Memorial address of 1938 to the (Bose) Institute "to bring our call to science herself to rescue the world from the clutches of the marrauders who betray her noble mission into an unmitigated savagery". Rabindranath did not live to witness what extremes of destruction of human life and human constructions became possible with the release of atomic bomb, which is another destructive application of pure science.

During the last twenty years of uneasy peace following the signing of the treaty of 1945, science based technology again was more than effective in restoring the war devasted areas of industrially advanced nations of the world not only to its pristine prosperity, but also an unanticipated height of affluence. In the underdeveloped regions of the world in Asia, Africa, South America where the new technology spread, it has somewhat raised the standard of well being and continued application of these methods hold out promise of still further improvement in the living conditions of the inhabitants. These instances of the power of technology not only to rapidly obliterate the destructive effects of war but also to quicken the pace of material prosperity of the country forces us to recognize the double edgedness of the weapons which science has placed in our hands both as a creator of material prosperity as well as a potential destroyer of the latter, and even of human existence in this world. Along with this destructive and recuperative power of technology on the material

content of human culture, there is another aspect of the effect of war, the loss of inhumerable human lives, the sufferings, the cruelty of man to man the destruction of human values which technology is powerless to avert and which future generations try their best to forget. It is this aspect of war that prophets like Rabindranath have denounced.

Another double edged concept with which we have to come to term is nationalism. The world nationalism was coined in the 19th century to represent the legitimate struggle of suppressed people to recover their identity; as such as a great deal of good will was attached to it. In course of time the word has come into disrepute, when many powerful nations used it to cloak their naked aggression to destroy their weak neighbours and extend their empires. It is to this aspect of Nationalism that Rabindranath denounced in the lecture on Nationalism he gave in Japan.

There is however another facet to this picture. At certain stage in the evolution of human society preparation for conflict and conflict may have had some influence in forging the unity of a nation.

In 1947 India achieved independence through peaceful means, thereby a loosely formed independent state arose which under the inspiration of Jawaharlal Nehru accepted the ideal of a welfare state, secular in character, aiming to raise the economic and social status of the people through education, science based development of industry and agriculture; her foreign policy was based on non-alignment. On the expectation that the neighbouring countries would take her lead in accetpting the ideal of *Panchsil*, India did not think it necessary to devote a sizeable portion of her resources to strengthening national defence. But things have not turned out as expected.

During these 17 years of independence many of us while watching the frittering away of our national efforts in fruitless controversis over languages over the particulate regional interests against all efforts at national integration have felt keenly the absence of an over-riding consceiousness of a sense of common destiny which nations can achieve only when they have to fight and suffer for independence. Things did not happen with us in this way.

The challanges from the north eastern and western frontiers during the last three years have created in this country an upsurge of combative spirit for defending our national unity and the ideals on which it is based. For this purpose the nation is prepared to face all sacrifices which a prolongation of conflict may bring with it. The depth of this upsurge of feeling has surprised many of us. Even amongst some of the western nations not too well disposed towards us, some individual observers who have visited the scene of recent fighting have observed something of this. One of them has testified that this upsurge of spirit amongst the people of India is comparable to what occurred in Britain in 1940 when that country single handed decided to face the German invasion of their island. We should feel proud at this comparison and strive in our efforts, during the dreary days of the coming struggle, to live up to this spirit.

The loss of prestige which his country had suffered during the 1962 invasion revealing the state of our unpreparedness to face external aggression has been more than amply retrieved by the splendidelan with which our Army and Air forces have countered the Septem-

ber aggression from Pakistan; the superior strategy the tactical resources of the high command, the skill bravery and spirit of adventure shown by our jawans, against the much superior land and air frig armaments supplied by foreign powers to our oponents, has inspired our people with a renewed faith in our capacity to defend the country's integrity and the ideal of a secular state on which our national unity is based.

There are many urgent problems facing the country, on the agricultural and industrial fronts which require to be solved in the same way the defence problems of the country has been met. The same kind of sense of urgency is needed for decision making and for mobilising the people's will to overcome the many complex problems facing us. We have allowed the problems to accumulate without showing the will to solve them. I have in my address on the last anniversary meeting of the Bose Institute summarised the problems as follows :

"... how soon the present policy of depending so largely on foreign aid, loans, food supply, supply of capital goods and technical know-how from foreign countries, which though easing the present economic situation, will enable the country ultimately to reach its goal of self sufficiency in technology, industry and agriculture. We have so long been comforting ourselves with the belief that with such foreign aid the country will be spared the terrible struggle which some of the totalitarian countries had to undergo and some are still undergoing to enable them to build up out of their own resources viable capital industries and defence organisations. The question which many of us find disturbing is whether we are not getting too habituated to this dependence on foreign aid".

Events which have taken place in course of this year have forced the Government to the decision of making the country self sufficient in agriculture, industry and defence products.

Of the many factors which has led to this decision, the need for achieving self sufficiency in food supply appears to be very urgent, specially to an organisation like the Bose Institute which is mainly devoted to biological research and to the utilisation of research results to problems of agriculture. India is largely dependent on the USA for meeting her foodgrain deficiency under the PL-480 agreement. Mr. Chester Bowles, the American Ambassador in his recent Ahmedabad address informs us that during the last 14 years the USA has delivered to India 35 million tons of food grains which is about 7 per cent of the total food grain production in India. India's moderate attempts to increase her annual food grain production is nullified by an annual growth of population of about 8 millions. No country should be dependent on another country however friendly to supply her with increasing quantum of food grains for an unspecified period.

The requirements for achieving self sufficiency in food products without restricting the cultivation of cash crops on which we are dependent for earning part of our foreign exchange, are many.

Some of these are (i) supply of improved quality seed so that for the same area of cultivation a larger yield of food crop is possible, (ii) an increased supply of fertilizers which is in acute short supply at present, (iii) improved irrigation facilities, (iv) larger supply of pesticides, (v) adequate distribution of land amongst the small cultivators and the agro-industries.

We are honoured that Prof. P. Maheshwari has accepted the invitation of the Council of the Bose Institute to deliver the 27th Jagadis Chandra Bose Memorial Address, for which he has selected the subject 'Botany and the Food Problem of India', a very important subject to discuss in view of the present acute crisis in food supply through which we are passing at present in this country. We shall hear his address with great deal of attention and shall profit from it. Prof. Maheshwari who has recently been elected a F.R.S. of London, is probably now the most widely known Botanist of this country at home and abroad. He is at present Professor and Head of the Department of Botany of Delhi University, the author of several important botanical monographs. He has visited many western countries as visiting professor and is the receipient of many foreign academic distinctions. We are fortunate in having him to speak here this evening on this important subject.