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REPORT

OF

THE BOSE INSTITUTE

FOR

1970–71

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

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The Bose Institute was founded by Acharya Jagadis Chandra Bose in 1917 for 'the fuller investigation of the many ever opening problems of the nascent science which includes both life and non-life'. The Bose Institute provides facilities for fundamental and applied research in wide range of scientific subjects. Besides the main laboratories and Workshop 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 Chemistry and Biochemistry; Botany, including Plant Physiology, Plant Biochemistry, Tissue Culture, 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.

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Governing Body

Three meetings of the Governing Body were held during the year. Some of the principal decisions taken were :—

i) Approved the expenditure to an extent of Rs. 14,000/- for the repair of the fire damaged Phytochemistry Laboratory.

ii) Passed the Budget Estimate for 1970-71.

iii) Accepted the resignation of Shri Amiya Nath Mitra due to painful illness.

iv) Eelected Prof. A. K. Sharma and Prof. A. K. Saha as Governing Body representatives on the Council of the Bose Institute.

v) Approved the Plan and Estimate for a multistoried J. C. Bose Centenary Laboratory Building prepared by M/s. Ballardie Thompson and Mathews at an estimated cost of Rs. 19·57 lakhs.

vi) Sanctioned a grant of Rs. 2000·00 to the Indian Science News Association out of the income of the J. C. Bose Endowment Fund for popularisation of Science.

vii) Constituted Nilratan Sircar Memorial Prize Fund for award of a Prize to a Scientist of the Bose Institute working on biological problems.

viii) Sanctioned an ex-gratia payment of Rs. 500/- to Shri Dhinrendra Nath Das an employee of Birati Experimental Station, who had to retire owing to protracted illness.

Council :

Two meetings of the Council were held during the period under review.

The Council approved—(*i*) the revision of the scales of pay and allowances of the non-research staff of the Bose Institute as per scales of pay and allowances prevalent in the Calcutta University with effect from 1st April 1970 on the line envisaged by the Government of India in the Ministry of Education and Social Welfare (vide letter No. F.15(70)/67-SR.II dated 2nd February 1971. And the revised scales of pay have been given effect to from 1st April, 1971.

ii) Accepted the resignation of Prof. B. M. Sen from the Chairmanship of the Finance Committee of the Bose Institute and placed on record the singular services rendered by him as Ex-officio Chairman of the Finance Committee.

iii) Considered a draft Ordinance relating to the terms and conditions of services of employees of the Bose Institute and forwarded the draft rules to the Govt. of India, Heads

of the Departments and the Bose Institute Employees Association for observation; and constituted a Sub-committee with Prof. S. Mukherjee, Prof. A. K. Saha and Prof. A. K. Sharma to review the Ordinance.

Acharya Jagadis Chandra Bose Memorial Lecture :

The thirty second lecture of the series, entitled "Science and life as cultivated in Ancient India" was delivered by Prof. Priyadaranjan Ray, M.A., D.Sc. (Hon.), F.N.I. on November 30, 1970. The lecture is being published in the Transactions of the Bose Institute.

Acharya Jagadis Chandra Bose Endowment Lecture :

The thirty-fourth, thirty-fifth, thirty-sixth and thirty-seventh—Endowment Lectures of the series were delivered during the year.

a) Prof. G. O. W. Kreemp, Dept. of Geochronology, University of Arizona, USA on "Examination of Earth's oldest sediments of stony meteorites and of moon rocks and their relation to an inquiry into the origin of life—a fascinating puzzle" on 13th July 1970.

b) Prof. M. Levine, Dept. of Human Genetics, University of Michigam, U.S.A. on "DNA Replication in phage P.22" on 7th August 1970.

c) Prof. Knut Faegris, Director, Botanical Museum, University of Bergen, Norway, on "Quaternary Pollen Analysis—Past, Present and Future" on 11th January 1971.

d) Sir Frederick Bawden, F.R.S. Director, Rothamsted Experimental Station, Herts, England, on "Plant Viruses and Virus Diseases" on 9th February 1971.

Special Lecture :

a) Prof. H. S. Loring, Dept. of Biochemistry, Stanford University, California, U.S.A. on "Reversible partial inactivation of Tobacco mosaic virus".

b) Dr. Usha Ranjan Ray of Princeton University, U.S.A. on "Alkylation damage repair and Mutagenesis in T_4 phage".

Degree awarded :

The following were awarded the D.Phil. (Sc.) degree of the Calcutta University :

1.	Shri Swapan Kumar Ghosh	Dept. of Chemistry.
2.	Sm. Rama Chatterjee	Dept. of Chemistry.
3.	Shri Sunil Kumar Pal	Dept. of Chemistry.
4.	Md. Azizul Islam	Dept. of Chemistry.
5.	Shri Shyamal Kumar Roychowdhury	Dept. of Chemisrry.
6.	Shri Subhendu Narayan Ganguli	Director's Unit

Acknowledgement

The Council takes the opportunity to express its grateful thanks to the Government of India, the Government of West Bengal, the Department of Atomic Energy, the Indian Council of Agricultural Research, the Council of Scientific and Industrial Research. US. PL-480 authorities for their grants. The Council also records its thanks to the Governing Body, Bose Institute and to the members of the various Selection Committees.

The grant of $ 1000·00 per annum from the World Health Organization for the year 1969 and 1970 for the development of an Immunochemistry Unit is gratefully acknowledged. Apart from some chemicals, an LKB Electrofocussing instrument, spares for the Ananlytical ultracentrifuge and other small equipments have been obtained.

Gift of some chemicals and equipment from Royal Netherland Fermentation Industries worth about Rs. 3000·00 is gratefully acknowledged.

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NON-TECHNICAL REPORT

CHEMISTRY

A comparison of the amino acid composition of goat and bovine trypsins shows notable differences in individual amino acids. The molecular weight of goat trypsin is somewhat lower than that of bovine trypsin. Such differences are a reflection of variations in primary structure.

Turtle egg white proteins from different species show that egg of all species do not show that presence of lysozyme. Lysozyme from eggs of one species has been purified and crystallized. In enzymatic as well as immunological properties it shows distinct difference from those of hen egg white lysozyme. Phosvitin of turtle egg yolk shows higher *P* content.

Haemogglutinating activities of *Abrus precatorius* as well as different seeds of different leguminous plants are being studied.

A new alkaloid has been isolated from the plant *Adhatoda vasica* (known as Vasaka) which is widely used in the treatment of cough and bronchitis.

A number of phenolic compounds have been isolated from the leaves of the plant *Eupatorium odoratum* and *Careya arborea*. A few new acids have also been isolated from the latter.

The chemical structures of a number of bitter principles isolated from *Mollugo hirta*, which is commonly used as green vegetable in India, have been elucidated.

The chemical structure of rubidin—an antibiotic isolated from a Streptomyces species has been partly elucidated.

A number of new non-steroidal antifertility agents have been synthesized.

The extract of the seeds of the plant *Elaeocarpus Ganitrus* (known as Rudraksha) has been found to have marked inotropic effect on intact and isolated mamalian heart.

The synthesis of a plant antibiotic and the first pyranocarbazole girimimbine reported from this laboratory has been accomplished; the structure and synthesis of a new carbazole alkaloid has been reported. A new plant antibiotic from *G. xanthochymus* has been reported.

The inhibition of tryptophan pyrrolase by psoralene puts the use of psoralene in vitiligo on a rational basis.

The fatty acid compositions of linseed and castor oils containing polyunsaturated and hydroxy fatty acids respectively have been elucidated in fine detail by using a suitable combination of the techniques of urea adduct formation and gas-liquid chromatography. A systematic study of the fatty acid and glyceride compositions of seed oils obtained from plants belonging to different species included in the *Cucurbitacea* family is in progress. Some of these seed oils have been found to contain significant high drying properties. In another systematic study the nature and composition of oils obtained from six different

species of sweet water fishes of India known as cat-fishes have been investigated. The effects of infection by different bacteriophages on the complex liquid composition in bacteria *E.* coli are being studied. Significant quantitative changes in phospholipid composition due to phage infection have been observed. A simple apparatus for rapid microhydrogenation of unsaturated lipids has been designed. constructed and standardised with fatty acids. The specificity of egg white lysozymes of hen and turtle have been studied by immunochemical methods of gel-diffusion and immunoelectrophoresis. The results indicated that the two antigens did not contain any common determinants. Standardisation of "Complement fixation test" a very sensitive immunochemical method for the characterisation of antigens and antibodies is in progress.

Phosphotransferase system probably mediates sugar transport in bacteria. The correlation between the phosphotransferase system and the sugar transport has been studied generally in *E. coli* K12 and *V. cholerae*.

BOTANY

Studies on informational macromolecules revealed that in eukaryotic cells the informations encoded in the hereditary material i.e., DNA are decoded by several protein factors and RNA polymerases isolated from the chromosal acidic proteins. One factor (factor B) has been found to act as an initiation factor for the synthesis of RNA *in vitro* while the other (factor C) seems to behave as termination factor, thus determining the specificity of transcription process. Another type of regulation in the synthesis of RNA has been found to be mediated through hormone i.e., Indole acetic acid (IAA) in case of plant cell. It is suggestive that the action of IAA is mediated through an acceptor protein. IAA and acceptor protein from a complex with DNA at some specific site. The initiation factor and RNA polymerase interact with these sites and form initiation complexes. RNA synthesis seems not to occur at these sites in absence of IAA acceptor protein. Any irregularities at any point in this regulation of transcription may lead to uncontrolled growth.

Metabolism of phytic acid (inositol hexaphosphate acid) plays a great role during seed formation and germination. A new enzyme system, phosphoinositol kinase has been discovered in the seed which can phosphorylate inositol-1-phosphate to inositol hexaphosphate in a stepwise fashion using ATP as phosphate donor. The failure in detecting phosphoinositol kinase activity in the ungerminated seeds seems not to be due to its actual absence, on the other hand, it is a cumulative result of the enzyme being present together with an inhibitor, thus conferring a definite role to the inhibitor which is also a protein in nature, in regulating the biosynthesis of inositol phosphates.

Investigations on the effects of X-rays on Indian Spinach indicated that X-ray dosages in general produced decreased survival, lateness in flowering, long flowering period, increase in frequency of plants, with morphological abnormalities, in M_1 generation. However, it was possible to isolate some early flowering and tall plants from the treated populations. Repeat dosages on M_2 seeds in some cases yielded interesting results. The radiosensitivity

in five species, as measured by survival, height and chromosomal abnormalities has been shown to be correlated with ICV. Chromosomal abnormalities has been found to be responsible for the killing effect, seedling height and plant height at maturity.

Cytological studies on five varieties of *Abelmoscus esculentus* (L) Moensch have been made. In each variety higher and/or lower chromosome numbers than the normal complement were observed regularly. In three of these varieties there were six pairs of chromosomes which had secondary constructions while the remaining two had four pairs of such chromosomes.

Effects of increasing doses of X-rays along with lethal doses have been tried in *Lens esculenta* Moensch with a view to study the nature of cytohistodifferentiation of component cells of leaf and stem anomalies. Shoot apical cells have been found to be less affected in comparison with root cells. Most of the plants failed to survive due to heavy damages in root cells. As a result no manifestation of leaf and stem abnormalities could occur. All these indicated that changes in cellular level in root system disturbs the growth rate of plant. X-ray irradiation on *Lathyrus sativus* yielded a decrease in the height of plants as well as number of seeds in the pod.

The effects of alkylating agents (ethylmethane sulphonate, ethylene-imine) on 3 varieties of paddy namely Taichung Native I, Latisail and Kumargore have been studied with respect to cytology and cytogenetics. Changes in germination rate, growth rate, tillering, flowering time, morphological changes, sterility etc. have been observed.

There are isolated mutants induced in rice variety Marichbati, after X-ray treatment and in Satika variety after ^{32}P treatment. The floral morphology of these mutants are very different from that of *O. sativa* L. Many mutants of rice obtained from different treatments have been analyzed for seed protein. No significant increase in protein value has however, been noted.

Tissue culture from a number of plant species has been raised to study the synthesis of some pigment probably of anthocyanin type, synthesis of carbazoles, breakdown products of Istin as well as the process of differentiation. The root growth and differentiation in three species belonging to Umbelliferae have yielded promising results.

Aeropalynological investigations in Calcutta and Falta show a considerable fall of pollen grains incidence in air during rainy seasons. *Mimusops*, *Antidesma*, *Colophyllum*, *Cocus* and other grains were abundantly captured from air in different seasons. Pollen morphological studies of Phytolaccaceae (28 species from 12 genera), Rubiaceae (20 species from 15 genera), Geraniaceae and allied taxa (7 species from 7 genera), Verbenaceae and allied taxa (246 species from 109 genera in 9 families), Boraginaceae and allied taxa (200 species from 72 genera), etc., in relation to taxonomy have been done. Pollen morphological studies have supplied interesting data concerning phylogeny and affinity of different plant groups. Pollen grains of some gymnosperms (with special reference in Coniferales), Indian and extra-Indian, have been studied, some of them are found to be saccate and show interspecific variation. *Nyctanthes* pollen were found to be dominant in Bolpur honey samples and some typical mangrove pollen have been recorded from Sunderban honey samples.

Some peat samples, possibly of late Quaternary deposits, have been collected from Darjeeling and neighbouring places. The results of subsurface analysis show good potentiality of this project in relation to vegetational history and phytogeography. Condiospores of *Aspergillus* have revealed differential characters with regard to their spore morphology. These characters together with the differences in size have led to the formation of various groups.

ANIMAL PHYSIOLOGY

Amphibian Physiology : The physiological consequence of thyroxine-protein binding was studied in tadpoles. The action of thyroxine in the cells depends on the availability of free or unbound thyroxine. In addition to usual thyroxine binding proteins in blood, e.g. albumin, thyroxine binding globulin, etc., the thyroglobulin immunized rabbit's serum contains a new thyroxine binding gammaglobulin, the anti-thyroglobulin. When thyroxine is complexed with albumin or antithyroglobulin and injected in tadpoles, no action of the hormone is found, while injection of only free thyroxine produced dramatic changes characteristics of the morphological as well as biochemical differentiation in these larvae. The results have significant implications in human subjects showing circulating autoantibodies which have been demonstrated in various forms of thyroid disease and even in normal subjects.

Thyroxine in higher doses causes catabolic changes in tadpoles as well as in higher animals. But distinct anabolic action of this hormone with physiological dose is noted in tadpole, fish and also in mammals. An interesting finding was made on the effect of thyroxine on melanin metabolism in tadpole and toad liver. This is the first evidence that thyroxine stimulates the synthesis of melanin in tadpole and toad liver. This finding may help in the treatment of leucoderma in the long run.

Piscine Physiology : Thyroid hormone action in poikilotherms has not yet been established. Studies are being made to evaluate the physiology of thyroid hormone in fish as well as in adult amphibia. Attempts are being made also to increase the protein content and stimulate spawning in latea fish (*Ophicephalus punctatus*).

The treatment of latea fish with antithyroid drug, thiourea, produces goiter. The thyroid hormone causes both anabolic and catabolic action in latea fish, depending on the dose and environmental conditions. In normal animals rise of environmental temperature increases both urea-N and NH_3-N excretion. The nitrogen excretion is reduced at lower temperature. Thyroxine treatment causes anabolic action (reduced nitrogen excretion) at lower temperature and catabolism (increased nitrogen excretion) at higher temperature. The results suggest the temperature-specificity of thyroxine action in fish.

Gerontology : Studies are being made on the physiology of ageing of rats. The nucleo-cytoplasmic interactions with special reference to nucleo-cytoplasmic ratio and the synthesis of protein, RNA and DNA in important organ like liver are being studied in rats of different age groups. Attempts are being made also to define age from the morphological and biochemical changes of the cells. There are experimental indications that cell area and nuclear area and also protein and RNA contents in liver of rats vary as the animals age.

A. PHYSICS

A.1. Nuclear Physics Unit

I. GAMMA RAYS

I.1. *Determination of Electronic Charge Density Distributions of Lead.*

The method of determining the radial electronic charge density distributions of heavy elements reported in the previous year has been applied in case of lead with certain modifications. In this method the charge density $\rho(r)$ has been evaluated using the following relation

$$\rho(r) = \frac{1}{2\pi^2 r h^2} \int_0^\infty F(Z, q) \sin \frac{qr}{h} .q.dq \qquad \ldots (1)$$

where, $q = \frac{2E}{c} \sin \frac{\theta}{2}$; and $F(Z, q)$ is the atomic form factor.

In this present method the atomic form factor $F(Z, q)$ which takes into account the charge distribution of an atomic was determined from the coherent scattering cross-section data of gamma rays obtained experimentally. Initial attempt along this line, made by Woollan for neon and argon using X-ray scattering data, revealed that it was not possible to resolve the K and L group of electrons for high Z atoms (even for Argon $Z = 18$) due to the longer wavelength of radiations used. Therefore the scattering data of gamma rays having much smaller wavelength may be used as a successful tool to determine the electronic charge distribution of heavy atoms like lead.

The differential cross-section for the co-operative or Rayleigh scattering for a deflection of the photon through an angle is related with the atomic form factor $F(Z, q)$ by,

$$\sigma_R(\theta) = \tfrac{1}{2} r_0^2 (1+\cos^2\theta) |F(Z, q)|^2. \qquad \ldots (2)$$

where, r_0 is the classical electron radius and q is the change of the momentum of the photon and is given by $\frac{2E}{c} \sin \frac{\theta}{2}$, where E is the energy of the incident photon. The equation (2) which is valid for non-relativistic cases extended upto relativistic range semi-empirically in our laboratory by Nath and Ghose from the observations of coherent scattering cross-section data for various values of E by introducing a new term $\phi(E, q)$ depending on E and q in equation (2). The factor $\phi(E, q)$ bears the following relationship

$$\phi(E, q) = (13{\cdot}5E - 4{\cdot}4)^{-0{\cdot}35q} \qquad \text{for } E \geqslant 0{\cdot}4 \text{ Mev}$$
$$= 1 \qquad \text{for } 0 \leqslant E \leqslant 0{\cdot}4 \text{ Mev}$$

It has been observed that the relationship agrees within experimental error with experimental data upto $q = 2{\cdot}5$ mc units. Thus the form factor $F(Z, q)$ is determined from the

following relationship

$$F(Z, q) = \frac{1}{r_0} \left[\frac{2\sigma_R(\theta)}{(1+\cos^2\theta)\phi(E, q)} \right]^{\frac{1}{2}}$$

The radial electronic charge density is determined by carrying numerical integration of equation (1) using Simpson's 1/3rd rule/Gauss quadrature formula for $q = 0$ to $1{\cdot}5$ mc units.

The result obtained in this way is compared with the values obtained from Hartree self-consistent field model. $\rho(r)$ has been calculated from the following expression

$$\rho(r) = \sum_{j=1}^{z} \psi_j^*(r)\psi_j(r).$$

using the self-consistent wave functions tabulated by Herman and Skillman. The result is in good agreement with the results obtained from Hartree model. Anamolies obtained previously using integration upto $q = 2{\cdot}5$ mc unit for lead and tin indicates certain cut off to the limit of integration q is essential due to larger experimental uncertainties at higher momentum transfer region. The calculation for tin indices that much more accurate measurement is needed for low and intermediate Z elements.

I.2. *Angular Dependence for Pair Annihilated Radiation.*

Calculations based on the quantum theory of radiation indicates that annhihilated pairs should in general have an angular dependence with the direction of the incident photon. The experiments performed in the past to measure pair production cross-section have been carried with the assumption of isotropic angular distribution. The intention of the present work is to measure the pair production cross-section at different angles to check the validity of the assumption of the isotropic angular distribution of the pair annihilated radiation with respect to incident gamma rays.

In the conventional pair production cross section measuring system the difficulty arises in separating the Compton continuum from the photopeak due to annihilated radiation. In the present experiment the Compton continuum was eliminated very efficiently employing a special method of photopeak sharpening in scintillation spectrometers. In this method the detector employed is a single crystal two photomultiplier combination and the spectrum is scanned from one photo-mu.ltiplier coincidence gated by other. Prloper gating eliminates the Compton continuum from the photopeak to a large extent. The scatterers used were cylindrical in shape to adopt the cylindrically symmetrical disposition of the experimental set up.

A 100 mCi Co^{60} source is placed within a massive lead block, the radiation from the former is extracted using a tapering rectangular slit. The scatterer is placed directly in line with the source at the centre of an iron table. The iron table is graduated to facilitate the placing of the detector at any desired angle. The detector is surrounded with lead bricks to reduce the unwanted background. The detector was first calibrated with a standard Na^{22}

source and the proper gate was set so that the photopeak of 0·511 MeV was completely separated. Figure 1 shows the single spectrum of Na^{22} without coincidence and the properly gated coincidence spectrum eliminating the compton continuum to a large extent. The scatterer was then placed in proper position removing the Na^{22} source and the coincidence spectrum for annihilated pairs was obtained directly from a 128-channel analyser.

The photopeaks obtained experimentally due to annihilated pairs from lead scatterer at five different angles clearly indicates that the intensity of annihilated radiation has a decreasing trend towards lower angles. The experiment was repeated with scatterers of different thickness and placing the detector diametrically opposite to each of the angle obtaining the same decreasing trend. From this we conclude that pair annihilated radiation has an angular dependence with respect to the incident photon and the re-evaluation of pair production cross-section is essential at this energy region.

Absolute pair production cross-section using the method is in progress.

II. NEUTRON PHYSICS

II.1. *Construction of apparatus for the measurement of reaction cross-sections by the continuous flow method.*

This is a new method of measuring interactions of neutrons with sample nuclei. This method can record the activity of the sample continuously with irradiation. And if there occur any competitive reactions in the sample than the sample activity is recorded in separate forms simultaneously. We designed and constructed an experimental arrangement for this experiment to avail of all its advantages.

The experimental arrangement consists of a polyethelene disk of diameter 24 inches and thickness $\frac{1}{2}$inches. A groove was machined on the disk leaving a space of $\frac{1}{4}$ inch from the rim of the disk. The groove is extended along the peripheri of the disk and it was a width of 1 inch and depth $\frac{1}{4}$ inch. The groove is highly polished so that the powder sample after irradiation may be wiped out easily. The disk is mounted on a wooden stand and pulley. This pulley is again connected by means of rubber cord to gear and motor arrangement. With the help of this motor and gear box the rotating speed of the disk can be controlled easily. The disk containing the sample powder is shielded from the neutron beam by means of heavy circular lead block of diameter 22 inches and thickness $1\frac{1}{2}$ inches. A hole of diameter 1 inch was drilled near one end of a diameter of the lead block. This hole limits the region of irradiation on the sample. The lead block is mounted on a heavy iron structure. The sample holder disk is placed at a distance of 3 inches from the focussed point on the tritium target and the lead block at a distance of 1 inch from the source. The detector is a G.M. counter it is placed at a place diametrically opposite to the region of irradiation. The detector is heavily shielded to bring down the background counts during the time of irradiation. The testing experiments of this arrangement is bring carried out in our neutron generator laboratory.

II.2. *Absolute* $(n, 2n)$ *cross-sections*

We had also a programme for extending the measurements of absolute $(n, 2n)$ cross-sections in the 14 MeV region. We had to drop this programme because the neutron generator could not be switched on. The neutron generator requires for its proper operation the rooms to be maintained at low temperature, nearly at 15°C and humidity controlled within 50 percent. The air conditioning machines which are used to maintain the above mentioned conditions of the room are overhauled during this one year and some few more months period. So we cannot carry out any measurement in the $(n, 2n)$ measurement programme.

III. APPLIED RADIOACTIVITY UNIT

This unit is associated with the application of Nuclear radiations for the study of physical properties of different substances. It is chiefly involved with the development of tracer techniques for the study of transport phenomena in liquids. As it has been described in earlier reports, an accurate method has been developed for the investigation of self-diffusion in liquids. The working formula has been formulated on the basis of Fick's second law which has been solved for a particular case of restricted diffusion where the solute and solvent columns are of equal length. The basic assumption was that the diffusion flow was a unidirectional one. But our experimental condition was such that this assumption was not true, since the diameter and the length of the diffusion column were comparable. Hence the best way was to solve the Fick's equation for a radial flow but then the mathematical steps were very complicated because of the complicated nature of the boundary conditions. Hence this idea was given up and the effect of finite radius of the diffusion cell was tested in a direct manner. Several diffusion cells were constructed having diameters 0·6 cm, 0·8 cm, 1·0 cm and of lengths in the range 2 to 3 cms. Then the self-diffusion of liquid mercury was measured at constant temperatures using cells of different diameters and a different radii. The results with different radii did not vary appreciably and they were within experimental error. We did not try to make the radius smaller because of the curvature property of the liquid surface.

Our next programme was to develop the technique more and use it for the study of transport properties of the other metals. But several difficulties prevented us from undertaking this project. The most important among them is the irregularity in the functioning of the air-conditioning plant of our laboratory. It did not function for most part of the season, and even when it did work, it was so regularly irregular that it was useless. It could bring the temperature of the laboratory only 2°C below the outside temperature so that it was ineffective. It hampered the work because of the following reasons : (1) one particular set of data recording in the present investigation requires good stability of the electronic equipments for more than 40 hours, but as the room temperature was varying over a considerable range the electronic stability was far from satisfactory. (2) For temperature control, a thermostat was used. But as the room temperature was high, after a few hours work, the pressure pump of the thermostat would become so hot that for its safety it had

to be switched off. These were the princpal reasons for the rather unsatisfactory progress of work in this unit.

Another programme in this unit is the study of defects of solids produced by nuclear radiations. Some progress has been made for keeping the radiation source in a shielded house for irradiation facilities.

A.2. Accelerator Physics

1. *Capture Cross Sections*

Last year's report was made on the acquisition of data on the capture cross-section for 14 MeV neutrons for a number of samples. This year the data have been published in the Indian Journal of Physics (p. 204–211), 1970 titled—"Capture Cross Sections for 14 MeV neutrons for ^{71}Ga, ^{75}As, ^{127}I, ^{138}Ba and ^{141}Pr".

2. *Proton Angular Distribution*

A vacuum camera of Allen type (Allen 1961) has been developed. With the help of this camera, we are irradiating K_2 photographic emulsion plates with protons ejected from (n, p) reactions of elements ^{28}Sr, ^{32}S, ^{55}Mn and ^{58}Ni when exposed to 14 MeV neutron beam. Irradiations were made with neutrons to obtain proton tracks in the photographic plates and the tracks are scanned to study the angular distribution. Data obtained are being processed.

3. *Isomeric Cross Sections*

We are ow interested in studying the isomeric cross sections for the nuclei ^{111}Cd, ^{113}Cd, ^{59}Co, ^{121}Sb and ^{123}Sb. Accurate calculations of the detector efficiency is in progress. Experiments have been done with radioactive (usually β^-) sam'ples having various energies and thicknesses. To account for self-absorption and self-scattering effects, variation of sample thicknesses is allowed from a few micrograms (very thin) to a few milligrams/cm^2. We are confronted with the problem of making very thin samples uniformly. We have applied the electrospraying technique to prepare very thin samples. The necessary modifications of the apparatus are being done to meet requirements.

A.3. Acoustics, Solid State Physics and Microwaves (Sri J. C. Bose Unit)

The research projects in the above mentioned sections were outlined in the Annual Reports, Bose Institute 1967-68 and 1968-69. It was scheduled at that time that significant progress would be made during the Fourth Five Year Plan.

Accordingly all out efforts were made for complete utilisation of then available resources. Inspite of the fact that promising progress was made, further work could not be

1. D. L. Allan, *Nucl. Phys.*, 24, 274, 1961.

made for want of required funds and personels. All the projects came to a stand still position. It is still in the same situation. Developments made so far have been reported in the annual reports 1967-68 and 1968-69.

In the meantime with the limited available resources, two problems were studied in the ultrasonic division.

1) On the absorption of ultrasonic waves in water and alcohol series by the streaming method.
2) On the absorption of ultrasonic waves in water/alcohol mixtures by the streaming method.

Data acquisitioned on these two projects are being processed. From the absorption data, bulk viscosities for these liquids have been calculated.

Findings of these projects would be included in the two theses to be submitted for the Ph.D. degree in science of Calcutta University. Theses are being written now.

The attention of the authorities are hereby drawn to provide the necessary facilities, funds and personels, so that the work on the half finished planned projects may be resumed without further delay.

B. CHEMISTRY

B.1. Protein Chemistry

B.1.1. *Studies on goat trypsin*

Effect of enzyme concentration on the reaction velocity. The effect of enzyme concentration on the rate of proteolysis was studied using casein as substrate at *p*H 8 and at 30°. The proteolytic activity (increase in absorbance at 280 nm/min/mg enzyme) of goat trypsin was 5·5 as compared to the value of 4·7 for bovine and porcine trypsins.

Effect of substrate concentration on the reaction velocity. A study of the effect of different concentrations of substrate on the rate of enzyme catalysis was made in 0·1*M* borate buffer, *p*H 8 at 30° using casein as substrate. The substrate saturation curve of trypsin was of hyperbolic form. The Michaelis-Menten constants calculated from Lineweaver and Burk plots were $2{\cdot}9\times10^{-5}M$ for goat trypsin and $3{\cdot}5\times10^{-5}M$ for bovine trypsin.

Dissociation constants of trypsin-inhibitor complexes. Ovomucoid, soybean, lima bean and pancreatic inhibitors reacted with goat trypsin to form inactive complexes which apparently contained molar ratios of inhibitor/enzyme close to unity. Inhibitors differed to the extent of their association with trypsin. The departure from stoichiometric inhibition in the neighbourhood of equivalence point indicated the dissociation of all four inhibitor-typsin complexes. The dissociation constants were approximately $10^{-10}M$ for pancreatic, soybean and lima bean inhibitors and $10^{-9}M$ for ovomucoid.

Molecular weight determination by gel filtration. The molecular weight of goat trypsin in acetate—NaCl buffer, *p*H 5·1 was determined by gel filtration on Sephadex G-100 at room temperature. The column was calibrated with dilute solutions of α-lactalbumin, β-lactoglobulin and bovine serum albumin. The void volume of the column was measured with blue dextran. The ratio of effluent volume was determined and from the plot of this ratio against the logarithm of the calibration molecular weights, the molecular weight of goat trypsin was estimated as 20,417 daltons. The result suggests that like bovine trypsin, goat trypsin exists as a monomer at low protein concentration at *p*H 5·1.

Amino acid composition. Amino acid analyses were performed on a Spinco Model 116 amino acid analyser in the Department of Biochemistry, Yale University, Connecticut, U.S.A. Serine and threonine were determined by extrapolating the values obtained from the three hydrolysates to zero time values. Valine and isoleucine were calculated from 72-hour hydrolysate. The values of other amino acids were averages of those found in 20-, 48- and 72-hour hydrolysates. Tyrosine and tryptophan contents were determined spectrophotometrically. Cystine was determined by reduction with sodium borohydride in the presence of 8*M* urea and the liberated sulphydril groups were estimated with 5-5′ dithiobis (2-nitrobenzoic acid).

The amino acid composition of goat trypsin has been compared with that of bovine trypsin (Walsh and Neurath, Proc. Natl. Acad. Sci. U.S., **52**, 884 (1964)) in Table 1. The total number of acidic amino acids is practically same in both enzymes, but goat trypsin has 3 fewer basic amino acids than bovine trypsin. Significant differences in the levels of other amino acids, including histidine, serine, valine, methionine, isoleucine and leucine have been noted. The molecular weight of goat trypsin calculated from its amino acid composition is 22,479 daltons which is somewhat lower than the molecular weight (23,254 daltons) of bovine trypsin.

TABLE 1

Amino acid composition of goat and bovine trypsins

Amino acid	Residues per molecule	
	Goat trypsin	Bovine trypsin
Lysine	12	14
Histidine	2	3
Arginine	1	2
Aspartic acid	21	22
Threonine	10	10
Serine	28	33
Glutamic acid	16	14
Proline	9	9
Glycine	25	25
Alanine	14	14
Half cystine	12	12
Valine	16	17
Methionine	4	2
Isoleucine	12	15
Leucine	15	14
Tyrosine	10	10
Phenylalanine	3	3
Tryptophan	4	4
Total residues	214	223

B.1.2. *Studies on Egg White Proteins*

Comparison of proteins of egg-white from three different species of turtle with those of hen egg-white has been carried out by polyacrylamide gel electrophoresis at *p*H 4·5 and starch gel electrophoresis at *p*H 8·6. Considerable differences were noted in the number of protein constituents and their electrophoretic mobilities. Polyacrylamide gel electrophoretic patterns showed 10, 8, 7 bands with three types of turtle egg-white and 4 bands with hen egg-white. Only one of the three types of turrtle egg-white possessed lytic activity against *M. lysodeikticus* and showed a band in the electrophoretic patterns corresponding to a lytic enzyme. The other two egg-whites were devoid of any lytic activity. It was

also noted that one of the species showed the presence of two electrophoretically distinguishable lysozymes occurring singly in individual animals. No animal was found to possess both the types.

Isolation, Purification and Enzymatic Properties of Turtle egg-white lysozyme. Turtle egg-white possessing lytic activity against *M. lysodeiktecus* was obtained from eggs collected from local market. Lysozyme rich material was prepared by adsorbing the proteins on CM-cellulose according to Canfield and McMurray (Biochem. Biophys. Res. Commun. **26**, 38 (1967)). The $(NH_4)_2CO_3$ eluate was dialysed free from salt and freeze-dried.

Attempts were made to purify lysozyme by column chromatography on CM-cellulose column (48×1·5 cm) previously equilibrated against 0·01*M* phosphate buffer, *p*H 5·5. Elution was carried out in two stages, first with 0·01*M* phosphate buffer, *p*H 5·5 when a small peak separated; at this stage elution was commenced under a salt and *p*H gradient (0·01*M* to 0·015*M* PO_4; *p*H 5·5 to 7·5). The absorbancy of the fractions were read at 280 nm. The fractions containing the lytic activity were pooled. The protein was precipitated at 90% $(NH_4)_2SO_4$ saturation, collected by centrifugation, dissolved in water, dialysed free from salt and freeze-dried. This material however contained some minor impurities as shown by polyacrylamide gel electrophoresis.

Biogel P-30 Filtration. An improved method of purification of lysozyme was devised by gel filtration through Biogel P-30. CM-adsorbed lysozyme rich material as well as CM-chromatographed lysozyme obtained as described above were purified in a single step by gel filtration through a Biogel P-30 column (95×3·2 cm) in acetate buffer, *p*H 5·5, $\mu = 0{\cdot}1$. This method has also been used in the case of hen egg-white lysozyme with excellent results. The preparations thus obtained were electrophoretically homogeneous. A commercial grade hen egg-white lysozyme (3×crystallized) was freed from trace contaminants by this method.

Enzymatic Properties

Assay Method : The lytic activity of lysozyme was measured by measuring the rate of decrease in the absorbancy at 540 nm of a suspension of *M. lysodeikticus* (30 mg/100 ml) at *p*H 7·0, $\mu = 0{\cdot}05$ during the first five minutes of the reaction.

pH optimum : Turtle egg-while lysozyme shows maximum activity at *p*H 7·0 as found by making measurements in the *p*H range 4–10 in different buffers of molarity 0·05 (Haenger, J. Bact., **95**, 376 (1968)).

Effect of ionic strength at constant pH : The enzyme is activated or inhibited strongly as a function of ionic strength at *p*H 7·0. The activity was measured in 0·01*M* phosphate buffer, *p*H 7·0 and the ionic strength was varied by adding NaCl dissolved in the same buffer in the reaction mixture. Maximum activity was obtained at ionic strength of 0·05.

Effect of Cu^{2+} ion : Cu^{2+} ions cause inactivation of both turtle and hen egg-white lysozymes, but to different extents. Lytic activity gradually diminishes with time and

reaches 4·3% and 19·2% of initial activity of turtle and hen lysozyme respectively after 25½ hr. in $1{\cdot}6\times10^{-4}M$ $CuCl_2$ solution at 0·45 mg/ml enzyme concentration at 37°.

Effect of substrate concentration on reaction velocity : At low substrate concentration the velocity of lysis of *M. lysodeikticus* increases linearly with the increase of substrate concentration but gradually the rate of increase of velocity diminishes and reaches a flat zone. Similar observation has been made by others with hen egg lysozyme.

Heat stability : Heat stability of turtle egg-while lysozyme was studied by exposing a lysozyme solution in 0·01*M* phosphate buffer, *p*H 7·0 for 15 minutes at different temperatures in the range of 25–100°. The activity of this solution falls slowly as the temperature is raised from 25 to 80°. The protein solution loses 20% of its initial activity at 80° but it retains only 10% remaining activity at 100°.

B.1.3. *Spectrophotometric Titration of Buffalo β-lactoglobulin in presence of 6M guanidine hydrochloride*

Spectrophotometric titration of buffalo β-lactoglobulin (Ann. Rep. 1969-70) was done at 25° at 0·15 ionic strength. Further experiments were done to note the time dependency and effect of Gu-HCl on the ionization of tyrosyl phenolic group of buffalo β-lactoglobulin. The measurements were made at 290 and 295 nm in a Hilger Uvispeck spectrophotometer. Spectrophotometric titrations in 6*M* Gu-HCl (ultrapure, Mann Research) was carried out at 295 nm. The *p*H measurements were made an a Radiometer PHM 26.

The ionization of the phenolic hydroxyl groups of buffalo β-lactoglobulin in presence of 6*M* Gu-HCl started at *p*H 8·4 and was complete at *p*H 11·6. In the absence of Gu-HCl the change in molar extinction co-efficient, $\Delta\epsilon$, in going upto *p*H 13 was noted as 15,000 when *p*H readings were taken within 10 min. after mixing. This corresponds to a value of approximately 6·5 tyrosine residues per dimer assuming an $\Delta\epsilon$ increment of 2300 per residue. At higher *p*H values, time-dependent measurements (after 24 hrs.) gave a total change in $\Delta\epsilon$ of 17,000 giving 7·3 residues/mole. This is in agreement with Gorbunoff's observation (Gorbunoff, Biochemistry 6, 1606, (1967)) in the case of cow β-lactoglobulin-B that 3 of the tyrosine residues/chain of 18,000 mol. wt. are exposed below 12 while the last one ionizes only slowly at *p*H 1·3. However, complete ionization of the last group does not appear to have occurred. The titration curve in Gu-HCl shows that 8 groups/dimer become available for titration at about *p*H 12. The total change in $\Delta\epsilon$ of 19,500 gives 8 tyrosine residues per molecule taking an $\Delta\epsilon$ increment of 2450/residue.

B.1.4. *Studies on Toxic Proteins of Plant Origin*

Isolation and characterization of the toxicprotein of Abrus precatorius. It is known that the seeds of *A. precatorius* contain toxic proteins which are quite fatal in small doses and possess strong hemagglutinating activity with human RBC which is non-specific in respect of blood groups. Studies on this material was made in this laboratory several years ago and has been undertaken again for further detailed work.

(a) *Abrus precatorius* seeds after decortication and crushing in a grinding mill were extracted by shaking in a mechanical shaker with 5 times its weight of 0·15*M* NaCl (0·9% NaCl) in the cold room for 16–18 hours. The extract after filtration and centrifugation was saturated with 80% $(NH_4)_2SO_4$ to precipitate the total protein. It was noted that the protein responsible for agglutination of red blood cells was completely precipitated within 70% saturation with $(NH_4)_2SO_4$. The precipitate of the total protein was dissolved in 1·0*M* NaCl and dialysed exhaustively first against water and then against 1·0*M* NaCl. The gummy precipitate was discarded by centrifugation and the supernatant (1) was chromatographed on Sephadex G-200.

(b) *Column chromatography on Sephadex G-200.* The supernatant (1) was chromatograhped on a column (55×4 cm) of Sephadex G-200 equilibiated with 1·0*M* NaCl. Three different protein peaks and a peak for non-protein material were obtained. Of the three protein peaks, peak 1 showed the highest agglutinating activity.

The non-protein fraction was only slightly hemagglutinating (upto a dilution of 1 : 4). It gave a coloration with Folin reagent and a peak at 270 nm.

(c) *Polyacrylamide gel electrophoresis.* In polyacrylamide gel electrophoresis at *p*H 4·5, it was found that for peak 1, the bands did not move at all and in the case of peak 2 and 3, the bands moved only a very small distance but were not sufficiently resolved.

At *p*H 8·3, for peak 1, four bands were obtained—one major and three minor bands. For peak 2, the pattern was almost the same as for peak 1. For peak 3, one major and six minor bands were obtained.

(d) In the above method of extraction it was found that the amount of non-protein material present in the supernatant (1) was very high. So in order to decrease the amount of non-protein material, the $(NH_4)_2SO_4$ precipitate of the total protein was dissolved and dialysed against 0·1*M* PO_4—0·2*M* NaCl buffer, *p*H 7·0, and the chromatography on Sephadex G-200, was performed in the same buffer.

(e) *Test for carbohydrates.* Molisch test for sugar was performed for the four different peaks obtained by chromatography on Sephadex G-200. It was found that all the three protein fractions contained sugars, but the non-protein fraction was free from sugar.

(f) *Further studies with peak* 1. Peak 1 was chromatographed on DEAE column (18×2 cm) in 0·01*M* PO_4—1·0*M* NaCl buffer, *p*H 7·0. A single but skew peak was obtained. The fraction contained carbohydrates.

The protein was freed from carbohydrate according to the method of Rigas and Johnson; (Ann. N.Y. Acad. Sci. **113**, 800, (1964)). It was found that after the separation of carbohydrate the hemagglutination power of the protein was not changed but the solubility was changed. The protein was completely soluble upto *p*H 3·0 and almost insoluble at *p*H 2·0.

In the polyacrylamide gel electrophoresis at *p*H 8·3, it was found that one major and two minor bands were present.

B.1.5. *Preparation of phosphoprotein 'phosvitin' from Turtle egg yolk*

The phosphoprotein 'phosvitin' from egg yolks of three varieties of turtle was prepared by the method of Joubert and cook (Can. J. of Biochem. and Physiol., **36**, 399, (1958)).

Egg yolks were first rolled on a cheese cloth to free from adhering white. The yolk membranes were then punctured and yolk fluid sieved through a single layer of cheese cloth into a beaker. Weighed amount of yolk fluid were then treated by the method of Joubert and Cook. In the last step the volume of salt free phosvitin solution was reduced by pervaporation. The yield of the freeze-dried material was 0·2–0·3% of the total egg fluid.

Polyacrylamide gel electrophoresis. The purity of the preparation was checked by polyacrylamide gel electrophoresis at *p*H 8·3. A major broad band with a sharp and thin faster moving band appears for the three species.

Phosphorus content. Phosphorus content of the protein was estimated by Fogg and Wilkinson's method. The result obtained for the three species were 12·06%; 11·76% and 9·0%. Phosphorus content of hen's egg yolk phosvitin is known to be 9·6%.

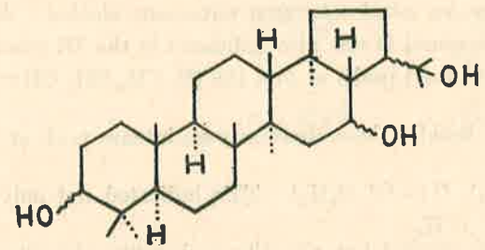
B.2. Organic and Plant Chemistry

B.2.1. *Chemical investigation of Mollugo Hirta*

Four new triterpenoid sapogenins, mollugogenol A, B, C, E were isolated from this plant. The structures of mollugogenol A, B and C were established earlier (vide earlier reports).

Another new sapogenin called Mollugonenol F was isolated from this plant. Mollugogenol F, $C_{30}H_{52}O_3$, m.p. 211–12°, on treatment with AC_2O and pyridine furnished a diacetate, $C_{34}H_{56}O_5$, m.p. 225–26°. The I.R. spectrum of the diacetate still showed the band of a hydroxyl group, showing thereby that the three oxygen functions are present as two secondary and one tertiary hydroxyl group.

On oxidation with CrO_3-Py complex mollugogenol F furnished two compounds. Fraction A, m.p., 228–29° (major) and fraction B, m.p. 231–34°. Fraction B gave positive Zimmermann's colour reaction for 3-keto group, and when subjected to acid (5% EtOH-HCl) treatment for 3 hours furnished a compound which showed u.v. absorption maximum at 256 mμ (α,β unsaturated ketone).



I

Mollugogenol F on treatment with 5% EtOH-HCl for 3 hrs. furnished a compound which showed u.v. absorption maximum at 240, 251, and 260 mμ similar to that of hop-15, 17, 21 diene system. Therefore from the above discussion, mollugogenol F is considered to be a hopane or isohopane derivative with three hydroxy functions at 3, 16 and 22 positions (I). Further work to confirm the proposed structure (I) is in progress.

Mollugogenol D

Mollugogenol D, m.p. 268–71° (Corr.) $[\alpha]_D^{23}+49°$ ($CHCl_3$) was found to be a completely saturated pentacyclic triterpene, containing an ether linkage and a number of hydroxyl groups. Its molecular formula was established as $C_{32}H_{56}O_4$ from combustion data and molecular weight determination by mass spectrometry. On treatment with acetic anhydride and pyridine at 0°, mollugogenol D furnished two diacetates : diacetate A, $C_{36}H_{58}O_5$, m.p. 266–69° (d) (minor fraction) and diacetate B, $C_{36}H_{60}O_6$, m.p. 237–40° (major fraction) which were formed in the ratio 1 : 3. Diacetate A moved faster than diacetate B in thin layer chromatography over silica gel G, but reverse was observed when thin layer chromatogram was impregnated with aq. silver nitrate solution (12·5%), i.e., diacetate B moved faster than diacetate A. This indicated that the diacetate A might be an unsaturated compound and this was further proved by its positive reaction with tetranitromethane and its u.v. absorption maximum at 204 mμ (log ϵ 3·76). Diacetate B did not develop any colour with tetranitro methane.

Mollugogenol D, on oxidation with CrO_3-pyridine complex yielded a colourless diketone, $C_{32}H_{52}O_4$, m.p. 250–52° (d). It gave positive Zimmermann's colour test for 3-keto group. It did not develop any colour with alcoholic ferric chloride. Therefore it could not be an enolizable α or β-diketone. The I.R. spectrum of this compound showed bands at 1720 (six membered ring ketone) and 3440 cm^{-1}. The latter was due to a tertiary hydroxyl group.

Since mollugogenol D formed two diacetates (diacetate A and B) and a hydroxy diketone, it was concluded that it contains one tertiary and two secondary hydroxyl groups.

The mass spectrum of mollugogenol D showed the molecular ion peak at m/e 504 which corresponded to the molecular formula $C_{32}H_{56}O_4$. Thus mollugogenol D appeared to contain C_2H_4 unit more than an usual saturated triterpene alcohol. Absence of any ester or carbonyl group in mollugogenol D was also indicated in the IR spectrum. The mass spectrum of mollugogenol D showed peaks at m/e 458 $(M-CH_3.CH_2.OH)^+$ and m/e 418 [M–(CH_3.CH–O–C$\langle^{CH_3}_{CH_3}$)+H.]. Besides these there was an intense peak at m/e 87 which corresponded to the ion $[(CH_3)_2\ C=O^+\ C_2H_5]$. This indicated not only the possibility of the presence of a meioty (–C$\langle^{CH_3}_{CH_3}$–OC_2H_5) but also the probability of mollugogenol D being either an hopane or isohopane derivative.

Moreover the N.M.R. spectrum of mollugogenol D also indicated the presence of nine methyl groups instead of the usual eight methyl groups in the region 0·75 to 1·32δ. Further the signal at 3·55δ (2H, q, J = 7 c.p.s.) ($O.CH_2.CH_3$) in the NMR spectrum of mollugogenol D clearly indicated the presence of an ethoxy group in it. This was further supported by the fact that mollugogenol D on treatment with hydrobromic acid and acetic anhydride in chloroform-phenol mixture furnished a saturated acetate m.p. 201–5°, different from the diacetate A and B, which on saponification yielded a tetrol, $C_{30}H_{52}O_4$, m.p. 250–55°.

Presence of an oxygenated (ethoxy) isopropyl side chain indicated that mollugogenol D may be either a hopane or isohopane derivative. The mass spectral fragmentation pattern of this compound was also found to be compatible with those observed for hapane or isohopane derivative which undergo two types of cleavages (*i* and *ii*) through rinc C.

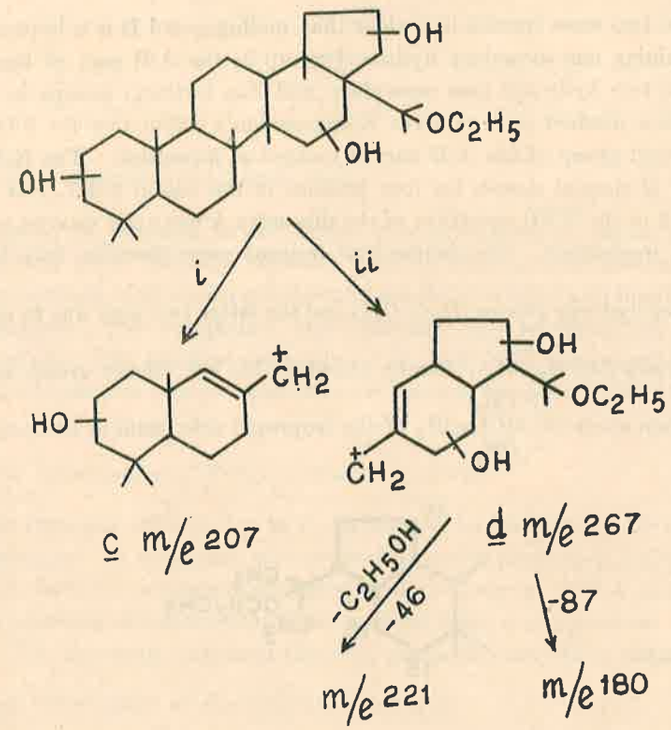
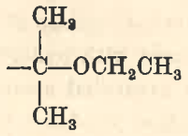


Fig. II

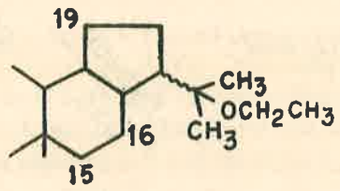
The mass spectrum of mollugogenol D showed two ions at m/e 207 and m/e 267, the former was attributed to the part containing rings A and B (formed by *i* type cleavage) and the

latter to the part containing rings D and E (formed by ii. cleavage) because loss of the moiety, (87 mass units) or one mole of ethanol (46 mass units) from the latter gave rise to the peaks



at m/e 180 and 221 respectively. Thus appearence of ions *c* and *d* at m/e 207 and 267 clearly showed that in mollugogenol D only one hydroxyl group is present in the A/B part of the molecule and the remaining two hydroxyl groups and the ethoxyl groups are present in the other part of the molecule. Mass spectrum of the oxidation product (diketone) of mollugogenol D showed a weak peak for molecular ion at m/e 500, four mass units lower than that of mollugogenol D obviously due to the oxidation of the two secondary hydroxyl groups to the corresponding ketone.

From these two mass spectra it is clear that mollugogenol D is a hopane or isohopane derivative containing one secondary hydroxyl group in the A/B part of the molecule and one ethoxyl and two hydroxyl (one secondary and one tertiary) groups in the D/E ring. Since its oxidation product gave positive Zimmermann's colour test for 3-keto group, the secondary hydroxyl group of the A/B part is located at 3-position. The N.M.R. spectrum of mollugogenol D showed signals for four protons in the region 3–4·7, two of which were shifted to 4·5–5·5 in the NMR spectrum of the diacetate A but a 2H quartet at 3·55δ (J = 7 c.p.s.) remained unchanged. The former two protons were therefore attached to carbons bearing secondary hydroxy groups (*H*–C–OH) and the latter two were due to the two protons of the ethoxy group (O.C*H*$_2$.CH$_3$), thereby showing that the ethoxy group was attached to the tertiary carbon atom—C(CH$_3$)(CH$_3$)–OCH$_2$CH$_3$ of the isopropyl side chain in the part structure A.



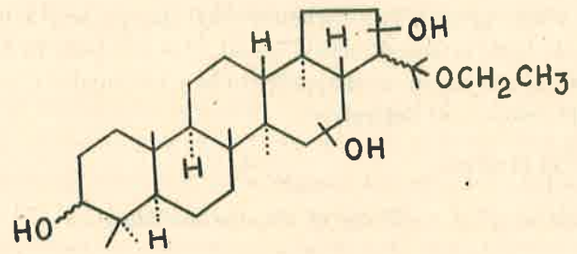
A

Fig. III

One the basis of the discussion made so far a tentative structure (II) may be proposed for mollugogenol D,

A sterol glycoside was isolated from the same plant. On treatment with 5% EtOH–HCl, the glycoside furnished an aglycon which may be β-sitosterol or stigmasterol. Further work is in progress.

The acid part of the sapogenin of *Mollugo hirta* was esterified with CH_2N_2 and the crude methyl ester on purification through chromatography furnished methyl oleanolate, compared with an authentic sample. Further work is in progress.



II

Fig. IV

B.2.2. *Chemical investigation of Adhatoda vasica*

The possibility of isolating further active principles from *A. vasica* which is widely used in asthma and other bronchial troubles from ancient age has been explored. From the young influorescence of *A. vasica* a compound has been isolated and found to be different from other alkaloids. This compound, m.p. 198–200° (d), on treatment with AC_2O-Py furnished an acetate. The spectral data (u.v., I.R., and NMR) suggested that it may be an alkaloid of 4-quinazolcne group. Another new alkaloid has been isolated from the leaves of *A. vasica.*

B.2.3. *Chemical examination of Phlogocanthus thyrsiflorus*

Systematic chemical examination of *P. thyrsiflorus* was taken up with a view to isolating its active principle. A compound was isolated from this plant, m.p. 152–55°. From the mass and N.M.R. data the compound appeared to be a diterpene with a number of oxygen function The presence of unsaturation was evident from u v spectrum Further work is in progress. I.R. spectrum indicated that the compound may be a lactone.

B.2.4. *Chemical examination of Barringtonia acutangula*

Studies on the constitution of Acid B : In the previous report the isolation of another new acid called Acid B, from the fruits of *B. acutangula* was described. It formed a dimethyl ester $C_{32}H_{50}O_7$, m.p. 253–4°. On the basis of chemical degradation and U.V., I.R., N.M.R. and mass spectral studies the constitution of this acid, now called barrigenic acid, has been established as 2α, 3β, 19β-trihydroxy-olean-12-en-23, 28-dioic acid.

B.2.5. *Chemical examination of Careya arborea*

The isolation of two acids called arboric acid and arborinic acids from the leaves of *C. arborea* was reported earlier. These two acids will henceforth be referred to as careyagic acid and careyagenic acid respectively. It has now been possible to isolate a number of phenolic compounds from the same source and these are being studied. Some studies on the constitution of careyagenic acid has been made. It formed a dimethyl ester, m.p. 168–70°, which furnished a diacetate, $C_{36}H_{52}O_8$, m.p. 244°. Its NMR spectrum showed the presence of two acetyl groups, two carbomethoxyl groups and a homoannular diene system having a double bond attached methyl group. On the basis of U.V., I.R., N.M.R. and mass spectral studies, careyagenic acid appears to be a 18-dehydro-ursolic acid derivative or a 18-dehydro-20-epi-ursolic acid derivative.

Further work is in progress.

B.2.6. *Chemical examination of the leaves of Eupatorium odoratum*

The isolation of three phenolic constituents from the leaves of *Eupatorium odoratum* was reported earlier (vide previous Ann. report). One of these has been characterized as isosecuranetin. A new tetramethoxy chalkone and another new dimethoxy flavone have also been isolated. Besides the above phenolic constituents, two low melting colourless compounds have also been isolated.

Further work is in progress.

B.2.7. *Chemical investigation of microbial metabolites*

The chemical investigation of rubidin—an antibiotic from a Streptomyces sp. is being carried out in collaboration with the Microbiology Department of this Institute (vide earlier reports).

The mass spectrum of rubidin has been studied in detail. It showed molecular ion peak at m/e 444 and another peak of almost equal intensity at m/e 446 which is undoubtedly the M+2 peak due to reduction of the quinonoid carbonyl groups of rubidin by moisture in the mass spectrometer. This was evident from the fact that the reltative intensities of the above two peaks varied with change in the temperature of the ionization chamber. When the mass spectrum of rubidin was recorded at an inlet temperature of 260° (direct inlet system was used), only a single peak was observed at m/e 446 (M+2) and the m/e 444 ($M^{\cdot+}$) peak completely vanished. This experiment left little double that the peak at m/e 444 was the $M^{\cdot+}$ peak. On the basis of this observation and the combustion data, rubidin appears to have the molecular formula $C_{22}H_{20}O_{10}$. The mass spectrum of rubidin also showed peaks at m/e 400 and 356 corresponding to loss of one and two molecules of CO_2 from the molecular ion. There were also peaks at m/e 402 and 358 due to the loss of two molecules of CO_2 from the M+2 ion. The loss of two molecules of CO_2 indicated the presence of either two carboxyl or lactone rings in rubidin. There were also peaks at m/e 338 and 323 due to the ions $[M^{\cdot+}-2CO_2-H_2O]^+$ and $[M^{\cdot+}-2CO_2-H_2O-CH_3]^+$ respectively. The IR spectrum

of rubidin in Nujol mull showed bands at 3400 cm^{-1}_q (hydroxyl groups), 1780, 1770 and 1615 cm^{-1}. The bands at 1770 and 1780 cm^{-1} are indicative of the presence of two γ-lactone rings rather than two carboxyl groups in rubidin. The band at 1615 cm^{-1} is most probably due to the two chelated quinone carbonyl groups.

Rubidin on heating with acetic anhydride and sulphuric acid on steam bath furnished a bright yellow tetra-acetate, $C_{30}H_{28}O_{14}$, m.p. 243–245°, in very poor yield. Its I.R. spectrum showed band at 1665 cm^{-1} for free quinone carbonyl groups thus suggesting the presence of at least two hydroxyl groups peri to the quinone carbonyl groups. The mass spectrum of the tetra-acetate showed $M^{\cdot+}$ peak at m/e 612 and a M+2 peak at m/e 614, which was far more intense than the former. The data so far discussed indicated that most probably rubidin is a quinone having four hydroxyl groups and two lactone rings. At least two of the hydroxyl groups must be peri to the two quinone carbonyl groups.

Rubidin is sparingly soluble in most organic solvents like $CHCl_3$, CCl_4 etc. Rubidin is soluble in $CHCl_3$, but it was obtained in very poor yield. So the NMR spectrum of Rubidin was studied as a pyridine D_5 solution. Unfoutunately the spectrum was not satisfactory due to decomposition of the sample during NMR studies. However, the presence of two secondary methyl groups in rubidin was evident from the spectrum.

Rubidin was found to be active against *Staph. aureus*, *Bacillus subtilis* and *Streptococcus faecalis*.

Further work is in progress.

B.2.8. *Chemical and pharmacological studies on Indian medicinal plants*

A collaborative programme has been undertaken with the Pharmacological Department of Medical College, Calcutta, to carry out Chemical and pharmacologiccal studies on Indian Medicinal plants. Pharmacological tests has been carried out on the seeds of the plant *Elaeocarpus ganitrus* commonly known as rudraksha.

The seeds of the plant *E. ganitrus* was collected from Nepal. The ethyl acetate extract of the defatted seeds was subjected to pharmacological tests. The extract was found to have significant myocardial stimulating action in both intact and isolated mamalian heart. Work is in progress to isolate the active principles.

B.2.9. *Synthesis of new potential antifertility agents*

The synthesis of a number of potential antifertility agents were reported earlier. Two more oxazolidine-2-thione derivatives have been synthesized.

B.2.10. *A new method for the conversion of chalcones to flavones*

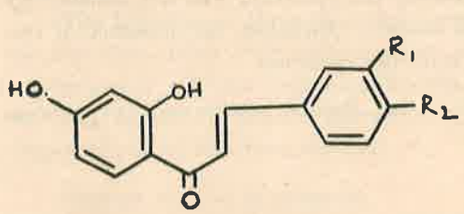
Chalcones, flavones and flavanones frequently co-exist in nature. The interconversion of chalcones to flavanones is well known and the methods are simple. Conversion of chalcones to flavones is not easily achieved as in the previous case. A new one step method has now been developed for the conversion of chalcones to flavones and this is described below.

Description of the method

An intimate mixture of chalcone and palladium-charcoal (10%) in equal proportion (w/w) was taken in a sublimation tube and the mixture was heated under vacuum at a temperature 40–50° above the melting point of chalcone for 1 hour. The product was trapped in one of the bulbs of the sublimation tube by cooling. The products were identified as flavones by their colour reaction with melting points and u.v. spectral data.

The following chalcones were prepared by the usual procedure. 2′,4′-dihydroxy chalcone (I), m.p. 150–51°, 2′,4′-dihydroxy,-4-methoxy chalcone (II), m.p. 185°; 2′,4′-4-trihydroxy-3-methoxy chalcone (III) m.p. 207–8°, 2′,5′-dihydroxy chalcone, 175–76°, (IV); 2′,5′-dihydroxy-4-methoxy chalcone (V), 172–73°.

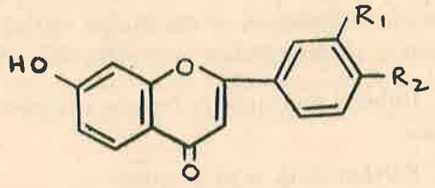
The above chalcones under the above experimental conditions furnished the following flavones respectively : 7-hydroxy flavone (VI), m.p. 238–40°; 7-hydroxy-4′-methoxy flavone (VII), m.p. 262–63°; 7-4′-dihydroxy-3′-methoxy flavone (VIII) 292–3°. 6-hydroxy flavone (IX) m.p. 228–30°; 6-hydroxy-4′-methoxy flavone, 202–4° (X).



I, $R_1 = R_2 = H$

II, $R_1 = H$; $R_2 = OCH_3$

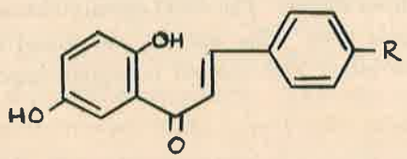
III, $R_1 = OCH_3$; $R_2 = OH$



VI, $R_1 = R_2 = H$

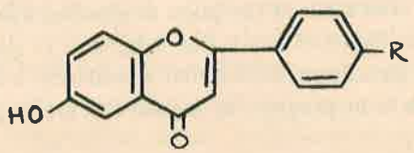
VII, $R_1 = H$; $R_2 = OCH_3$

VIII, $R_1 = OCH_3$; $R_2 = OH$



IV, R = H

V, $R = OCH_3$



IX, R = H

X, $R = OCH_3$

Fig. V

B.3. Comparative Phytochemistry

B.3.1. *Chemotaxonomic studies*

(i) *Studies on the constituents of the family Rutaceae.*

(a) *Synthesis of mahanimbine* : During the synthesis of mahanimbine by citral

condensation other intermediates and end products were obtained The characterisation of these compounds are in progress

(b) Work on the ozonisation products of mahanimbine is in progress

(c) *Synthesis of girinimbine* : The synthesis of girinimbine has been accomplished starting from 2-hydroxy-3-methyl carbazole and $\beta:\beta$-dimethyl acrolyl chloride The acrolyl ester on Fries migration and acidification yield a chromanone. The chromanone on reduction furnished the alcohol. The alcohol on dehydrotosylation furnished girinimbine identical with the natural compound.

(d) The work on the structure of murrayazoline is in progress.

(e) Some constituents of *Murraya exotica* (Syn. *M. paniculata*) are being examined.

(f) *Synthetic investigations in connection with glycozolidine* : Further studies have resulted into synthesis of several dimethoxy methoxy carbazoles : structure of glycozolidine has been settled.

(g) Some crystalline constituents have been isolated from *Toddalea aculeata*.

(ii) *Studies on the constituents of the family Meliaceae*

(a) The work on the crystalline constituents of *S. mahagoni* previously reported are in progress.

(b) Some progress has been made in the studies of the triterpenes reported from *S. mahagoni*.

(c) Progress in the triterpene constituents of *Hayenea trijuga* are in progress. A new heterocyclic product has been isolated from the *H. trijuga*.

(iii) *Besides the above report other members of the order Rutales are being examined.*

B.3.2. *Antibiotics from higher plants*

(a) A new xanthone having a c-methyl phloroacetophenone skeleton has been obtained from *Garcinia xanthochymus*. The compound is being examined for its antibiotic action and its structure.

(b) The synthetic studies on the plant antibiotic mesuagin and mesuol are in progress.

B.3.3. *Biochemical and chemotheraputic studies in relation to vitiligo*

We reported previously that there is unusual break down of tryptophan in vitiligenous subjects. This was documented by detection of 5-hydroxy anthranilic acid. This presupposes that there will be more tryptophan pyrrolase activity in vitiliginous subjects. In order to have some information tryptophan pyrrolase activity *vis-a-vis* psoralene drug, we carried out some experiments with toad liver. The tryptophan pyrrolase enzyme in the toad liver was detected. It was then found that the tryptophan pyrrolase activity of the

psoralene treated animal and normal subject differed. The treated animals showed lower activity.

B.3.4. *Synthesis of biochemically active constituents*

(a) Characterisation of products of Friedal-Craft's reaction on the heterocyclic compounds are in progress.

(b) Studies involving the introduction of a monoterpene or C_5- unit to a heterocyclic compound like indole and carbazole are in progress.

B.4. Analytical Chemistry & Instrumentation

B.4.1. *Fatty acid composition of oils & fats by gas-liquid chromatography*

As reported in last Annual Report, the gas chromatographic analysis of fats and oils in this laboratory is being supplemented with prior fractionation of the mixed methyl esters. This improves the analysis to a great extent. Of the methods of fractionation used in this laboratory fractionation by urea adduct formation was found uptil now to be most suitable. The following is a report on the studies of fatty acid compositions of some complex types of seed lipids and lipids of fresh water fishes commonly available in our country, using the above method prior to G.L.C. analysis.

(i) *Linseed oil* : Fatty acid methyl esters obtained from linseed oil has been fractionaed using modified urea fractionation procedure. The fractions have been analysed by gas-chromatography. The fatty acids thus identified found to contain some polyunsaturated acids of 16, 18, and 20 carbon chain length which were hitherto unreported. The work on the determination of the positions of the double bonds by oxidative cleavage and gas-chromatography of the oxidation products are in progress.

(ii) *Castor oil* : This oil is known to contain recenoleic acid, a hydroxy unsaturated acid (hydroxy oleic acid) as the major component fatty acid. The gás-chromatographic analysis of the mixed methyl esters from this oil in our and in other laboratories showed only minor differences, compared to the reports by other techniques. A survey of the biochemical pathways of the synthesis of oxygenated fatty acids in plants, reveal that there is possibilities of the formation of other oxygenated acids than the major one. With this view, castor oil fatty acid methyl esters were urea fractionated and the fractions were analysed gas chromatographically, using special techniques for hydroxy acids. Some minor hydroxy acids other than the major one i.e., recinoleic acid were found to be present in castor oil. These have been found in castor oil for the first time.

(iii) *Seeds oils of some member of Cucurbitaceae family commonly used as vegetables in India* : Presence of fatty acids containing 3 conjugated double bonds in the seed oil of one member of the cucurbitaceae family has been reported by workers in this field. Seed oils of 8 different members belonging to different species of this family, the fruit of which are commonly used as vegetable in India have been studied for their fatty acid compositions

by gas liquid chromatography supplemented by prior urea fractionation as well as U.V. and I.R. spectrophotometry of each fraction. The results showed that most of the above seed oils contain conjugated trienoic acids as one of their major component. Isolation of some of the members of this class of fatty acids in pure state from these seed oils has also been achieved. The isolated fatty acids are now being studied for their chromatographic behaviours on gas liquid columns, adsorption columns and thin layers plates as well as for their U.V. & I.R. absorption spectra. It has aloso been revealed during this study the urea adduction is not very suitable technique to be used for the fractionation of mixed methyl esters containing the above acids.

(iv) *Fatty acid compositions of some fresh-water fishes common in India* : 6 Different varieties of fresh water cat fishes has been choosen for this study these are Wallago attu (Boal), Clarias betrachus (Magoor), Heteropneustes fossilis (Singhi), Mystus cavasius (Tengra) and Tachysurus Spp. (Aor). The lipids were extracted and purified by the method of Bligh & Dyer (Canad. J. Biochem. Phisol., 37, 911, 1959). The mixed methyl esters obtained from the oil after saponification and methylation were subjected to fractionation by urea adductation method. The different fractions have been analysed by gas-liquid chromatography. The results showed that these oils are composed of very complex polyunsaturated fatty acids containing as much as 6 double bonds. The urea adductation method alone has been found to be inadequate for proper fractionation of the factty acid methyl ester required for the positive identification of the polyunsaturated fatty acids. This difficulties have been proposed to be solved by other fractionation methods which will fractionate the mixed methyl esters according to the number of double bonds present in their structures. Thin layer and column chromatography with specially treated adsorbents have been taken recourse to for this purpose. Works on the standardisation of these methods with known polyunsaturated fatty acids are in progress.

B.4.2. *Studies on bacterial lipids* : *Effects of phage infection on bacterial lipid*

It has been observed that the surface structure of bacteria *E. coli* alters after infection with bacteriophage. Phospholipids as a class constitutes a major component of bacterial cells membrane and the aim of this study is to investigate the effect of bacteriophage infection on the phospholipid composition of bacterial cells. Four batches of *E. coli* cells, one healthy and the other three infected with bacteriophages M-13, 5H3 & 8H1 respectively were grown in peptone medium at neutral *p*H. The bacterial cells were harvested at log phases of their growth. Lipids from the wet cell pallets obtained by centrifugation were extracted with methanol chloroform mixture. The mixed lipids were dried in vacuo, weighed and analysed for their phosphorus content to determine the amount of phospholipid per gram of bacteria. The lipid extracts were fractionated on silicic acid columns by batch-wise elution method. Pure chloroform was first used to elute the neutral lipids. The phospholipids were then fractionated using increasing amounts of methanol in chloroform as solvent. The phospholipids in each fraction were tentatively identified by thin layer and silica impregnated paper chromatography according to Marinetti *et al.* (Fed. Proc., 16, 837, 1967). Both specific spraying reagents and available standards were used for identification purpose.

The silica-impregnated paper had been prepared in the laboratory from Whatman No. 1 chromatographic filter paper. The compounds in each fraction thus tentatively identified was further fractionated by preparative thin layer chromatography and each component collected was subjected to chemical methods of degradation for further conformation of its identity. In the healthy bacteria seven compounds were detected. The 1st column fraction contained two compounds, cardiolipin and phosphatydic acid the 2nd column fraction contained a ninhydrine positive phospholipid which could not be definitely identified. Phosphatidylethanolamine and phosphatidylglycerol were present in the 3rd fraction. The 4th fraction also contained two compounds which could be tentatively identified as bysophosphatidylglycerol and a glucosamine derivative of phosphatidylglycerol. Works are being carried on the final confirmation of their identities. Of these phospholipids phosphatdylethanolamine and phosphatidylglycerol were the major components found in healthy *E. coli*. Investigations on the phospholipids in infected *E. coli* are in progress. The preliminary results so far obtained showed that at least in the case of one particular phage the amount of the glucosamine derivative of phosphotidyl glycerol has increased significantly.

B.4.3. *A simple apparatus for rapid micro-hydrogenation under moderate pressures*

The micro-hydrogenation technique is extremely important for lipid analyses. The conventional apparatus is generally operated at atmospheric and higher pressures. The disadvantages of such technique lies in the facts that (a) large amount of samples rquired, (b) only one sample may be hydrogenated at a time and (c) sufficient time is taken for complete hydrogenation, when worked at atmospheric pressures.

The present apparatus has been designed for hydrogenating samples at the level of 5–20 mg of each and 10–20 samples or more at a time. Upto 5 samples containing trienoic acid, have been successfully hydrogenated in 20 minutes when operated at a pressure of about 250 mm of mercury. The apparatus for hydrogenating upto 20 samples at a time is under test.

B.4.4. *Immunochemistry*

Immunochemical studies on the specificity of Egg white Lysozymes of hen and trutle : Male white rabbits were immunized each with 10 mg of the lysozyme in 5 divided doses and the resultant antibodies were allowed to react with the antigen (lysozyme) by Ouchterlony gel diffusion and Immunoelectrophoresis. Hen egg white lysozyme was a commercial preparation (Worthington); turtle egg white lysozyme was prepared in the department. Two rabbits were used for each lysozyme. Initially 2 mg of the lysozyme in 1 ml of saline (0·85% NaCl) was emulsified with 1 ml of Freund's complete Adjuvant and the emulsion was injected subcutaneously on the back of the animals. One month after the initial immunization each rabbit was given an intravenous injection of 2 mg of the protein in 1 ml saline. Sera were collected 11 days later. Two months later following the first intravenous injection each rabbit received intravenously 2 mg of the protein in 1 ml saline on alternate days for a total of three injections. After seven days sera were collected.

Preliminary ring test was performed to detect the presence of antibody in the sera collected twice. Both the sera (antiserum to hen lysozyme and antiserum to turtle lysozyme) showed positive ring test. The sera collected after the final dose of both lysozymes showed greater antibody titre in subsequent gel diffusion tests.

Immunodiffusion test was carried out in petridishes containing 1% Ionagar gel. One percent gel was prepared in 0·02*M* sodium phosphate buffer (*p*H 7·1) containing 0·01% merthiolate and 0·14*M* NaCl. Antigens and antisera were placed in the wells in optimum proportion (20 μl of 0·0125% antigen solution in each peripheral wells and 20 μl of undiluted antiserum in the central wells). Diffusion was carried out at room temperature. Within 48 hrs. the precipitin arc appeared. Each antiserum was found to react only with the corresponding antigen and no cross reactions were observed even on using different antigen antibody proportions. In case of hen egg lysozyme one sharp and two faint arcs and in case of turtle egg lysozyme one sharp and one faint arc appeared.

Immunoelectrophoresis of the lysozymes was carried out on microslides in phosphate citrate buffer (*p*H 6·9) at room temperature with a voltage gradient of 4 volts/cm measured across the gel. Ninety minutes after the electrophoretic run of the lysozymes, antisera were added to the slots. Within 24 hrs. after the addition of antisera only one precipitin arc appeared at the cathodal end of the gel in each homologous reaction and there was no evidence of cross reaction with turtle and hen egg lysozymes. When in electrophoresis the lysozymes were substituted by total egg white protein of the respective species more than one arc appeared in each case. In addition to the arc for the lysozyme at the cathodal end three more arcs appeared near the anodal end of each species.

From the above experiments it appeared that the antigens used for immunisation were not homogeneous. Both lysozyme preparations seemed to contain other minor antigenic components which were normal protein constituents of the egg white. Failure of the antigen itself (protein used for immunization) to reveal its heterogeneous nature in immunoelectrophoresis might have been due to very low concentration of the antigenic impurities which were too insufficient to exhibit any precipitin reaction with its antibody. Substitution of the antigen by total egg white protein caused sufficient rise of those components as a result of which the antibodies to those components met the requisite amount of antigen to form the precipitin arc. This was also confirmed by raising the load of the antigen (lysozyme preparation) to as much as 200 μg when the heterogeneous nature of the antigen was to some extent revealed.

Both Ouchterlony and immunoelectrophoretic experiments indicated that the two antigens did not contain any common determinants and each one was specific for its own system.

Gel diffusion tests were further extended for comparison of the lysozymes with α-lactalbumins of cow, buffalo and goat's milk. Antisera to both lysozymes were exposed to α-lactalbumins in different antigen-antibody ratios. Neither of the two antisera showed

any evidence of precipitin reaction with the α-lactalbumins. Thus from gel diffusion tests no evidence of genetic resemblance between the lysozymes and α-lactalbumins was observed.

For better immunochemical studies the lysozymes have been further purified and the immunization of the rabbits with these puirified antigens are in progress. In the meantime 'Complement Fixation Test' is being standardised and this sensitive test for the characterisation of antigens and antibodies will be employed in the study of the purified lysozymes along with the gel diffusion tests.

B.5. Biochemistry

B.5.1. *Phosphotransferase system in bacteria*

Wild type *E. coli* K12 can transport and utilize all metabolisable sugars. Earlier it was generally accepted that the sugars are transported through the membrane by the corresponding specific permeases. After the discovery of phosphotransferase system (PTS), which can phosphorylate sugars in several bacteria, it was generally assumed that it might have a significant role in sugar permeation. Attempts are being made to correlate the permease and the phosphotransferase system in *E. coli* K12 by genetic studies.

A mutant has been isolated from wild type *E. coli* K12 and this cannot utilise glucose, galactose, fructose, lactose and maltose. It is probably due to a single point mutation and this has been confirmed by reversion studies. It is possible to have a wild type revertant ($1 : 10^6$). Assay of β-galactosidase is also consistent with this idea. Both the mutant and the parent have the same enzymatic make up, indicating that this gene has not been mutated.

Mapping with *E. coli* HfrH shows that the mutation is at 21 min. for galactose and 82·5 min. for glucose. Studies are in progress to explain why mutation studies show that there are two mutated sites.

Several revertants of the mutant on different sugars have been isolated. The reversion spectrum indicates that the reversion may be due to the wild type or it may be due to constitutiveness. Lactose constitutive revertants are essentially lactose positive. Reversion property has been shown to correspond the uptake of specific sugars.

Galactose permeation is thought to be mediated earlier by TMGI or TMGII permeases. As the mutant has no mutation in *y* gene and it can revert to Lac 0^c, the TMGI permease is not operative. It has also been found from the studies with parent, mutant and the revertants, that the TMGII permease is not involved. So probably a third pathway, the PTS, is operative for effective transport of galactose.

The mutant has been shown to be Enzyme I (of PTS) defective. Consequently it has been found that the mutant is unable to permeate radioactive sugars. It is probable that the defect in sugar utilization is at the permeation level and that is due to the defect in PTS.

B.5.2. *Studies on a PEP-hexose phosphotransferase system of Vibrio cholerae*

The presence of PEP-hexose phosphotransferase system in *Vibrio cholerae* and its analogy with *E. coli* and *S. typhimurium* PEP-hexose phosphotransferase system were

previously reported. The present aim is to study the biochemical role of this phosphotransferase system. A mutant of *Vibrio cholerae* has been isolated. It can not grow on glucose, galatose, fructose, maltose, mannitol and mannose, but it can grow on very simple synthetic medium containing K_2HPO_4, NaCl, NH_4Cl, $MgSO_4$ and glycerol as sole carbon source. Enzyme analysis shows that none of the three components of PEP-hexose phosphotransferase were present. Uptake measurements of different sugars like glucose, galactose, fructose and maltose shows that uptake of these sugars have been cut by about 75%. These genetic experiments reveal that this phosphotransferase system plays a vital role in the transport of different sugars in *Vibrio cholerae*.

A two-step pattern of growth (diauxic growth) was found in *Vibrio cholerae* when glucose-galactose and glucose-fructose was used as the only carbon source. It was also found that during first phase of growth glucose was utilized and during the second phase of growth galactose and fructose were utilised. Uptake measurements revealed that galactose uptake was inhibited by glucose to the extent of 90% but glucose uptake was inhibited by galactose and fructose only to the extent of 25–30%. Similar inhibition pattern was obtained for the phosphotransferase system. PEP-dependent phosphorylation of galactose and fructose was inhibited by glucose to the extent of 90% while that of glucose was inhibited to the extent of 30% only by galactose and fructose. It appears, therefore, that diauxic growth is due to competition at the level of sugar uptake and PEP-dependent phosphorylation of sugars. This is an indirect evidence which suggests that PEP-hexose phosphotransferase system plays an important role in sugar transport and phosphorylation of sugar is needed for transport.

C. BOTANY

C.2. and C.3. Biometry and Mutation and Plant Breeding

C.3.1. *Changes in the aestivation of the floral parts*

There are isolated mutants induced in rice variety namely Marichbati, after X-ray treatment and in the Satika variety after P^{32} treatment. These mutants are S.221 and S.12 respectively. The floral morphology of these mutants are very peculiar and different from the normal floral morphology of the *Oryza sativa* L. The observed morphological results are given below :

TABLE 1

The floral morphology of the control and mutant rice

Selection	Glume	Lemma	Palea	Lodicule	Anther	Ovary
Marichbati	2	1	1	2	6	1
S. 221	2	1–2	1–2	0–4	3–12	1–6
Satika	2	1	1	2	6	1
S. 12	2	1	1	2	3–6	1–3

In normal rice there are two glumes, one lemma, six anthers and one overy. From the table it can be observed that in the mutant S.221, the number of lemma and pelea were increased, number of anthers were 3–12, and the number of ovaries were 1–6. Similar changes in the floral parts were observed in the mutant S.12. There the number of anthers were 3–6 and number of ovaries were 1–3. This new arrangement of the floral parts are important from the taxonomic out look.

C.3.2. *Agronomic behaviour of rice mutants*

Several mutants in rice were selected for minor changes in the morphological characters. These mutants were maintained for several generations. In the ninth generation these mutants were put in a yield trial to assess there performances against their control. Along with the yield per plant, other important characters were considered for comparative study. The analysed data is presented in the table 2.

TABLE 2

The mean values of agronomic characters of the mutant and their control

Selection	No. of tillers	Height of plant (cm)	Length of panicle (cm)	Flowering time (days)	Yield per plant (gm)
Chinsurah Boro I	12.60±0.43	106.50±1.95	21.72±0.21	84.00±0.27	11.77±0.45
S. 316	13.53±0.44	109.50±2.37	22.16±0.64	86.87±0.18	11.33±0.82
Chinsurah Boro II	10.56±0.21	106.40±1.86	21.28±0.72	85.34±0.16	12.20±0.28
S. 317	10.33±0.42	102.86±5.64	18.88±0.22	86.57±0.25	12.11±0.34
S. 322	8.60±0.79	96.57±2.56	20.37±0.53	87.94±0.36	10.43±0.25
S. 324	9.93±0.56	99.34±3.13	18.76±0.14	85.55±0.78	9.51±0.40
S. 325	9.20±0.50	98.81±1.95	18.55±0.36	88.04±0.93	9.45±0.56
S. 326	10.10±0.41	95.30±1.37	19.63±0.20	85.30±0.21	9.18±0.88
S. 328	10.00±0.69	106.53±3.18	21.95±0.53	88.68±0.24	10.98±0.60
S. 336	8.26±0.72	108.88±3.62	20.69±0.63	87.69±0.30	6.64±0.72

From the table 2, it is observed that the yield per plant has decreased, the mean time flowering has increased in all the mutants when compared to their respective parents. The changes in the other characters of the mutants shows both increase and decrease of their mean value.

C.3.3. *Studies on some selections of rice in the P_2 and S_2 generations*

In the variety Chinsurah Boro II, several healthy plants were isolated after 4 mc, 8 mc treatments of radio active sulphur and phosphorus. Agronomic behaviour of these isolated plants in P_2 and S_2 generations were analysed and given below.

TABLE 3

Mean agronomic values in the second generation after treatment with radio active sulphur and phosphorus

Selection	Height of plant (cm)	No. of tillers	No. of panicles	Length of panicle (cm)	Length of flag leaf (cm)
Chinsurah Boro II (Control)	108.44	12.17	11.51	21.03	31.53
4 mc P^{32}	106.24	11.26	10.80	20.81	31.84
8 mc P^{32}	107.32	12.40	12.17	21.45	29.81
Chinsurah Boro II (Control)	109.91	12.02	11.86	20.91	31.53
4 mc S^{35}	105.57	10.48	11.04	21.44	30.98
8 mc S^{35}	109.19	12.62	12.24	20.96	30.19
S.E.	0.43	0.71	0.62	0.48	0.65

From the table 3 it is observed that the mean values of the plant height were reduced in the treated population The changes in the other characters after radiation were at random.

C 3 3 1 *Studies on hybrids*

Some hybrids were selected after the cross between the rice varieties namely Chinsurah D G and Marichbati These were maintained from year to year to get homozygous lines for stiff character of the rice stem In the sixth generation these selections were grown in the field along with their parents to study their agronomical characters The observed results are given below.

TABLE 4

The agronomic studies of the hybrids and parents in the sixth generation

Selection	Height of plant (cm)	Number of panicle	Length of panicle (cm)	Mean time of flowering (days)
Marichbati	172.26±1.33	5.60±0.53	21.85±0.47	100.12±0.20
Chinsura D.G.	195.18±2.88	4.66±0.22	22.51±0.43	120.26±0.16
S. 8	202.23±2.23	6.20±0.48	26.26±0.55	108.56±0.43
S. 9	193.63±2.77	6.43±0.29	22.69±0.93	113.07±0.36
S. 15	195.04±2.11	8.63±0.87	21.33±0.27	103.11±.014
S. 16	168.32±1.42	7.33±1.02	23.46±1.35	111.35±0.25

It is clear from the table 4 that the hybrids are both higher and lower in their mean values of different agronomic characters when compared to their parents.

C.3.3.2. *Studies on segregation of characters*

In a hybrid plant (Marichbati × Chinsurah D.G.) some abnormalities were observed in the nature of lemma and palea of rice. Some seeds of the selected plant had closed lemma and palea but other had normal lemma and palea. For detailed study of this character, (closed lemma and palea), progeny of the selected plant were maintained. The segregating results of this character can be seen from the table 5.

TABLE 5

Family	Normal grain	Closed grain	X^2
1	901	34	2.55
2	617	22	1.03
3	470	12	0.28
4	148	6	0.70
5	194	7	0.37
6	326	8	1.68

From the Table 5 it is clear that the closed rice and normal rice is segregating in a ratio 35 : 1. Further studies on this peculiar character is in progress.

C.3.4. *Protein value of the mutants*

Many mutants were analysed for their seed protein value according to the microkjeldhal method. The analysed values are presented in the following table.

TABLE 6

Protein values of the rice mutants and their control

Selections	Mutagens used	Protein value (%)
Jhanji	Control	8.50
S. 154	X-rays	8.52
S. 154/8	X-rays	8.45
S. 11/6	P^{32}	8.10
Marichbati	Control	8.80
S. 218	X-rays	8.67

From the Table 6 it is clear that there are very little differences between the control and mutants.

C.3.5. *Effect of Alkylating agent on Paddy*

The effects of alkylating agents (Ethyl methane Sulphonate, Ethelene Imine) on 3 vars of Paddy namely Taichung Native I, Latisail and Kumargore had been observed in relation to Cytology and Cytogenetics.

In Taichung N I observations were taken on percentage of germination, rate of growth, tillering, flowering, number of days taken in flowering, morphological changes, sterility etc. 10 mutants with following characters were isolated from C_2 & C_3 generations as—

i) *Compact panicle* : Total number of flowers per panicle is higher than that of the normal one, seeds are more compactly set and slightly course.

ii) *White Husk* :—husk is white, highly sterile.

iii) *Long Plant* :—Height generally normal but after flowering this plant becomes double in length than the mother plant, seeds losely arranged on the panicles.

iv) *Round leaf* :—Leaves round almost hollow, cylindrical, thicker and dark green.

v) *Red husk* :—Husk-red to brick-red, highly sterile, seeds losely arranged on the panicle.

vi) *Dwarf* :—Height of the plants is much longer than the control and high number of tillers are noted.

vii) *Fuzzy-Panicle* :—Highly sterile, vary small, ireegular shaped grain, major part of the flowering head transformed to fuss; irregular number of floral parts are also observed.

viii) *Spreading type* :—Tillers are spreading and slightly dwarf.

ix) *Whirled leaf* :—2-small leaves arise from each node, dichotomously branched and completely sterile.

x) *Fine grain* :—Grain size and shapes are much reduced and slightly beaked, highly sterile.

C_2 and C_3 of Latisail, Kumargore treated with EIM EMS and C_3 treated with EIM are in the field and further selection and isolation work are in progress.

C.4. Cytogenetics

C.4.1. *Effects of x-rays on Indian Spinach* :

The investigations on the effects of *x*-rays on Indian Spinach were continued during the year under report.

Self fertilised seeds of three varieties of spinach were exposed to 22·5, 27, 45, and 54 kR *x*-rays after keeping the seeds immersed in water for 24 hours. At the end of this period the seeds had about 47% moisture content. M_2 seeds were similarly treated with the same dosages. In the third series, dry seeds were first kept immersed in water for 24 hours. The moist seeds were dried in air to reduce the moisture content to 18% and then irradiated with 22·5 and 45 kR *x*-rays. Treatments with 27 and 54 kR were also made on F_1 and F_2 seeds after crosses were made using red and green varieties as the parents.

In the M_1 generation the survival of plants till maturity decreased with increase in dosage excepting in 45 kR treatment which recorded the maximum killing effect in all the three varieties under investigation. During early stages of growth the treated plants manifested various forms of morphological abnormalities. The frequency of plants with morphological abnormalities increased with increase in dosage excepting in 45 kR treatment when a decrease on the occurrence of such plants were recorded. With repeat dosages on M_2 seeds the frequency of abnormal plants increased further with increase in dosage. In the variety Banerjee's Giant all the surviving plants manifested morphological abnormalities when 54 kR repeat dosage was applied on M_2 seeds. In all cases repeat dosages had higher frequency of abnormal plants than single dosages in any season.

There was no significant difference in plant height at maturity between single and repeat dosages.

Treatments in general produced lateness in flowering and longer flowering period indicating increased variability. The magnitude of difference in mean time of flowering between single and repeat dosages was low.

The M_1 population raised after decreasing the moisture content to 18% from 47% and followed by treatments with 22·5 and 45 kR manifested lateness in germination, reduced survival, lateness in flowering and decreased plant height.

In the M_2 generation, the plants, grown from seeds bulked treatmentwise, manifested earliness in flowering in the varieties red stem and Banerjee's Giant following treatments with 22·5 and 45 kR in the former and with 27 kR in the latter and increase in ultimate plant height also in the latter after treatments with 27 and 45 kR.

Out of 77 selections made for earliness in flowering from the treated populations 27 manifested significant earliness over their respective controls. A number of these selections again had long flowering period. Six single plant selections were significantly taller than their respective controls.

The selections made in the M_2 generation were grown without any further treatment. While, selections for tall plants proved to be promising in some cases, there was no significant difference in mean time of flowering in the selections when compared to their respective controls.

The behaviour of the chromosomes during meiosis in the four parental types and their reciprocal crosses has been studied in detail. In the F_1 generation, the frequency of abnormal cells was more when crosses were made between the two colour groups than those observed in the hybrids within any of the colour groups.

C.4.2. *Radiosensitivity in plant species :*

Attempts were made to correlate radiosensitivity with ICV. Three parameters viz., survival of plants at maturity, ultimate plant height and chromosomal abnormalities were taken into consideration. In *Beta vulgaris*, *Nigella sativa*, *Allium seepa*, *Impatiens balsamina* and *Vinca rosea* all the three parameters were found to be closely related to ICV.

In *I balsamina*, the frequency of cells with chromosomal abnormalities during root tip mitosis increased with increase in dosage In field experiment it was possible to correlate the decrease in seedling height and plant height at maturity with increase in chromosomal abnormalities.

C.4.3. *Cytological and Cytotaxonomical Studies in the Family Malvaceae*

During the year under report cytological and cytotaxonomical investigations were carried out in five varieties of *Abelmoschus esculentus* (L.) Moensch. This is commonly known as lady's finger or okra and is cultivated throughout India and in all tropical countries. The pods are used as pot herbs and sometimes for therapeutic purposes. The plants are mainly insect pollinated which may be one of the reasons that plants grown from seeds procured from reputed farms show hybrid characteristics and hence correct determination of $2n$ chromosome number straightway from such seeds becomes a problem.

$2n = 72$ chromosomes has been reported earlier (Ann. Rep. 1964-65) in this species. Also, polyploid numbers like 116 and 130 were observed. Earlier, Teshima (1941), Purewal and Randwa (1947), Joshi and Hardas (1953), Medvedeva (1936) reported the number in the $2n$ complement of this species as 72, 120, 130 and 132 respectively, while, Dutta and Naug (1968) observed in three strains namely, long fruited, short and broad fruited, and the third one as the intermediate between the two, the number as 120, 108 and 144 respectively. Accepting 9 as the basic number as suggested by Darlington and Wylie, the above mentioned numbers may be considered as polyploids.

With a view to establish interrelationships, if any, among the different varieties available in the market, five varieties based on their fruit characters mentioned below were chosen for the present and their chromosome morphology in $2n$ complement was studied. The fruit characters of the varieties are as follows :

1. Long, narrow and green.
2. Long, narrow and brown.
3. Medium-long, narrow and green.
4. Medium-long, broad and green.
5. Short, narrow and green (dwarf variety).

Somatic chromosome studies were made from root tip squash preparations following propiono-orcein schedule. As the chromosomes were small in length and high in number, some modifications were needed in both prefixation and fixation stages. Aesculin gave the best result as a prefixative.

Variety No. 1.

The $2n$ complement shows 120 very small chromosomes. Cells with 112 and 130 chromosomes have also been observed. The length of the chromosomes varies from 1·0μ

to 3·0μ and the primary constriction from median to submedian. The maximum number of chromosome pairs with secondary constrictions have been found to be six.

Variety No. 2.

This variety has been found to have 120 chromosomes in its 2n complement. But higher numbers upto 144 were also observed. Due to large variation and small size the number of chromosomes with secondary constrictions which for the present has been found to be six pairs needs confirmation after further investigations. The length of the chromosomes varied from 0·75μ to 2·50μ. The chromosomes have mainly median to submedian primary constriction.

Variety No. 3.

The somatic complement had 144 chromosomes but lower numbers like 120 and 130 were also observed. The chromosome length ranged from 0·50μ to 2·00μ approximately. Secondary constrictions were observed in six pairs while the location of primary constrictions varied from median to submedian.

Variety No. 4.

This variety has been found to have 72 chromosomes in its 2n complement but higher numbers upto 130 were also recorded in some cells. The chromosome length varies from 0·75μ to 2·50μ and the primary constrictions were from median to submedian. Four pairs of chromosomes with secondary constrictions were observed in complements with 72 chromosomes.

Variety No. 5.

108 chromosomes have been observed in its 2n complement. Also, variation in number like 72 and 120 has been recorded. Four pairs of secondary constricted chromosomes and the length of the chromosomes varying from 0·50μ to 2·50μ have been observed. Primary constructions ranged from median to submedian.

Detailed studies of the varieties to confirm the above findings and to establish interrelationships, if any, among them are in progress.

C.5. Tissue culture

C.5.1. *Pigment Producing Callus Cultures*

Effect of different growth substances-2,4-D, NAA, IAA and kinetin—on tissue growth and formation of green pigment in *Hibiscus esculentum* callus isolated previously was studied. 2,4-D at 0·5 mg/l was found to be the best auxin for growth as well as pigment formation in presence of coconut milk (15% v/v). The tissue could not be grown in synthetic media.

Along with this green pigmented mutant callus strain of *Hibicus*, a pink-red pigment producing strain was isolated from *Croton* callus tissue last year. Of the different auxins

tried, the best growth was obtained in complex medium containing 2,4-D (0·35 mg/l). Pigment formation was enhanced when the medium was fortified with ammonium nitrate (50 mg/l). Attempts were made to isolate and identify the pigments in both the cases.

In the case of *Hibiscus*, green callus tissues dried over sodium sulphate over night were extracted with a mixture of pet.ether : methanol : benzene (90 : 10 : 32). A control sample from fresh leaves of *Hibiscus* was made in the same way. The thin-layer chromatography plates were prepared with silica gel G. Solvent used was pet-ether : acetone (10 : 1). Two spots, yellow coloured and pale green coloured, were separated in both the samples. Spots were identified as carotene and chlorophyll respectively.

In case of *Croton* callus, pink-red callus tissues were extracted with methanolic solution of 0·1% HCl. A control sample was also prepared in the same way from *Croton* leaves. Silica gel G was used as the adsorbent for thin-layer chromatography plates. The chromatograms were developed with solvent mixture ethyl acetate : methyl ethyl ketone : formic acid : water (50 : 30 : 10 : 10). Only one faint red spot was detected in both the samples. With 1% KOH solution in ethanol the red spot turned blue. The pigment is most probably anthocyanin. Further work on pigment synthesis in both the tissues could not be carried out for want of spectrophotometer essential for quantitative measurement.

C.5.2. *Effect of Light On Growth And Pigment Formation In Callus Cultures*

For this purpose, normal and crown gall tissues of *Althaea rosea*, *Coreopsis lanceolata* and *Vicia faba*, normal tissues of *Nigella sativa*, *Mimosa pudica*, *Murraya koenigii* and *Dalhia tuberosa* and gall tissue of *Ixora indica* were used. All these tissues were isolated previously in Tissue Culture Laboratory at Bose Institute. Tissues were grown in their respective media solidified with agar. One set of tissues was grown in a photo-period of 8 hrs. light and 16 hrs. dark at 25°C. Light (250 f.c.) was provided by white fluorescent tube and one 40 watt incandescent lamp. In the control set tissues were grown in dark. Tissues were grown through ten successive passages of 30 days each. Beneficial effect of light was observed in *Althaea* and *Ixora* gall tissues. Gradual reduction in growth in successive passages was observed in light-grown coreopsis and vicia gall tissues. On the other hand *Nigella* and *Mimosa* tissues failed to grow in presence of light. In rest of the tissues there was no difference in growth under the two experimental conditions. Only in *Ixora* gall tissue a light yellowish green pigment was developed. Whether porphyrin was synthesised in light grown tissues could not be examined for want of spectrophotometer. Light-grown tissues were fragile in texture.

C.5.3. *Application of Tissue Culture Technique*

It is one of the aims of this laboratory to use plant tissue cultures for production of chemical compounds of importance and also to apply the tissue culture technique for determination of site of synthesis of such compounds.

Site of synthesis of carbazoles in *Murraya koenigii* was determined in this laboratory using callus tissues obtained from different parts of the plant. Even after five years of growth

in culture the stem callus tissue cells have retained the capacity to synthesise carbazoles. The unidentified spot which gave brilliant sky blue colour with antimony pentachloride-carbon tetrachloride spraying reagent was found only in actively dividing cells. This compound was isolated from a number of TLC plates and its spectrum showed one major peak at 290mμ and one minor peak at 320mμ.

The degradation products of Isatin are Anthranilic acid and catechol of which former is known to play an important role in carbazole synthesis. For this reason experiments were conducted to find out whether Isatin has auxin-like activity in callus tissue and to find out the effect of Isatin on carbazole synthesis. Different concentrations of Isatin in presence or absence of 2,4-D were tested using the method of successive subcultures of 35 days each. Isatin (4 mg/l) could replace, 2,4-D upto fourth passage only but the tissue growth was at reduced level. Even an increase in concentration of Isatin upto 25 mg/l. could not replace 2,4-D. When Isatin and 2,4-D were used together maximum growth was obtained and it was even higher than that in 2,4-D alone. The best combination was 5 mg/l Isatin and 0·05 mg/l 2,4-D. This suggested synergistic effect of the two substances. From thin-layer chromatographic analysis it was found that carbazole synthesis was increased in presence of Isatin. This indicated that the tissue cells could absorb and utilise Isatin. Experiments are in progress to detect Isatin and its break down products in callus cells.

C.5.4. *Differentiation in callus tissues of 3 species belonging to family umbelliferae*

Growth and organogenesis in callus tissues of *Cuminum cyminum* (Jira), *Trachyspermum amni* (Jowan) and *Foeniculum vulgare* (Mouri) in agar medium was studied. For this purpose, tissue response to different substances viz., 2,4-D, NAA, IAA, kinetin, coconut-milk, adenine, raised level of phosphate and tryosine at different concentrations and combinations was examined.

Jira : In presence of coconut-milk best callus growth was obtained with 0·25 mg/l NAA in White's medium. In all the substances tested, organogenesis was not observed in presence of coconut-milk. Jira callus also grew satisfactorily in synthesis medium supplemented with $N(\cdot5)+k(\cdot05)$. Callus showed nodular out growth but no root. When kinetin was omitted and the concentration of NAA was increased to 1·0 mg/l number of roots formed on callus. From the observation of different experiments it appeared that coconut milk or kinetin inhibited root formation. Other substances like adenine, raised level of phosphate, tyrosine etc. which were found to induce bud in many other tissues, could not initiate bud in any of the treatments.

Jowan : Maximum callus growth was obtained in Tobacco medium fortified with 15% (v/v) coconut milk and 0·5 mg/l NAA. In this medium, tissue was brown and compact but without any signs of differentiation. When Tobacco medium was replaced by that of White though callus growth was poor, there was vascularization with formation of nodular outgrowths. In subsequent passage roots were found to develop from nodular outgrowths. All these roots were positively geotropic which grew inside the agar. Rooting was also

observed when 2,4-D (2·0 mg/l) was used instead of NAA (0·5 mg/l). However, frequency of root formation was less. Bud formation was not observed even in presence of adenine, increased phosphate level and tyrosine alone or in different combinations.

Mouri : Best callus growth was sustained in White's medium supplemented with coconut milk and 0·5 mg/l 2,4-D. In this medium, root-less shoots developed during 2nd passage in 40% of callus but in none of the callus root was found to initiate. Several experiments were conducted to initiate roots in the callus bearing the shoot. In no case roots could be developed and for the lack of efficient transport system the shoots ultimately died out. When the callus grown on 2,4-D (0·5 mg/l) medium was transferred to NAA (0·5 mg/l) medium, there was abundant root formation. These roots were long, thin and some of them were positively geotropic while others were negatively geotropic. It was found that the capacity of 2,4-D grown callus to form roots on transferring to NAA containing medium was retained even after ten subcultures. In subsequent passages callus growth was gradually reduced. It was of interest to note that for obtaining roots again in NAA medium, it was essential to take explants from mother pieces grown in 2,4-D. Copious root formation was also observed with IAA (1·0 mg/l) in presence of coconut milk but callus growth was poor in all the concentrations tested.

Anatomical studies—

Anatomical observations revealed that callus of all the three tissues before showing differentiation were solely composed of parenchymatous cells with afew isolated lignified cells scattered throughout the callus mass. In Jira growth of the callus took place by localized meristematic areas distributed randomly throughout the callus mass in contrast to the continuous marginal meristematic activity manifested by Jowan and Mouri callus. The meristematic areas were identified by dense cytoplasm and prominent nuclei.

Anatomical sections of the callus where root development took place showed that the roots initiated not at the periphery but from the deeper inner region of the callus. At first, a group of meristematic cells was formed, which differentiated growing point of root. This gradually pushed out of the callus forming a root. Although the roots in a callus were developed independently, at later stages the initiating regions of roots appeared to be interconnected to each other by the development of vascular strands in the intervening parenchymatous tissue. This pattern of root development was observed in all the roots formed in *in-vitro* showed typical diarch structure. This structure in all the three tissues was similar to that of respective roots *in-vivo*.

C.5.5. *Cytological study of Nigella sativa tissue culture*

This study was undertaken to determine the stability of chromosome number throughout the culture regime of one year and also to find out whether there is any difference in the karyotype of diploid cells of *in-vivo* and in *in-vitro* culture of the *Nigella sativa* new tissue strain (Ni S-5) isolated in the year 1970. *Shift in ploidy level with age of culture* : The most common observation was the divergency in chromosomal complement in the cell population as reported earlier by different authors. In the present study initially 78 percent of

the cells were diploid and about 4 percent aneuploids with chromosome number ranging from 3 to about 96 per cent while the rest were either haploids or tetraploids (Tab.e-1). After about 12 months of culture the percentage of diploid cells decreased to 47 per cent, while the percentage of aneuploids increased. It was interesting to note here that with aging of the culture the frequency of eupolyploid cells decreased with the subsequent increase of aneuploid cells.

TABLE 1

Per cent distribution of different ploidy cells at three month interval

(total counts 250 cells in each determination)

Tissue age in months	*n*	2*n*	3*n*	4*n*	5*n*	6*n*	7*n*	Aneuploid cells (including hypo-haploid and higher than 7*n* cells)	Cells with cytological irregularities+
1	6.10	78.09	—	12.20	—	—	—	3.66	5.22
3	7.18	64.41	1.31	13.07	—	1.31	1.31	11.46	9.48
6	2.76	66.81	3.70	7.41	—	2.78	—	16.51	13.66
9	4.90	56.86	7.84	10.78	—	—	1.20	18.52	15.13
12	5.33	46.66	4.00	14.67	—	—	6.67	22.67	20.62

+ Total percentage of cells with unequal separation of chromosomes, lagging chromosomes and bridges at anaphase and micronuclei formation at telephase stage.

Polyploidy : Of the 15 to 20 per cent polyploid neuclei, neuclei were mostly at triploid and tetraploid level with 18 and 24 chromosomes, respectively, though the cells with higher number of chromosomes were not infrequent. The occurrence of polyploidy may be due to pre-existing polyploid nuclei in the initial explant or could have occurred due to endomitosis or by nuclear fusion in a multinucleate cell and also may be due to persistant chromosome bridges through telophase.

Aneuploidy : In addition to the cells falling in the groups of euploidy there was about 10–20% cells with variable chromosome number. In the tissue culture of this strain aneuploidy was regularly occurred, but in *in-vivo* condition these were of very rare occurrence. Possibly this may be due to the absence of suitable mileu and opportunity for cell division in *in-vivo* system. These aneuploid cells possibly may have arisen due to mitotic irregularities during cell division.

Haploidy and Hypohaploidy : In *Nigella sativa* tissue culture percentage of hypohaploid and haploid cells varied between 3 to 6 and 5 to 10 respectively. It is interesting to note here that in all the haploids, all the characteristic six chromosome of the genome of this species was not respresented. The longest medianly constricted chromosome with secondary constriction was missing in about 30 per cent of the total haploid cells counted. Haploids and hypohaploids are generally considered as non-viable, but their presence at metaphase and anaphase stages in the present study indicate that these cells remain viable at least for a few cell divisions. Again haploidy and hypo-haploidy generally considered

to be of rare occurrence in tissue culture by different workers, but their regular occurence in considerable percentage in the present study indicate that these may not be so rare.

Karyotype study : The comparative karyotype study of diploid *Nigella sativa* tissue culture cells and root tip cells showed that there was no significant difference between the length and shape of corresponding chromosomes and also between the homologous chromosome pairs within a material. The analysis also showed that there was no significant difference between the lengths of corresponding chromosomes of the root-tip and tissue culture cells studied. This study then clearly showed that the diploid karyotype of *in-vivo* cells is also maintained in tissue culture cells.

Chromosomal abnormalities : Chromosomal abnormalities such as lagging chromosomes, bridges at anaphase and micronuclei formation during telophase stage occurred regularly and their frequency varied between 2·5 to 10 per cent and 2·8 to 11 per cent respectively. In about 2 to 4 per cent cells double the usual number of chromatids were observed at early metaphse stage, suggesting the occurrence of endomitosis in Nigella tissue culture cells.

C.6. Plant Biochemistry

C.6.1. *Studies on Phytin and Phosphate metabolism in germinating Mung Beans.*

That phytin phosphorus plays an important role during ripening and germination of mung bean seeds has already been shown.

C.6.1.1. *Characterization of phosphatase associated with phytase from cotyledons*

At least twelve phosphatases have been detected in the soluble fraction of the mung bean cotyledon by polyacrylamide gel electrophoresis among which three are acidic in nature. Among the three acid phosphatases, one has been found to be associated with phytase. Activity of this phosphatase was measured at different stages of germination both in cotyledon and embryo portion of the seedling. In the cotyledon, the enzyme activity is found to be accelerated with increasing period of soaking upto 48 hr. after which the activity declines, but in case of embryo, the activity increases gradually from 12 hr and attains maximum value at 72 hr. Acid phosphatase from cotyledon (12–16 hr soaked seed) mentioned above has been isolated, separated from phytase and purified approximately 85 fold with a recovery of 22·6%. The ratio of phosphatase and pyrophosphatase activity of the enzyme at different steps of purification was tested. The ratio of the two activities vary from 14·0 to 0·85 throughout the purification steps thus suggesting a possibility of the presence of pyrophosphatase as a separate entity. Phosphatase is stable at 0°C, has a *p*H optimum at 6·0 when tested with different substrates and has optimal temperature at 55°C, whereas maximum pyrophosphatase activity was shown at *p*H 5·5. The release of phosphorus increases in a linear fashion with the increase of the enzyme concentration, and also with the increase of time upto 60 min. Divalent cations are not absolutely required for the

phytase activity; however, the phosphatase activity has been found to be inhibited completely by Cu^{2+} (2 μmoles/ml) and partially by Zn^{2+} ion (43%). Monovalent cations and tartarate have no effect, either inhibitory or stimulatory on the phosphatase activity. 100/ inhibition of phosphatase activity and 55% inhibition of pyrophosphatase activity are apparent when 5 μmole EDTA is present in the incubation mixture; the activity thus lost, however, could be recovered by 100 μmoles/ml Mg^{2+} in case of phosphatase. The effect of thiol reagents on the activity of phosphatase was tested. Phosphatase is inhibited by pCMB (47% at 5 μmole/ml) and F^- ion (19%). Slight stimulation is observed with reduced glutathione. Phosphatase activity can be protected from heat inactivation both by Na-β-glycerophosphate and G-6-P. P_i can also protect the activity to the same extent. Phosphatase and associated pyrophosphatase are found to be nonspecific. p-nitrophenyl phosphate and β-glycerophosphate are found to be the best substrates for phosphatase. The affinity towards G-1-P is 1/3rd of that of G-6-P and potassium pyrophosphate has been found to be most susceptible to the pyrophosphatase. RNA, DNA, casein and 3′–5′ cyclic AMP are not hydrolyzed at all. P_i has been found to inhibit both the phosphatase and pyrophosphatase activity. The phosphatase activity is more susceptible to inhibition by P_i and completely inhibited at 2 μmoles P_i/ml when pyrophosphatase activity is only partially inhibited. Inhibition of phosphatase and pyrophosphatase activity have been found to be competitive in nature and the K_i values for P_i with different substrates have been calculated to be in the range of $0{\cdot}3$–$0{\cdot}5\times10^{-3}M$ where K values with different substrates are recorded to be between $1{\cdot}1-3{\cdot}9\times10^{-3}M$. Phosphatase preparation when subjected to polyacrylamide gel electrophoresis (5% gel) has been resolved into two bands, one major having phosphatase activity, and the other minor having pyrophosphatase activity as revealed by the assay of the gel slices. When polyacrylamide gel electrophoresis (10% gel) was carried out both with phytase and phosphatase preparation the latter gives only one band in the same posit'on as was found with phosphatase associated with phytas indicating that those two phosphatases are identical. This phosphatase preparation recorded two bands, one for phosphatase and the other for pyrophosphatase when the electrophoresis was done with 5% gel.

C.6.1.2. *Characterization of Inositol hexaphosphate-GDP-phosphotransferase from cotyledons of Mungbeans*

Inositol hexaphosphate-GDP phosphotransferase activity was measured both in the cotyledon and the embryo at different hours of germination. It has been found that the activi y is maximum in the ungerminated seeds and this level gradually declines with the advent of time of soaking, whereas in embryo the phosphotransferase activity is increasing gradually from 24 hr onward. The enzyme, inositol hexaphosphate-GDP phosphotransferase has been isolated from cotyledons of 36 hr soaked seeds, purified and characterized. Purification achieved is 86 fold with a recovery of 33%. pH optimum of this enzyme has been recorded at 7·0. The act'vity has been found to increase in a linear fashion with time upto 20 min.; the reaction is also directly proportional to the enzyme concentration. The enzyme requires divalent cations such as Mg^{2+} or Mn^{2+} for activity; when Mg^{2+} is replaced by Mn^{2+}

the activity is almost doubled. The purified preparation of the enzyme shows a marked specificity towards GDP; neither dGDP, GMP nor ADP, UDP, CDP could replace GDP. High GTP concentration inhibits the enzyme activity. ATP cannot influence the reaction A stimulation observed with EDTA at a concentration of 4 μmoles/ml but when the concentration of EDTA is increased further (8 μmoles) the phosphotransferase activity has been found to be inhibited (46% approximately). 3′, 5′ cyclic AMP inhibits the phosphotransferase activity to the extent of 50%. The inhibition by 3′, 5′ cyclic AMP is competitive in nature and the K_i value for 3′, 5′ AMP has been calculated to be $0{\cdot}55\times10^{-4}M$. The enzyme phosphotransferase can catalyze the transfer of phosphate from GTP to IP_5 to a very limited extent. ATP is found to be inactive as a donor. Phosphotransferase has got very little affinity towards IP_3, though besides IP_6, IP_5 and IP_4 are also reacted by the enzyme and the affinity towards the latter two compounds is also half that of IP_6. IP polyphosphate and P_i cannot act as donors. K_m values for GDP has been calculated to be $1{\cdot}09\times10^{-4}M$ at 37°C and that for IP_6 is $0{\cdot}155\times10^{-5}M$ at 37°C. Mechanism of phosphate transfer from inositol hexaphosphate to GDP has been elucidated. That transefer of phosphoryl group to GDP is mediated through phosphoprotein has been documented. Both the crude and purified preparaton of inositol hexaphosphate-GDP phosphotransferase have been subjected to polyacrylamide gel electrophoresis. The bands obtained from the crude preparation was assayed for inositol kinase, phosphoinositol kinase and phytate-GDP phosphotransferase, and these three activities have been located at 3 different bands. The position of band for phosphotransferase obtained from the purified enzyme preparation has been found to be identical with that from the crude preparation. More than one bands were obtained with purified enzyme preparation indicating that the enzyme is not homogeneous, but the other bands do not coincide with the positions of inositol kinase and phosphoinositol kinase suggesting that in the purified preparation the latter two enzymes are absent. This has been confirmed by assaying the purified phosphotransferase for the latter two activities as well.

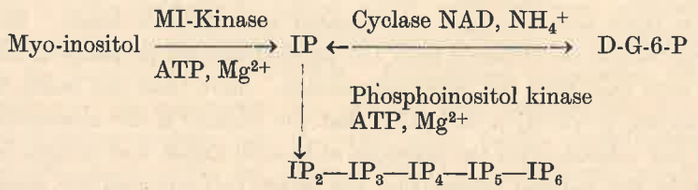
C.6.1.3. *Phosphoinositol kinase and its regulation during ripening and germination of Mungbean seeds*

While working with the ripening mungbean seeds for tracing out the pattern of phosphoinositol kinase activity, a steady decline after a certain stage was noticed. This lowering of the phosphoinositol kinase activity was found to be due to the accumulation of an inhibitor which was maximum in the ungerminated seeds. The inhibition was not due to any phytase, ATPase or protease activity. Preliminary observation and further confirmation thereafter suggested a protein-nature of the inhibitor. Attempts were then made to purify the inhibitor from the ungerminated seeds. The crude proteins after 0–40/ ammonium sulphate saturation was subjected to back ammonium sulphate fractionation. The soluble fraction in 20% saturated ammonium sulphate was further purified on a DEAE cellulose column using NaCl salt gradient. The phosphoinositol kinase and the inhibitor were resolved in two different protein peaks. The inhibitor thus obtained, when subjected to polyacrylamide gel electrophoresis yielded a single protein band. These observations suggest that

the failure in detecting phosphoinos'tol kinase activity in the ungerminated seeds was not due to its actual absence; on the other hand, it is a cumulative result of the enzyme being present together with its inhibitor, thus conferring a definite role to the inhibitor in regulating the biosynthesis of inositol phosphates.

C.6.1.4. *Characterization of Phosphoinositol kinase from cotyledons of Mungbeans*

Phosphoinositol kinase (Adenosine triphosphate-inositol-monophosphate phosphotransferase) has been isolated from cotyledons; about 300 fold purification has been achieved, with a recovery of 11%. The enzyme has a *p*H optimum at 7·4. It can mediate phosphorylation of lower inositol phosphates to their corresponding higher homologoues, ATP being the phosphate donor. ATP cannot be replaced by other phosphocompounds. However, UTP and PEP are partially effective. The enzyme requires divalent cations for the reaction. Mn^{2+} has been found to be twice as effective as Mg^{2+}, Ca^{2+} being inhibitory. Phosphoinositol kinase has been found to be different from inositol kinase. This is also consistant with the fact that another enzyme myo-inositol kinase can synthesize myo-inositol monophosphate (IP). Furthermore, IP is also obtained as an intermediate during the biosynthesis of myo-inositol from D-glucose-6-phosphate. All these evidences taken together we formulate the following pathway for the biosynthesis of phytic acid in plants.



C.6.2. *Factors that regulate RNA polymerases isolated from chromatin of coconut nuclei*

Three RNA polymerases (I, II, III) and several protein fractions (A, B and C) have been isolated from the chromosomal nonhistone proteins of coconut endosperm nuclei. The method involves disruption of nuclei in low salt medium, centrifugation to sediment the chromatin, solubilization of chromatin in high salt, dialysis of soluble chromatin to low salt to precipitate DNA-histone complex and centrifugation to get the nonhistone protein in the supernatant. Further purification involves ammonium sulphate fractionation and chromatography on DEAE-cellulose and QAE-sephadex. RNA polymerase I purified through QAE-sephadex step appears to be homogeneous and gives a single band on polyacrylamide gel electrophoresis. In gels containing SDS, this enzyme shows multiple bands indicating its subunit nature. Optimum *p*H for both the RNA polymerases is recorded at 8·0. Both the RNA polymerases require either Mg^{2+} or Mn^{2+} for activity. However, RNA polymerase I is maximally activated by Mn^{2+} (2 mM) while polymerase II by Mg^{2+} (10 mM). The activities of both the RNA polymerases are stimulated several fold by the protein fraction B. The stimulation is observed only when native coconut DNA is used as a template, but not with denatured coconut or native λDNA. In the absence of factor B, RNA polmerase show only minimal activity. On electrophoresis separately in polyacrylamide gels,

the stimulatory factor B moves much faster than RNA polymerase I. However, when factor B is run along with polymerase I, the two move together indicating that factor B binds with the enzyme forming a complex. From sucrose density gradient centrifugation experiments, it has been found that RNA polymerase I can bind to DNA, but factor B alone can not. However, RNA polymerase I factor B complex can bind to DNA. Factor C promotes the incorporation of β, γ-^{32}P-ATP into RNA and this incorporation has a plateau rather quickly while the incorporation of ^{14}C-ATP into the interior regions of RNA chains still continues linearly. The activity of RNA polymerase I is inhibited by rifampicin which is known to act at the initiation step. The inhibition is absent if factor B is added to the enzyme first, before the addition of rifampicin. The inhibitory effect of the drug can be completely reversed by high concentration of factor B, indicating that rifampicin probably binds at the same site of RNA polymerase I, where factor B also can bind. Polyacrylamide electrophoresis of radioactive RNA product have revealed that RNA molecules synthesized in presence of factor B are of larger size (10S–20S) than those synthesized in its absence (about 4S). All these evidences taken together show that B acts as the initiation factor in RNA synthesis. Fraction C, when aded at zero time of incubation, inhibits RNA synthesis by RNA polymerase I factor B combination. This inhibition is less and less as the time of addition of factor C to the incubation mixture is delayed. If the addition of factor C is sufficiently delayed, a stimulation of RNA synthesis is obtained instead. The inhibitory activity has been found to be associated with another component present in factor C. When factor C is added in a system where RNA synthesis has reached a plateau in the presence of limiting amount of template DNA, fresh synthesis of RNA begins. This reinitiation is analogous to that obtained at high ionic strength, the effect of which is known to be the release of newly synthesized RNA chains from the template. These evidences suggest that factor C may act as the termination factor. Factor C when added on top of factor B does not influence the size of the synthesized RNA molecules. Both factor B and C are free from any nuclease activities. The significance of the finding of RNA polymerases and its factors in the chromatin complex along with the template DNA in higher cells has been interpreted in line with the fact that the structure of chromatin itself during different stages of growth may regulate RNA synthesis in eukaryotic cells.

C.6.3. *Action of RNA polymerases as affected by Indole acetic acid*

That the Indole acetic acid (IAA) can influence nucleic acid synthesis in plant cells has been reported from a number of laboratories. However, the actual mechanism of IAA on RNA synthesis by isolated RNA polymerase system is yet to be elucidated. Recently from our laboratory RNA polymerase and factors for initiation and termination from the chromatin of coconut nuclei have been isolated and purified. This RNA polymerase requires the initiation factor (factor B) for proper transcription. IAA added to this system does not influence the reaction. Another protein fraction was isolated from nuclei and purified to homogeneity. This protein fraction can bind IAA thus forming a complex. When this acceptor protein and IAA were added to RNA polymerase system two to three fold stimulation of RNA synthesis has been recorded. There might be three possibilities as to the

mechanism of action of IAA on the RNA polymerase system. (1) Hormone acceptor protein modifies the initiation by interacting with the factor B. (2) It may interact with RNA polymerase or (3) with DNA so that the factor B can now initiate RNA synthesis from that portion of DNA which otherwise could not be transcribed. It has been shown that IAA acceptor protein cannot interact with RNA polymerase or factor B *per se*. However, when the binding of hormone acceptor protein with DNA was studied by passing the complex through membrane filter it has been noted that DNA can intereact with IAA protein complex in absence of RNA polymerase and factor B. That the RNA synthesized in presence of IAA acceptor protein complex is different from that of the control has been shown by hybridization of RNA with DNA as well as by electrophoretic separation of RNA species. All these evidences taken together suggest that the action of IAA is mediated through an acceptor protein. IAA and acceptor protein form a complex with DNA at some specific site. The initiation factor and RNA polymerase interact with these sites and form initiation complexes. RNA synthesis seems not to occur at these sites in absence of IAA acceptor protein.

C.7 Palynology

C.7.1. *Aeropalynological Survey of Calcutta and Falta* (24 *Parganas*)

Aeropalynological investigations upto the month of May, 1971 in Bose Institute and Falta show that there has been a considerable fall in the incidence of pollen grains during rainy season. The two years record of air borne pollen of the Bose Institute and $1\frac{1}{2}$ years record of Falta clearly indicate that the floating of pollen grains in air is affected by atmospheric condition.

There has been a considerable increase in the percentage of air borne pollen grain of *Mimusops* (Sapotaceae) in the month of April in Calcutta and also in Falta. *Antidesma* type Euphorbiaceae) pollen grains reached almost 48% of the total pollen during December, 1970–January, 1971. *Caloplayllum* (Guttiferae) pollen grains were abundantly present in February–April. Compositae (mostly cultivated variety) pollen grains and also typical tricolpate, reticulate, spheroidal pollen grains of Cruciferae were among the commonest types in winter season. A sudden rise in the percentage of cultivated grass pollen was quite considerable from late September to October in both Colcutta and Falta. Mention may be made of presence of *Casuarina* pollen grains (oblate, triporate with rugulate surface pattern) during February, 1971.

Instead of June-July, as it has already been recorded in the year 1969, the *Pinus* pollen grains were trapped in March.

Urticaceae, Chenopodiaceae and Amarantaceae pollen grains were recorded throughout the experimental period, their percentages however increased or decreased with time. Among the monocots besides Graminae and Cyperaceae, *Cocos* pollen grains have been recorded all through the year.

The work is still in progress.

C.7.2. *Pollen morphological studies in relation to plant taxonomy*

a) *Phytolaccaceae*

The family Phytolaccaceae display an eurypalynous nature in the 28 species from 12 genera those which have so far been pollen morphologically studied. A family of total 17 genera and 125 species (Lawrence 1965) Phytolaccacea is one of the centrospermous famlies to show various types of pollen grains. The pollen of *Phytolacca* are mostly tricolpate, punctitegillate and the thin tectum in turn is variously ornamented with proeesses. Presence of fine spinules on the surface of the pollen grains is a distinguishing character of some of the species. A noticeable feature is the presence of occurrence of both prolate and oblate pollen grains in the same species. Besides the tricolpate, tricolporoidate pollen type, as well as pollen grains where each colpus is associated with more than one ora (e.g. *Gallesia*) have been noticed. *Microtea* with its two species exhibit two different apertural conditions. *Microtea debilis* pollen are distinctly pantoporate seemingly allied to chenopodiaceous and amarantaceous pollen and *M. gracilis* pollen are tricolpate (phytolaccaceous). Presence of dodecacolpate pollen has been recorded in the genus *Hilleria*, *Petiveria* and *Rivina* (one species from each genus studied).

Further work is in progress.

b) *Rubiaceae*

The family Rubiaceae consist of herbaceous and woody plants and considered as an interesting family with regard to pollen morphology. Among the 20 species from 15 genera so far palynologically investigated dimorphic types have been encountered in *Hamelia* and heteromorphic in *Ixora*. The two species of *Hedyotis*, *H. scandens* and *H. auricularia*, show specific variation so far as their surface pattern is concerned. *Gardenia* is characterised by having pollen grains united in tetrads. However, it has been possible to make further distinction among the rest of the monad pollen types on the basis of their shape, size, apertural characteristic and surface pattern. The equatorial diameter varies from 11·6μ in *Mussaenda* to 56·4μ in *Morinda*. The shape varies from peroblate to prolate grains with all the transitory stages. Pollen grains of some genera possess simple apertures while others possess composite ones. The number of apertures vary from three to as many as nine.

Anotis calycina not only possesses 9–20 zocolpate peroblate grains but also a very characteristic exine which isolates it from the other types.

c) *Geraniaceae and allied taxa*

In course of the palynological investigation of the family Geraniaceae only seven species have been described. The pollen slides were prepared either from herbarium or from fresh material. The seven species are distributed under the following seven genera ;— *Tropaeolum*, *Geranium*, *Impatiens*, *Connaropsis*, *Pelargonium*, *Biophytum* and *Averrhoa*. Grains show shapes like oblate (*Tropaeolum*, *Impatiens*), spheroidal (*Geranium*), oblate-spheroidal to spheroidal (*Pelargonium*) and prolate (*Biophytum*, *Averrhoa*). Except the genus *Impatiens*,

whose grains are 4-colpate, grains of other genera are 3-colpate where colpa may be brevicolpate. The sexine may be gemmate, punctitegillate, reticulate; reticulum may be supratectal (*Impatiens*), homobrochate or heterobrochate.

d) *Verbenaceae and allied taxa*

Pollen morphology of 246 species from 109 genera in 9 families has been taken into consideration in order to evaluate the evolutionary trends in the sympetalous tubifloreae groups, with a special emphasis on the family Verbenaceae.

Pollen morphologically apertural forms have been taken as primary, surface ornamentation as secondary and other characteristics like size, shape, exine thickness, etc., as tertiary characters while establishing phylogeny. Again, eurypalyny is considered more primitive than stenopalyny. So far as the exine ornamentation is concerned, it is considered that those forms which serve best protection to germplasm are primitive and thus, echinate or spinuliferous groups are regarded as primitive than without excrescences but with depressions like puncta, scrobiculi, reticulum, etc.

The family Verbenaceae is distinctly eurypalynous, as it shows various apertural forms, surface pattern, size, shape etc. The family Labiatae, Selaginaceae, Globulariceae are stenopalynous in nature and thus more advanced then Verbenaceae. This has been proved by gross morphological characters also, as the stenopalynous families are in general herbaceous in habit while verbanaceous members are either herbs or shrubs or trees. The habit characters has led Hutchinson (1964, 1969) to establish two phyla among dicotyledons, the primitive herbaceae and the advanced lignosae.

There has been a considerable increase in the number of genera in the Verbenaceae. Bentham & Hooker (1876) recorded 59 genera, Engler (1895) made it 67 while Moldenke (1944) established 90 genera for the family. The increase in generic number is the result of morphological differences which warrant them to create new genera. Moldenke & Moldenke (1946, 1970), in this respect is well advanced in creating new families like Stilbaceae, Symphoremaceae, Avicenniaceae which were once considered as verbenaceous members. The segregation of *Lippia* into *Aloysia*, *Acantholippia*, *Phyla*, *Bertolonia* and *Lippia*, *Bouchea* into *Chascanum*, *Svensonia* and *Bouchea*, *Verbena* into *Junellia* and *Verbena* finds good pollen morphological support. When the status of the families are concerned, pollen characters advocate the segregation of Avicenniaceae, Symphoremaceae but it fails to provide much information as to warrant Stilbaceae to be a family.

The inclusion of *Phryma* in Verbenaceae by Bentham & Hooker (*l.c.*) do not find any pollen morphological support, as the aperture form displayed by *Phryma* is totally absent in the Verbenaceae. Thus, it helps Engler (*l.c.*) for establishing unigeneric monotypic Phrymaceae.

So far as the phylogenetic trends are concerned, it may be assumed that the 3-colpate pertectate and 3-colpate spinuliferous-punctetegillate verbenaceous members have evolved from the Boraginaceae-Ehretioideae members like *Rhabdia* and *Patagonula-Ethretia laevis*

stock respectively. During the course of evolution, this has further given rise to scrobiculate, rugulate, reticulate group alone in one line while the other made further progress to develope 3-colporate type of apertures with lalongate ora which give rise to lolongate ora, a type more advanced then lalongate ora. The genus *Avicennia* is an unusual member among Verbenaceae by having 3-colporate aperture, lolongate ora, reticulate sexine, etc. These combined characters are entirely absent in Verbenaceae and may be taken as the precursor of the family Phrymaceae which ultimately ends in Myoporaceae. The Phrymaceae and the Myoporaceae both display 3-colpo-diorate apertures, which in evolutionary sequence is advanced over 3-colporate aperture with lolongate ora. The link between *Avicennia* and *Phryma* has been evidenced from the occasional presence of *Avicennia* type of apertures in *Phyma* while the secondary character is common in them.

Gross morphologically, there is hardly any character to distinguish Verbenaceae from Labiatae except perhaps style form, which is terminal in the former and gynobasic in the latter. But the *Ajuga*-group of Labiatae display terminal style. Therefore, when similarity of structure was taken as a sure indication of genetic relationship, the problem promised an approximate evolution of Labiatae from Verbenaceae. This apparent genetic relationship is reflected in pollen characters in the monotypic *Teucridium parvifolium* of Verbenaceae and *Teucrium* of Labiatae (*Ajuga*-group). They display every similar pollen characters like colpi with operculum, pertectate sexine with excrescences in tectum, thicker polar exine, amb type, size, shape, etc.

That the Labiatae has emerged from Verbenaceae is further evidenced from the fact that the species of verbenaceous *Gmelina* have got characters, which are common to those members of Labiatae having supratectal reticulations, a feature dominant among 3-colpate Labiatae (e.g., *Galeopsis*, *Leucosceptrum*, *Anisomeles*, *Dysophilla*, *Pogostemon*, *Colquhounia*, *Ballota*, etc.) but occur in only *Gmelina* of Verbenaceae.

While pollen morphology rejects the view of Engler (*l.c.*) in keeping Selagineae as a mere tribe of Scrophulariaceae and support Lindley (1836), Wernham (1911), Bentham & Hooker (*l.c.*) and Moldenke (*l.c.*) in establishing Selaginaceae, it however, fails to provide as much data as to amalgamate Selaginaceae with Globulariaceae into Selaginaceae, as was done by Bentham & Hooker and Wernham.

The oleaceous member *Nyctanthes* has suffered a great taxonomic rearrangement. Airy-Shaw (1952), Stant (1952), Melchior (1964), etc. included it in Verbenaceae while others have either treated it as old oleaceous member or segregated to make Nyctanthaceae. Information from pollen morphology, however, are in accord with old treatment by Engler (*l.c.*) in keeping it close to the genus *Jasminum* of Oleaceae.

Embryological evidence has proved that unigeneric Callitrichaceae finds its closest ally with either Verbenaceae or Labiatae. The gross morphological characters have placed it into the members of the order Geranialea, near Euphorbiaceae. The inaperturate pollen grains with reticulate sexinal pattern of *Callitriche* has put it to make proper position near the crotonoid-group of Euphorbiaceae.

e) *Boraginaceae and allied taxa*

Pollen morphological studies of Boraginaceae and its allied taxa (Myoporaceae, Hydrophyllaceae) have been undertaken to trace an affinity and evolutionary trend within the group.

Study of about 200 species under 72 genera of Boraginaceae reveal some interesting and divergent pollen morphotypes. There are about 21 different types of pollen grains as regards apertural condition, *viz.*, 2-colpate, 3-colpate, 3-colpate with 3-pseudocolpa, 4-colpate, 6-colpate, 2-colporate with 2-pseudocolpa, 3-colporate, 3-colporate with 3-pseudocolpa, 3-colporoidate, 3-colporoidate with 3-pseudocolpa, 3-colporate with 3-colpoid streaks, 4-colporate, 4-colporate with 4-pseudocolpa, 5-colporate, 6-colporate, 7-colporate, 8-colporate, 10-colporate, 3-porate, 10-12-porate. anomotreme (in this type there are three ora alternating with 3-colpa), 3-pororate and 4-pororate types. Among these types 2-colpate condition is regarded to be the most primitive type and found in *Sericostoma* and *Hormuzakia*, etc. Among the members of the same type varied types of ornamentations are met with, ranging from obscure to reticulate.

According to Engler and Prantl the family Boraginaceae is divided into 4 subfamilies, within these subfamilies varied types of apertural conditions and ornamentations are noticed.

Cordioideae—3 genera studied

General pollen morphotypes are spheroidal to prolate 3-colpate (*Cordia macleodii, C. myra, C. sebestena*), prolate grains 3-colporoidate to colporate (*Patagonula americana*). Spheroidal 3-colporoidate (*Auxemma*). Punctitegillate (spheroidal grains of *Patagonula americana*). Spinuliferous and punctitegillate (*Cordia macleodii*), punctitegillate reticulate (*C. sebestena*) to reticulate grains (*Auxemma*).

Ehretioideae—7 genera studied—2-colporoidate with 2-pseudocolpa and 3-colporoidate with 3-pseudocolpa (*Ehretia cymosa*), 3-colpate (*Saccellium, Beureria*), 3-colporate (*Coldenia procumbens, Ehretia cymosa*), 3-colporoidate (*Coldenia, Rhabdia, Saccellium* and *Halgania lavandulacea*), punctitegillate spinuliferous (*Scaccellium, Ehretia*), spinuliferous (*Halgania cyanea*), scrobiculate (*H. lavandulacea*), finely reticulate (*Ehretia acuminata, E. silvatica*).

Heliotropioideae—2 genera investigated. They exhibit prolate to prolate-spheroidal condition with 3-colporate with 3-pseudocolpa alternating (except with 3-colporate condition in *Heliotropium indicum* which indicates a primitive nature in this respect). Both colpa and pseudocolpa perforating the sexine as well the nexine layers.

Subfamily Borraginoideae—Cynoglosseae—12 genera studied

This tribe is characterised by dumbbell-shaped grains with either 3-colporate or 3-colpora alternating with 3-pseudocolpate condition and with obscure ornamentation (e.g., *Suchtelenia acan'hocarpa, Pectocarya platycarpa, Omphalodes, Thyrocarpus, Lindelofia, Myosotidium, Paracaryum*). Prolate 3-colporate, striate punctitegillate (*Solenanthus*)

spheroidal-oblate grains with 3-colporoidate condition (*Caccinia*), 3-colporate (*Trichodesma*), 4-colporate dumbbell-shaped (*Rindera*).

Eritrichieae, 10 genera investigated. Like the grains of the tribe Cynoglosseae the grains of this tribe are dumbbell-shaped (except *Amsinckia* and *Gastrocotyle*). Either 3-colporate (*Eritrichium, nanum, E. strictum, Allocarya chorissiana, A. stricta, Plagiobothrys nothofulvus, P. kunthii, P. linifolius, P. canescens, P. campestris, P. pringleri, Oreocarya* sp. *Cryptantha, Microula*) or 3-colporate with 3-pseudocolpa (*Asperugo, Amsinckia* some *Cryptantha* sp., *Plagiobothrys scouleri, P. kingii, P. hispidus, P. arizonicus, P. californicus*), prolate grains with 3-colporate condition alternating with 3-colporoid streaks are obscure (*P. tenellus, P. torreyi, P. uncinatus*). Prolate, 8-colporate (*Gastrocotyle hispida*) *Amsinckia* grains are just like Heliotropioideae grains.

Tribe Anchuseae—8 genera studied. Shows an extreme line of evolution from 3-colporate to 7–10 colporate grains with obscure ornamentation to spinuliferous punctitegillate to scrobiculate condition.

In general the grains are prolate to subspheroidal (*Borrago officinalis*), anisopolar (*Alkanna*), 3-colporate (*Lycopsis arvensis, Alkanna, Trachystemon orientalis*), 4-colporate (*Anchusa, Nonnea*), 4–5-colporate (*Pulmonaria, Anchusa officinalis*), 7–10 colporate (*Symphytum* and *Borrago*), psilate to obscure (*Symphytum, Alkanna*), punctitegillate (*Anchusa strigosa, A. azurea, Nonnea, Pulmonaria*), spinuliferous, punctitegillate (*Borrago officinalis, Anchusa hybrida, A. capensis, A. officinalis, Lycopsis arvensis, Alkanna strigosa* and *A. tinctoria*. Some *Symphytum* sp. have spinuliferous, ornamentation, grains of *Nonnea* and *Pulmonaria* exhibit scrobiculate ornamentation near the aperture. Grains of *H. indicum* resemble the grains of the tribe in brevicolpate nature and aspidote condition in polar view.

Tribe Lithospermeae—12 species studied. Grains of this tribe are very interesting and show diverse type of pollen grains not only in apertural condition ranging from 2-colpate to 10–12 porate but also in shape.

Grains square in equatorial view oblate in polar view, 2-colpate grains with spinuliferous ornamentation (*Sericostoma*). Prolate grains are found in *Moltkia, Cerinthe minor, Lithospermum* and *Myosotis*, dumbbell-shaped in *Mertensia, Trigonotis, Lithospermum, Macrotomia, Arnebia* and *Cerinthe major*), anisopolar in *Onosma* and *Macromeria*, subisopolar in *Onosmodium*. Grains 4–6-colpate (*Arnebia*), 3-colporate (*Myosotis, Moltkia collosa, Onosma*), 4-colporate (*Macrotomia, Lithospermum*), 6-colporate (*Onosmodium, Macromeria, Lithospermum*), 7-colporate (*Moltkia petraea*), 8-colporate (*Lithospermum* and *Cerinthe minor*), 10–12 porate (*Cerinthe major*). Obscure ornamentation (*Myosotis, Mertensia, Trigonotis, Macromeria*), punctitegillate (*Onosmodium*), spinuliferous and punctitegillate (*Lithospermum, Macrotomia, Arebia, Onosma*), spinuliferous (*Cerinthe, Moltkia, Sericostoma*).

Borraginoideae—Tribe Echieae

Four genera studied. Grains subprolate to prolate (*Halacsya, Echiochilon*), anisopolar (triangular in equatorial view), 3-colporate (*Lobostemon* and *Echium*), 6–8 colporate

(*Echiochilon*), 3–4 pororate (*Halacsya*), punctitegillate (*Lobostemon*, *Echium*), striate punctitegillate (*Halacsya*), scrobiculate to reticulate (*Echiochilon*).

Borraginoideae—Tribe Harpagonellea, 2-genera studied.

This tribe is characterised by dumbell-shaped 3-colporate grains with obscure ornamentation (*Harpagonella* and *Rochelia*).

Borraginoideae—Zoellerieae (Single genus studied)

Zoelleria = *Trigonotis*

Grains dumbbell-shaped, 3-colporate with 3-pseudocolpa, psilate.

Wellstedia, a problematic genus having grains subspheroidal, spheroidal to slightly oblate, 3-colporate, finely reticulate.

Myoporaceae and Hydrophyllaceae are taken as allied taxa of Boraginaceae and a few species of both of the families investigated to show relationship with the family Boraginaceae.

Five species under 3 genera of Myoporaceae investigated. They exhibit 3 main types of apertural condition. 3-colpate (1st type of *Eremophila longifolia*). 3-colporate grains, each colpus diorate (*Eremophila rotundifolia*, *E. alternifolia*, 2nd type grains (*E. longifolia*, *Myoporum montanum*), 4-colporate grains observed in heteromorphic species (*Oftia africana*) punctitegillate ornamentation (*Eremophila rotundilfolia*, *E. alternifolia*) finely reticulate to reticulate (*E. longifolia*, *Myoporum montanum* and *Oftia africana*). Like Boraginaceae it also shows heteromorphism (e.g., *E. longifolia* and *Oftia africana* respectively).

Hydrophyllaceae. About 18 species under 11 genera have been studied.

The pollen morphological studies of these species shows that like Boraginaceae, in Hydrophyllaceae dimorphism is not an uncommon feature together with normal monomorphic forms.

There are 4 different pollen morphotypes as regard apertural condition, 2-colpate type (*Hydrophyllum virginianum*), 3-colpate (*H. virginianum*, 3-colpate with 3-pseudocolpa (*H. appendiculatum*), 3-colporoidate (*Eriodictyon trichocalyx*, *Emmenanthe penduliflora*, *Draperia systyla*, *Ellisia nyctelea*, *Hydrolea zeylanica*, *H. floribunda*, *H. nigricaulis*, *H. spinosa*, *N. demissum*, *Romanzoffia californica*, *Eriodictyon angustifolium*, and *Nemophila menzisii*) among the latter shows diorate condition as observed in some Myoporaceae grains. The different ornamentational types met with these members are punctilegillate (*Phacelia circutaria* and *Eriadictyon trichocalyx*), spinuliferous and punctitegillate (*Hydrophyllum appendiculatum*), to scrobiculate (*Emmenanthe penduliflora*), excepting these members all others are reticulate with finer reticulation near aperture.

f) *Gymnosperms with special reference to Coniferales*

A project has been undertaken on the world wide distribution of Gymnosperm flora with special reference to Coniferales based on pollen morphology. The work is in progress and the pollen grains so far studied are from northern hemisphere along with some Indian representatives which include *Pinus*, *Cedrus*, *Cupressus*, *Abies*, *Taxus*, *Juniperus*, *Cryptomeria* and *Thuja*.

Pollen grains of *Cedrus*, *Pinus* and *Abies* are saccate and *Cupressus*, *Taxus*, *Juniperus*, *Thuja* and *Cryptomeria* are non-saccate.

C.7.3. *Biostratigraphy of Darjeeling peat*

Some peat samples (4 profiles) possibly of Quaternary post-glacial deposits have been collected from different localities of Darjeeling district. The sub-surface analysis of a few of these samples reveal the presence of a number of fern spores, pollen grains of angiosperms and gymnosperms in a good preservation and frequency. The study of the present vegetation of this region, their ecological distribution and plant association have also been taken as a part of this study. Polliniferous material have been collected from the plants during April-May. These studies of the local vegetation will be of great help in producing the vegetational history of the region and their nature and mode of succession. For the complete record of flora of the region, further collection of plants in different flowering seasons is being undertaken.

C.7.4. *Melitttopalynology*

Further collection of honey samples from Bolpur and five samples from Sundarbans, all collected during April-May, 1970-1971, have been investigated. The samples of the Bolpur show the presence of *Nyctanthes* pollen grains as the dominant type. In Sundarbon samples a few typical mangrove pollen have been recorded.

C.7.5. *Fungal spore morphology* (*Aspergillus*)

Apart from its vegetative body and the parts connected with spore formation the conidiospores of *Aspergillus* have revealed differential characters with regard to its spore morphology. Not only the ascospores but the conidiospores also show some interspecific difference as is evident from these studies. Among the 15 species of *Aspergillus* the conidia are in general spheroidal, some are however oval or elliptical. Surface sculpturing shows that some are psilate, others spinulose and still others are prominently spinulose. Among the prominently spinulose forms further distinction can be made on the basis of arrangement of spinules. These characters together with the differences in size have led to the formation of various groups and a spore key.

C.8. Radiation Botany

3.8.1. *Irradiation of Lathyrus*

The experiments were conducted on different types of *Lathyrus sativus*, namely KH-2, KH-4, KH-13 and KH-19 seeds of types were originally obtained from the Economic Botanist, Berhampore Agricultural Firm, Government of West Bengal. Seeds of uniform size were sorted out and were kept in the growth room at 22°C for 2 months in order to stabilize the moisture content of the seeds. Before irradiation the seeds were soaked for 24 hours and after that the seeds were irradiated with different doses of X-rays. Before irradiation the seeds of the above types were arranged in single layer with the embryos facing the beam.

Irradiation work was done in the Botany Department with a therapy type of X-ray machine operated at 30 Kv with 2 mA. The doses applied were 1000, 2000, 3000 and 4000 r, and for the control the soaked seeds were utilized without irradiation.

After irradiation the treated seeds along with the controls were sown in replicated plots in the field. The distinguishing characters of the types observed are shown in Table 1.

TABLE 1

Type	Dose in r	Average		
		Height in cm	No. of pod per plant	No. of seeds per pod
KH-2	0 (control)	60.07	59.84	3.08
	1000	58.87	52.65	2.92
	2000	47.65	38.45	1.78
	3000	40.24	22.21	2.09
	4000	45.70	75.62	2.45
KH-4	0 (control)	61.35	90.63	2.26
	1000	55.06	76.44	2.21
	2000	43.96	41.84	1.71
	3000	32.57	21.14	1.45
	4000	48.67	59.60	2.06
KH-13	0 (control)	81.88	79.68	1.98
	1000	72.33	55.93	2.11
	2000	68.33	56.58	2.02
	3000	41.26	18.52	1.59
	4000	52.80	34.77	2.05
KH-19	0 (control)	72.66	89.04	2.92
	1000	59.44	64.08	2.18
	2000	42.65	30.30	1.85
	3000	40.60	31.00	1.68
	4000	26.30	3.00	2.00

Cytological observations were made from the X_1 root tips of type KH-2. After proper fixation and staining root tips squash preparations from the germinating seeds of treated as well as the control materials were done. A few observations were taken from each preparations at random. The Table 2 shows the different frequencies of observed data.

TABLE 2

Frequencies of abnormal anaphase in X_1 root tips of Lathyrus sativus (type KH-2)

Dose in r	Total No. of anaphase studied	No. of abnormal anaphase	% of abnormal anaphase
0	911	8	0.88
1000	449	108	24.05
2000	424	187	44.10
3000	355	219	61.69
4000	418	301	72.01

C.8.2. *Cytohistogenetical studies on the effect of X-ray on Lens esculenta Moench at various growth stages*

During the period under report seeds of *Lens esculenta* were treated with different dosages of X-rays, i.e., 10 kR, 15kR, 20 kR, 25kR, 30 kR and 35 kR to study the cytohistogenetical effects on plants at various growth stages. The X-rayed seeds along with control seeds were allowed to germinate in pots and petridishes. From control seeds, the growing embryos were dissected out and fixed in FAA in order to study the degree of differentiation of the growing embryos in the mature seeds, from their ontogenic behaviour. The degree of radio-sensitivity were measured from the study of percentage of abnormality occurred in root cells. Like previous observations in *Trigonella foenumgraecum* L. (var. 1978) here the rate of chromosomal abnormality in found to be directly proportional to the radiation dosages (Table 1).

TABLE 1

Showing the number of cells studied per treatment and percentage of abnormality occurred in root cells of Lens esculenta in X_1 generation

Treatment	Total number of mitotic cells studied	Normal cells		Abnormal cells			Bridge with fragment	Laggards	Percentage of Abnormal cells
		Metaphase	Anaphase	Metaphase with fragment	Anaphase with fragment	Anaphase with bridge			
0 (control)	1632	508	1108	6	5	2	—	3	0.1
10 kR	1901	601	900	176	180	36	88	—	21
15 kR	1969	597	713	302	230	77	18	32	34
20 kR	2034	522	602	348	218	210	119	15	45
25 kR	1673	302	401	402	212	175	141	40	58
30 kR	1374	215	210	448	208	55	176	62	69
35 kR	1022	100	65	575	104	20	119	39	84

But in comparison will the former experiment the morphological variations or abnormalities were very few. Most of the treated as well as control plants died prematurely, so in this case, the study could not be continued further. The rate of germination and germination

percentage in X_1 generation were recorded. Upto 20 kR X-rays, there was no effect on germination of *Lehs* seeds, but after that percentage of germination decreased with the increased dose rate.

TABLE 2

Showing the rate and percentage of germination in Lens.

Treatment / Date	Control	10 kR	15 kR	20 kR	25 kR	30 kR	35 kR
15.12.1969	6	22	30	40	36	34	0
16.12.1969	52	70	70	77	66	53	34
17.12.1969	82	86	80	80	60	55	34
20.12.1969	86	87	86	86	60	55	34
Percentage of germination+	72%	73%	72%	72%	59%	46%	28%

+ In all the cases 120 seeds were taken.

Collection of the shoot apices of plants from each treatment and control were made at regular intervals (weekly) and fixed in FAA. After fixation paraffin blocks of materials were made as per schedule. Microtome sections of shoot apices both longitudinal and tranverse were cut at 5–8μ thickness and stained with Heidenheins Iron Haematoxylin and Safranin. The changes occurred in the shoot apical organisation were studied critically on the basis of 'Tunica-Corpus' concept. Unlike root cells, the shoot apical cells were less affected and that was also supported by the occurrence of less number of stem and leaf abnormalities. This also indicates that here, the degree of radio-sensitivity in root cells was much more pronounced than that in the shoot apical cells.

The mature embryo of *Lens esculenta* is differentiated into cotyledons and apical meristem. The apical meristem appears to be a dome-shaped mass differentiated into two layered tunica and several layered corpus cells. The plants raised from irradiated seeds showed no morphological aberrations excepting few bifurcation of leaflets. At lower dosages of irradiation (i.e., 10 kR, 15 kR, and 20 kR) tunica-1 infrequently showed plasmolytic cells like that in *Trigonella foenum-graecum*. No severe damages were observed in shoot apical cells of plants treated with higher dosages of X-ray. The survivality percentages decreased and that was perhaps due to damages of root cells. The normal growth of roots somehow disturbed. As a result the plants under treatment dried out within a week or two after treatment because of damaged roots.

C.8.3. In the previous year seeds from irradiated plants of *Trigonella foenum-graecum* L. were collected were collected for the study of expectant mutants in the X_2-generation. hundred dry seeds from each treatment were sown in separate plots during the period under report. Like control seeds, seeds from 25 kR treatment in X_2-generation behaved normally and showed early germination and most of the seeds under 50 kR treatment failed to germinate, whereas, the 50% seeds of 75 kR treatment germinated. Germination and survivality percentage of the control and treated seeds have been recorded (Table 3).

TABLE 3

Showing the time taken for germination, percentage of germination and survivality percentage of Trigonella foenum-graecum L. *in* X_2 *generation*

Treatment (in kR)	Time taken for complete germination (in days)	Percentage of germination	Percentage of survivality
0×10^3	22.11.1969—17.12.1969 (19)	78	62
25×10^3	1.12.1969—17.12.1969 (18)	76	66
50×10^3	1.12.1969— 3.12.1969 (4)	5	nil
75×10^3	1.12.1969— 3.12.1969 (4)	50	24

Plants under 25 kR in X_2 generation showed stimulated growth. Except one plant under 75 kR treatment, all were quite normal. This plant grew vigorously and produced very few fertile fruits, each containing lesser number of seeds. Most of the fruits were seedless. This plant suffered highly from pollen sterility (nearly 85%). Pollen grains were of varying sizes.

In X_2 generation, seeds of plants under different treatments particularly of the plant producing seedless fruits were collected for the study of X_3 generation.

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Ipomoea fistulosa Mart.

Air dried leaves of *Ipomoea fistulosa* were first extracted with petroleum ether. The petroleum ether extracted plant material was further extracted with methanol. The methanolic extract was concentrated. The concentrated mass was taken up with ether (500 ml.). The ether solution was extracted with 5% $NaHCO_3$ for three times (200 ml. at a time). The total bicarbonate solution was acidified with hydrochloric acid in cold (4–5°C). The acidified solution was re-extracted with ether for four times (200 ml each time). The ethereal extract was washed with water till free from acid and dried over anhydrous sodium sulphate. The dried ethereal solution was distilled off and the yellow oil left was taken up with ethanol. The acidified solution after extracting with ether was further extracted with ethyl acetate for three times (150 ml each time). The combined ethyl acetate extract was washed with water till free from acid and dried over anhydrous sodium sulphate. The ethyl acetate extract was distilled off and the yellow oil left was taken up with ethanol. The two alcoholic solutions were mixed up and the total volume was made up with ethanol to 10 ml. It was then tested for the detection of the gibberellin present by paper and thin layer chromatography. The bioactivity of compound present was also studied by lettuce and cucumber hypocotyls test and also α-amylase of the endosperm.

The paper chromatography was carried out with the following solvents :

(1) Isopropanol : ammonia : water (10 : 1 : 1)

(2) *n*-Butanol : acetic acid : water (4 : 1 : 1)

(3) *n*-Butanol : ammonia (1·5*N*) (3 : 1)

The chromatograms were sprayed with 70 per cent sulphuric acid and viewed under UV chromatolight (253·7 mμ). The results are given in the following table

TABLE

	Rf of the chromatograms		Response to 70% H_2SO_4 spray under UV chromatography
	Isopropanol : ammonia : water (10 : 1 : 1)	*n*-Butanol : acetic acid : water (4 : 1 : 5)	
Acid fraction of *I. fistulosa*	0.74	—	Yellow green fluorescence.
Authentic A_3	0.52	0.70	-do-
Authentic A_5	0.74	—	-do-
Authentic A_{4+7}	0.70	0.46	-do-
		0.86	-do-

The results of thin layer chromatography with developing solvent and spraying reagent were given in the following table—

TABLE 1

Thin layer chromatography

Compounds	Rf. in the solvent		70% H_2SO_4 and observed under U.V. chromatolight	5% ethanolic sulphurice acid followed by heating at 120°C for 10 mins. and then observed under U.V. chromatolight
	Carbon-tetrachloride: acetic acid : water (8 : 3 : 5)	Carbon-tetrachloride: acetic acid : water (8 : 3 : 5) lower phase + 10% ethyl acetate		
Acid fraction *I. fistulosa*)	(1) No movement	0.27	—	Yellow green with bluish tinge.
	(2) 0.52	0.79	Faint yellow green	,,
Authentic A_3	No movement	0.15	Yellow green	,,
Authentic A_1	,,	0.24	—	,,
Authentic A_5	0.50	0.78	Faint yellow green	,,
Authentic A_{4+7}	0.06	—	—	,,
	0.64	0.84	Faint yellow green	,,

From the result of paper and thin layer chromatography it can be concluded that the acid fraction of the alcoholic extract of *I. fistulosa* contains GA_1 and GA_5.

Bioactivity was studied with the acid fraction. The acid fraction was chromatographed in isopropanol solvent for lettuce and cucumber bioassays. The acid solution showed activity in the 7th and 8th strips corresponding to the position of authentic GA_1 (7th strip) and GA_5 (8th strip). Activity was also found in the 6th and 9th strips. This was probably due to tailing of the compound as the acid fraction is not sufficiently pure. The α-amylase assay showed activity in 5th, 7th and 8th strips. The 7th and 8th strips activity is due to the presence of A_1 and A_5. The activity in 5th strip in the α-amylase assay may be due to the presence of other factor which did not show activity in lettude and cucumber assay as well as chemical spray specific for gibberellines. This may be an accelerating growth substance which could not be characterised.

The petroleum ether extract was concentrated and the concentrated mass taken up with ether. The ether solution was extracted with 3% NaOH. The alkaline extract was acidified with hydrochloric acid in cold and re-extracted with ether. The ethereal extract was washed with water till free from alkali and dried over anhydrous sodium sulphate. This dried ether solution was concentrated and the concentrated mass was taken up with chloroform and chromatographed over silica gel. The column was eluted successively with petroleum ether, benzene and chloroform. In early petroleum ether elute afforded much oil whereas latter petroleum ether gave a solid m.p. 85° crystallised from petroleum ether benzene mixture. The IR spectrum of the showed the presence of –COOH gr. The bioacitity of the compound was tested in different test material. In wheat coleoptile bioassay activity was found nil wheras in rice second leaf sheath test and bean second internode slight

inhibitory activity was observed. The gas chromatography examination of the solid was found to be a complex mixture of a no. of higher acids viz., Palmitic, Stearic, Arachidic, Behenic, Lignoceric, Cerotic and Montanic acid. (Gas chromatography data of the compound was obtained through the courtsey of Dr. Mandava of Plant Hormone and Regulator Laboratory, Beltsville, Maryland 20705, U.S.A.). The neutral fraction of petroleum ether extract was found to contain β-sitsterol.

Borassus flabellifer Linn.

The investigation for the purines was made with the fruit of *Borassus flabellifer* at four different stages of maturity. The first sample was collected when the endosperm consisted of the milk alone and there was no trace of meat. Then the consecutive three samples were collected every seven days after. The liquid endosperm is present only in the immature seeds and becomes exhausted during maturation. A decrease in the milk is generally associated with a corresponding increase in solid cellular endosperm. It is to be noted, however, that for each set of experiments, fruits were taken from a particular free.

The method followed for the isolation of purine compound was similar to the method described by Koshimizu *et al.* (1967). This method can be described as :

Fruits of *B. flabellifer* collected at different stages of maturity (2 kg in each stage) were extracted separately with methanol for 15 days at room temperature. The methanolic extracts for each batch of fruits were dealt with separately.

Isolation procedure : The methanolic extract was filtered and the filtrate was concentrated at 40–50°C vacuo and the aqueous concentrate after adjustment to *p*H 3 with 6*N* HCl was extracted with ethyl acetate to remove the soluble materials. To the aqueous layer, charcoal was added and the mixture was stirred for one hour at room temperature. Charcoal filtered and washed with water until the washing became neutral. Substances adsorbed in charcoal were then eluted with 70% acetone and with ethanolic ammonia (ethanol : liquor ammonia : water :: 50 : 5 : 45). Both effluents were collected separately and evaporation of solvents gave acetone elute and ethanolic ammonia elute respectively. The final elution was made by suspending the charcoal in a mixture of equal volume of pyridine and the above ethanolic ammonia eluent and incubating the suepsension at 70°C for 2 hours. The charcoal was removed by filtration and the elute was evaporated to give pyridine elute.

Purification of pyridine elute : Pyridine elute was dissolved in distilled water and the solution was adjusted to *p*H 3 with 6*N* HCl. It was then adsorbed in a column of Dowex 50 (H^+ form, 50–100 mesh). The column was washed with water until the effluent became neutral and then the column was eluted with 3*N* NH_4OH. The ammonia elute was then evaporated. The residue obtained on evaporation was dissolved in water and then *p*H of the solution was adjusted to 3 with $4NH_2SO_4$. 10% $AgNO_3$ solution was then added to this and the resulting mixture was kept at 0°C for two hours. The precipitate was then centrifuged and washed with ice cold 1% $AgNO_3$ solution. A suspension of the precipitate in 0·2*N* HCl was stirred for 2 hours at room temperature and then 30 mins. at 50°C and then centrifuged. The centrifuged acid extract was then further passed through a column of

Dowex 50 (H^+ form, 50–100 mesh), as described before and eluted with 3*N* NH_4OH. The resulting effluent was evaporated and the residue was dissolved in the mixture of ethanol and HCl [Ethanol : HCl(N) :: 10 : 1] and then it was treated with saturated solution of picric acid. The whole thing was concentrated and filtered through an alumina column. The column was eluted successively with petroleum ether, benzene, chloroform and 5% ethanolic chloroform. Each elute was subjected to thin layer and paper chromatography and tested for purine compounds. The purine compound was found in 5% ethanolic chloroform elute as this fraction gave positive response to $AgNO_3$, $Na_2Cr_2O_7$ spray followed by treatment with HNO_3 and then washed with water. This colour test was carried by spotting the compound in a filter paper and then treated with the spraying reagent. Purine compounds were detected in 5% ethanolic chloroform elute for the first three stages. Whereas it was absent in each of the elute in the 4th stage. For the identification of the purine derivatives in the palm fruit at different developmental stages, the above 5% ethanolic chloroform elute from alumina column was subjected to paper and thin layer chromatography.

RESULTS :

Chromatography of purine fraction of the purified palm extract

The purine fraction (5% ethanolic chloroform elute from the alumina column) of the purified palm extracts at different stages were subjected to paper and thin layer chromatography.

Paper chromatography :

For the paper chromatography Whatman No. 1 filter paper was used. The chromatograms were developed with (1) Isopropanol : ammonia : water (10 : 1 : 1) and (2) Butanol : acetic acid : water (4 : 1 : 5). The developed chromatograms were sprayed with 2% aqueous $AgNO_3$ solution followed by treatment with 0·5% $Na_2Cr_2O_7$ solution and subsequently the paper was treated with 0·5(*N*) HNO_3 and then the paper was washed with water.

TABLE

Compounds	Rf. in solvent system		Colour of the spot after spraying with $AgNO_3$ reagent
	Isopropanol : ammonia : water (10 : 1 : 1)	Butanol : acetic acid : water (4 : 1 : 5)	
Purine fraction of the palm fruit.			
Stage I	0.20	0.24	Bridk red
Stage II	0.20	0.23	,,
Stage III (a)	0.19	0.23	,,
(b)	0.81	0.84	,,
Stage IV Purine compound absent.			
Kinetin	0.77	0.80	,,
Cytosine	0.42	0.28	,,
6-Benzyl-amino-purine	0.81	0.83	,,
Adenine	0.67	0.44	,,
Guanine	0.20	0.24	,,

D. MICROBIOLOGY

D.1. Antibiotic production

D.1.1. *Studies on antibiotic-producing Streptomyces strain Ac* 28

While making a survey of antibiotic-producing Streptomyces from soil, a strain was isolated, found to produce an active substance in liquid medium and effective against a number of gram-positive, gram-negative human pathogenic bacteria. Attempts were made to isolate and purify the active material from the fermented broth. The strain was indexed as Ac 28 according to the convention of the laboratory.

D.1.1.1. *Isolation and purification of the active material from fermented broth.*

One litre of fermented broth freed from mycelium was distributed in 100 ml amount in different 500 ml Erlenmeyer flasks to each of which 3 gm of activated charcoal was added and shaken in a rotary shaker for 1 hour. The charcoal of each flask was separated and mixed together. The filtrate was tested for antibiotic activity but no activity was found in the filtrate indicating the total absorption of active material in the charcoal.

The active material was eluted from the charcoal by repeated washing with butanol. The butanol extract was concentrated under reduced pressure and then dried under vacuum. The dried material was dissolved in 5 ml of methanol and centrifuged. To the supernatant 20 ml of acetone was added and kept overnight at 4°C. A precipitate appeared and it was found to have no antibiotic activity. The supernatant was again dried while an oily material was obtained. The oily material was dissolved in acetone : chloroform solution (1 : 1) and passed through a column of silica gel (20×1·5 cm). The adsorbed material was eluted from the column with methanol and dried, and recolumned in the same manner. The eluates were dried and repeatedly washed with acetone. Finally orange plate-like solids appeared with melting point 164°C.

D.1.1.2. *Homogeneity of the antibiotic A-28*

The antibiotic obtained by the method metnioned above was indexed as A-28. To ascertain the purity of the antibiotic homogeneity was studied by paper chromatography in different solvents by flooding the spotted strip of paper (1·5×30 cm) for 6 hrs. at 30°C. Results are summarised in Table 1.

TABLE 1

Paper chromatographic studies of A-28

Solvent	Rf value
1. Ethanol : water (3 : 1)	0.824
2. Butanol : acetic acid : water (2 : 1 : 1)	0.944
3. Pyridine : acetic acid : water (2 : 1 : 1)	0.819
4. Isopropanol : water (7 : 3)	0.901
5. Water saturated butanol	0.559

D.1.1.3. *Antimicrobial spectrum of A-28*

The material was also tested for antibiotic activity against different bacteria. The spectrum is given in Table 2. 0·1 ml of A-28 solution (100 μg/ml) was applied in each cup having a diameter of 8·0 mm.

TABLE 2

Antimicrobial spectrum of antibiotic A-28

Test organism	Diameter of inhibition zone (mm)
1. *Staphylococcus aureus*	20
2. ,, *albus*	20
3. *Escherichia coli*	23
4. *Vibrio cholerae*	20
5. *Salmonella typhosa*	25
6. ,, *paratyphi*	25
7. *Streptococcus faecalis*	25
8. ,, *pyogenes*	25
9. *Aerobacter aerogenes*	27
10. *Klebsiella pneumonae*	25
11. *Proteus vulgaris*	26

D.1.1.4. *Solubility of the antibiotic A-28*

Solubility of the antibiotic was tested in different solvents. A-28 was highly soluble in methanol, ethanol, acetone, chloroform, benzene, butanol, isopropyl alcohol, ethyl acetate, butyl acetate, pyridine, acetic acid, slightly soluble in ethylene glycol, propylene glycol and insoluble in water, pet-ether and ether.

D.1.2. *Studies on antifungal antibiotic-producing Streptomyces strains*

D.1.2.1. *Taxonomic studies on the antifungal antibiotic-producing strains.*

Taxonomic identifications of two strains of Streptomyces were carried out by studying microscopic, cultural, and biochemical characters as well as their carbon utilization patterns. Microscopic examinations showed hyphal diameter 1·0 μ, spore diameter 1·5–3 μ, sporophores in the form of open hooks or loose spirals, spores produced in chains and round to oval in shape. Considering all the characters it was concluded that both the organisms belong to the genus *Streptomyces*. Ac_7 appeared to be related to *Streptomyces aureus* and Ac_2 resembled *Streptomyces griseoplanus*.

D.1.2.2. *Optimum conditions for antibiotic production by strains Ac_7 and Ac_2*

Ten different culture media were tested for the optimum antibiotic production and growth of Ac_7 and Ac_2. Of these, the medium suggested by Dhar and Bose (1968) (KCl

—0·32 g, NaH_2PO_4—0·32 g, $MgSO_4$, $7H_2O$ —0·016 g, asparagine 1 g., and glucose—10 g. and distilled water 1 litre) was chosen as the basal medium. Various nutrients in the form C, N, and vitamins were added to the basal medium, distributed in conical flasks in 25 ml quantities, sterilized, inoculated and incubated at 28°C. Antibiotic production was measured by the agar cup method of assay against *Saccharomyces cerevisiae* and growth was measured by taking the dry weight of the mycelium on the 8th day of incubation. After selecting the suitable C- and N- sources, their optimum concentrations for antibiotic production were determined. The medium was composed of the following ingredients, gm/lit; maltose 4·0; asparagine 0·175; for strains Ac_7 and sucrose 2·0; and $NaNO_3$ 0·3 g. for Ac_2. The results of the experiments are given in Tables 3 and 4. It will be seen that the strains Ac_7 and Ac_2 have specificity in utilizing the carbon and nitrogen sources in respect of production of antibiotics.

TABLE 3

Effect of different carbon sources on antibiotic production and growth of Ac-7 and Ac-2

Carbohydrate source	Test organisms (*S. cerevisiae*)			
	Ac 7		Ac 2	
	Zone of inhibition in mm	Dry wt. in mg/25 ml	Zone of inhibition in mm	Dry wt. in mg/25 ml
1. Glucose	19	65	17	70
2. Maltose	23	88	17	75
3. Arabinose	14	42	—	63
4. Mannose	12	55	10	47
5. Mannitol	10	60	—	26
6. Xylose	—	50	—	87
7. Lactose	—	80	—	40
8. Fructose	—	55	—	22
9. Sucrose	—	56	19	40
10. Sorbitol	—	5	—	15
11. Raffinose	—	60	—	40
12. Galactose	—	4	15	10
13. Dextrin	20	20	18	88
14. Starch	14	29	16	51

— absence of inhibition.

TABLE 4

Effect of different N-sources (Conc. of ·186 N_2/litre = 1 unit/litre)-on antibiotic production and growth of Ac 7 and Ac2

Name of the sources	Test organism (*S. cerevisiae*)			
	Ac 7		Ac 2	
	Zone of inhibition in mm	Dry wt. in mg/25 ml	Zone of inhibition in mm	Dry wt. in mg/25 ml
1. $NaNO_3$	—	25	20	44
2. NH_4NO_3	—	40	—	20
3. $(NH_4)_2SO_4$	+	25	—	10
4. KNO_3	—	—	16	20
5. Urea	—	30	—	25
6. Alanine	25	20	18	25
7. Proline	25	25	16	20
8. Serine	22	25	19	15
9. Aspartic acid	11	40	—	20
10. Glutamic acid	—	20	18	20
11. Valine	23	19	15	14
12. Tryptophane	15	10	14	29
13. Leucine	+	20	—	4
14. Methionine	12	29	13	12
15. Histidine	23	35	15	24
16. Asparagine	24	57	17	36
17. Arginine	15	44	12	32
18. Threonine	23	63	15	20
19. Phenyl alanine	—	54	12	18
20. Lysine	15	26	15	14

D.1.3. *Mode of action*

D.1.3.1. *Biochemical studies on the effects of boseimycin in* Bacillus subtilis ; *a comparison of the metabolic changes induced by boseimycin and streptomycin.*

Preliminary studies on the biochemical changes induced by boseimycin and streptomycin were carried out with a sensitive strain of *Bacillus subtilis*. Medium of the following composition : glucose, 10 g; glutamic acid, 5 g; KH_2PO_4, 1·76 g; $MgSO_4$ $7H_2O$, 0·02 g; KCl, 5 g; $MnSO_4.4H_2O$, 0·01 g; $FeSO_4.7H_2O$, 0·01 g. water, 1000 ml; was used in all experiments. The *p*H of the medium was adjusted to 7·1. Metabolic studies were performed by comparing the rates of utilization of various substrates. 250 ml Erlenmeyer flasks containing 80 ml of the medium were inoculated with 0·5 ml freshly grown (24 hr. old) *B. subtilis* culture and incubated at 37° under stationary condition. Antibiotic solutions

were added at subminimal inhibitory concentrations at zero time. Cells were separated from the broth by centrifugation, then washed twice with distilled water and finally dried overnight at 60° and the dry weight of the cells was determined. The culture filtrate was preserved below 4° for analysis. Estimation of glucose was done according to the method of Folin and Wu (J. Biol. Chem., 41, 367, 1920), lactic acid by Barker and Summerson (J. Biol. Chem., 138, 535, 1941), phosphate by Hertha and Ephriam's (J. Biol. Chem., 202, 675, 1953) methods and nitrogen by nesslerisation. Quantitative estimation of total nitrogen and phosphorus was carried out on aliquots previously digested with sulphuric acid.

In the above growth medium, under stationary condition, it was observed that a maximum growth in control culture reached after 93 hr. and subsequent loss of cell dry weight noted, was accounted for lytic action of the cells, whereas in both boseimycin and streptomycin treated cultures growth continued even after 93 hr. The growth rate in antibiotic treated cultures was low and this slower growth rate was comparable to the rate of utilization of different substrates in the medium, The medium maintained a steady lower *p*H value in the treated cultures all through the growth period. In control experiment maximum lactic acid production reached during 69–93 hr. of growth and subsequently lactic acid in the broth diminished probably due to its utilization owing to low availability of glucose. In antibiotic treated cultures lactic acid production increased steadily. Utilization of phosphate was comparatively higher during initial growth phase in control experiment but in both boseimycin and streptomycin treated cultures phosphate utilization was comparatively higher towards the initial and later growth phase. The rate of utilization of nitrogen was directly linked with the growth of both antibiotic treated and untreated cultures.

D.1.3.2. *Effects of boseimycin on some enzyme systems of* Bacillus subtilis.

To observe the effects of boseimycin on the formation of the enzymes cells grown in nutrient broth under shaking at 37° at early log phase were treated with 0·2 μg/ml boseimycin for 3 hr. This supports 70% growth of the bacteria compared to that of the control. A similar set without any antibiotic was done as control.

Crude enzymes were extracted from the cells after washing with Tris-HCl buffer (0·05 *M*, *p*H 7·0), by grinding with fine glass beads (Minnesota Mining and Manufacturing Co., U.S.A.).

Aconitase was measured according to the method of Anfisen (Methods in Enzymol. A.P., N.Y., 1, 695, 1955) using citrate as substrate. Lactic dehydrogenase was determined by measuring the oxidation of NADH in presence of pyruvate at 340 nM and isocitric dehydrogenase by measuring the reduction of $NADP^+$ in the presence of isocitrate. Glutamic and α-alanine dehydrogenases were assayed by measuring the oxidation of NADPH in presence of α-ketoglutarate and pyruvate respectively.

Boseimycin inhibited the activity of lactic dehydrogenase (10%). Glutamic, α-alanine, isocitric dehydrogenases and aconitase activities were without any effect. Boseimycin stimulated the synthesis of glutamic dehydrogenase (65%) but lactic dehydrogenase synthesis

was inhibited (50%). Molecular basis for the specific stimulation or inhibition of the enzymes is yet to be elucidated. It was reported earlier that protein was one of the macromolecules first inhibited by boseimycin followed by inhibition of nucleic acid synthesis in *B. subitlis*.

D.2. Induced mutation in microorganisms

D.2.1. *Antibiotic activity of Recombinants derived from* Streptomyces indicus.

The antibiotic activity of prototroph recombinants from two different crosses between biochemical mutants was studied. The biochemical mutants were obtained from *Streptomyces indicus* through UV-irradiation.

In all cases the activity of prototrophs from one and the same cross varied markedly as did their morphological features. In some cases, colonies similar in their morphology were also similar in their antibiotic activity. Therefore, the activity of only one colony from each morphological type of prototroph from the given combination was usually studied. Table 5 presents the data on the antibiotic activity of prototroph recombinants obtained

TABLE 5

Comparative antibiotic activity of the parent strains of Streptomyces indicus, *its biochemical mutants and their prototrophs.*

Strain		Antibiotic activity+ (Zone of inhibition in mm)
Wild type parent		
Streptomyces indicus		26.5
(a) *Biochemical mutants*		
66-w hist⁻		20.3
23-w gly⁻		22.6
14-y ribo⁻		18.6
(b) *Prototrophs* from combination 66-w hist⁻ × 14-y ribo⁻		
	H-5	20.0
	H-8	19.3
	H-14	—
	H-16	20.1
	H-22	14.3
	H-25	22.2
(c) *Prototrophs* from combination 23-w gly⁻ × 14-y ribo⁻		
	H-62	20.3
	H-86	12.2
	H-89	21.0
	H-91	13.3
	H-145	28.2
	H-152	28.0
	H-160	27.5

+ *Curvularia lunata* was used as test organism for assay of antibiotic.
—, indicates absence of activity.

from crosses between 66-w hist$^-$ × 14-y ribo$^-$ and 23-w gly$^-$ × 14-y ribo$^-$. As is evident from the Table 5, the activity of almost all these prototrophs exhibited difference than that of the biochemical mutants. Some of them produced the same, less or even the greater amount of antibiotic activity. In one case the activity was completely lost.

It was also noticed that variation in antibiotic producing capacity among the recombinants exists according to their requirements for the composition of the fermentation medium. The recombinants in media containing maltose as the carbon source gave enhanced antibiotic activity than those when grown in media containing glucose. The recombinant strains H-145, H-152 and H-160 were grown in Pridham and Gottlieb's basal medium supplemented with 4·0% maltose, allowed to incubate at 28°C for 14 days and then the antibiotic activity was observed. *Curvularia lunata* was used as the test organism for the antibiotic assay. It was found that the activity was increased moderately (Table 6).

TABLE 6

Comparative antibiotic activity of the initial strains of S. indicus *and the prototrophs in P & G+ media containing glucose and maltose*

Strain	Antibiotic activity (Zone of inhibition in mm)	
	Media with glucose	Media with maltose
Wild type		
Streptomyces indicus	26.5	27.0
Prototrophs		
H-145	28.2	29.0
H-152	28.0	29.0
H-160	27.5	28.5

*Pridham and Gottlieb's medium.

Obviously any conclusion concerning the prospect of the use of the recombinant phenomenon for obtaining highly productive strains of *Streptomyces indicus* apparently would be premature at this stage. However, these facts offer a possibility for the use of recombinants as materials for strain selection.

D.2.2. *Effect of UV-irradiation on proteolytic activity in* Streptomyces *strain RF*-81.

It was reported earlier that the *Streptomyces* strain RF-81 possesses strong proteolytic activity. This can be easily demonstrated by the process of gelatin liquefaction in culture media. The degree and rapidity of proteolysis also vary within the mutant strains.

The *Streptomyces* strain RF-81 was subjected to ultraviolet irradiation for 40, 80, 120 and 160 seconds at a dose level of 100 ergs/mm²/sec. The irradiated spore suspension was placed on CM agar media and the per cent survival was determined. To study the alteration in proteolytic activity among the mutants, each strain was inoculated into 5 ml gelatin media, incubated at 28°C and the intensity of gelatin liquefaction was noticed after 14 days of incubation. It was observed that the proteolytic activity varied greatly among the mutant strains due to UV-irradiation. The activity was either increased, decreased or remained

unchanged. Table 6A represents the experimental results. It was found that out of 630 strains tested, 39(6·2%) showed increased protease producing capacity, 62(9·9%) showed decrease in protease activity and the remaining 529 (83·9%) were more or less unchanged.

TABLE 6A

Alteration in proteolytic activity among the mutants induced by UV-irradiation in the Streptomyces *strain Rf*-81

Time of irradiation in secs.	No. of colonies tested	Proteolytic activity					
		Decrease		Increase		No change	
		No. of isolates	No. of isolates in %	No. of isolates	No. of isolates in %	No. of isolates	No. of isolates in %
40	150	12	8.0	6	4.0	132	88.0
80	180	18	10.0	10	5.5	152	84.5
120	200	22	11.0	14	7.0	164	82.0
160	100	10	10.0	9	9.0	81	81.0
Total	630	62	9.9	39	6.2	529	83.9

D.2.2.1. *Effect of physical and chemical mutagens on* Aspergillus amstelodami (17455)

UV and X-ray treatment : Spore suspension of *A. amstelodami* from 6–7 days old cultures was subjected to UV treatment for 1, 2, 3, 4 and 5 minutes. The dose was 100 ergs/mm²/sec. Per cent survival, per cent biochemical and morphological mutation were then calculated. 2 ml of spore suspension was taken for each of the five separate screw-capped aluminium capsules. These were then exposed to X-ray. The dosage was

TABLE 7

Survival and mutation percentage

Mutagen	Dose	Per cent		
		Survival	Bio-mutation	Morpho-mutation
(I) UV	1	36.2	1.7	2.6
	2	3.8	2.3	3.9
	3	2.2	4.6	5.4
	4	0.34	2.7	4.1
	5	2.28	1.3	3.5
(II) X-ray	10	48.9	1.1	1.8
	10	23.8	2.2	2.9
	30	22.6	3.5	4.2
	40	16.3	3.9	4.1
	50	1.0	2.7	3.1

(I) Dose in minute.
(II) Dose in Kr

10, 20, 30, 40 and 50 Kr respectively. Per cent survival, per cent biochemical and morphological mutation were then calculated. Table 7 shows the results.

D.2.2.2. *NTG treatment*

NTG solution containing 1000 μg/ml of NTG was added to 25 ml flask containing 9 ml of heavy spore suspension. The flask was then allowed to shake in a rotary shaker for 8 hours. After every 2 hours, 2 ml of suspension was pipetted out and centrifuged. After centrifugation the spore sample was washed thrice with normal saline and the final spore suspension was prepared in 2 ml of normal saline. Plating was done from the sample after proper dilution. Percent survival, percent morphological and biochemical mutation were calculated. Table 8 shows the result.

TABLE 8

Survival and mutation percentage of NTG-treated A. amstelodami

Mutagen	Dose (III)	Per cent		
		Survival	Bio-mutation	Morpho-mutation
NTG	2	55.7	2.0	2.6
	4	31.6	3.1	3.7
	6	28.5	3.3	4.1
	8	20.2	2.5	3.1

(III) Time of treatment in hours.

D.2.2.3. *Antibiotic activity of* A. amstelodami

For testing antibiotic activity, *A. amstelodami* was inoculated in 250 ml flask containing 50 ml CD liquid medium. The flasks were incubated for 6–7 days at 28–30°C. Assay plates were made with the bacterial suspension of *Vibrio cholerae*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhosa* with nutrient agar medium. The plates were incubated at 37°C for 18 hrs.

Antifungal assay tests were done with the metabolic filtrates of *Aspergillus niger*, *Alternaria solani*, *Curvularia lunata* and *Penicillium notatum*. The method is similar to that as with bacteria except CD agar was used for assay medium. The results are given in Table 9.

In Table 7 the results of survival percentage and frequency of morphological and biochemical mutations of physical and one chemical mutagens are given. The higher percentage of biochemical and morphological mutations occurred in the case of UV-treatment and killing rate was greater than X-ray and NTG treatments. From Table 9 it could be seen that the strain *Aspergillus amstelodami* produced an antibiotic in synthetic medium, which is active against both gram-negative and gram-positive bacteria. The antibiotic production was done at 28°C–30°C.

TABLE 9

Assay of antibiotic produced by A. amstelodami

Assay organism	Diameter of zone of inhibition in mm
Bacteria	
1. *Salmonella typhosa*	—
2. *Vibrio cholerae*	—
3. *Staphylococcus aureus*	35.5
4. *Escherichia coli*	25.5
Fungi	
1. *Aspergillus niger*	—
2. *Alternaria solani*	—
3. *Curvularia lunata*	—
4. *Penicillium notatum*	—

— indicates no antibiotic activity.

D.3. Microbial Genetics

D.3.1. *Biochemical studies with* P. vermiculatum

In continuation of the study of biosynthesis of riboflavin in *P. vermiculatum* experiments on the effects of K_2HPO_4, $FeSO_4$, $MgSO_4$, $NaNO_3$, organic nitrogen sources and *p*H were done in order to determine the optimum concentration of such ingredient in the medium encouraging riboflavin production. The medium used was Czapek-Dox slightly modified. Various concentrations of the different chemical constituents were used in the medium. The highest production of riboflavin was found at the concentration of 0·15, 0·0005, 0·0375 and 0·2% respectively.

*p*H 7·0 is the optimum *p*H level which gives better production of riboflavin. The *p*H range from 5·0 to 11·0 at steps of one was tried in the experiment. The inoculum used in the cultural experiments was generally 5×10^5 conidia/20 ml.

D.3.1.1. *Effect of inoculum on riboflavin biosynthesis*

Inoculum of various concentrations of conidia ranging from 5×10^2 to 5×10^7 were used in 20 ml Czapek-Dox medium having the *p*H level at 7·0 dispensed into 100 ml flasks. The cultures were incubated at 30°C for 5 days. The results are given in Table 10.

TABLE 10

The effect of concentration of spore inoculum on the production of riboflavin

Concentration of inoculum (conidia/20 ml)	5×10^1	5×10^2	5×10^3	5×10^4	5×10^5	5×10^6	5×10^7
Riboflavin production in terms of 0·1 (*N*) NaOH in ml/20 ml.	106	107	124	163	269	322	316

TABLE 12

Showing the percentage of normal acid in Shu and Johnson's and Doelger and Prescott's medium at different temperature. (Still and shake conditions)

Sucrose concentration (%)	Incubation temperature °C							
	25				28 to 30			
	Shu & Johnsons medium		Doelger & Prescott's medium		Shu & Johnsons medium		Doelger & Prescott's medium	
	Shake	Still	Shake	Still	Shake	Still	Shake	Still
4	11.00	5.80	5.00	1.74	2.00	1.82	2.75	8.80
6	14.20	12.40	7.86	10.62	5.12	1.62	3.50	8.25
8	30.00	13.00	24.24	10.36	13.12	14.00	2.25	24.75
10	14.20	10.20	14.36	12.24	5.60	13.00	2.00	18.50
12	12.80	11.20	13.12	10.12	5.74	20.86	4.38	17.38
13	18.20	9.20	10.50	3.50	5.74	12.40	4.13	21.13
14	14.20	11.00	8.00	8.24	5.74	11.20	4.75	12.25
15	9.00	7.00	15.62	8.86	8.74	16.50	5.00	12.00
16	8.00	6.60	4.50	7.74	5.86	6.50	5.75	13.63
Control (Sucrose—14%)	3.0	3.0	1.5	1.5	2.8	3.0	2.0	1.5

The above table shows some interesting results. Doelger and Prescott's medium gives 24·24% of acid at shake condition at 25°C with a scucrose concentration of 8%. On the other hand it produces only 2·25% of acid in shake condition at 28°C to 30°C with the same concentration of surcose. This medium, however, gives 24·75% acid at still condition at 28°C to 30°C against 10·36% acid in still condition at 25°C.

D.3.31. *Induced mutations with UV and X-rays*

UV-induced mutations Asp. niger strain 6N3 isolated from the soil of Naihati (West Bengal), was selected for mutational study.

The culture was grown in sporulating medium (Shu & Johnson, 1948) in petridishes at 30°C for 8 days. The spores were harvested in sterile calsolene solution (1 part calsolene : 10,000 parts water) filtered, centrifuged and adjusted to suitable concentration. Irradiation was done in 9 cm petri dishes containing 7 ml spore suspension. The spores were irradiated for 3, 6, 9, 12, 15, 21, 24 and 30 minutes at 25°C.

Irradiated spores were then plated out in complete medium (Pontecorvo, 1953) after proper dilution. Survival percentage was determined from viable counts of colonies appearing on petri dishes and the results are shown in Table 13.

TABLE 13

Showing the percent survival of conidia of Aspergillus niger *strain 6.N3 on UV-irradiation*

	Treatment of ultraviolet in minutes	Initial colony count	Average survival conidia/ml	Percentage of survival
Control (untreated)	–	210×10^5	210.0×10^5	100.00
	3		205.8×10^5	97.50
	6		76.5×10^5	36.19
	9		294.0×10^4	14.00
	12		116.3×10^4	5.52
	15		206.4×10^3	0.98
	21		29.2×10^3	0.138
	24		109.7×10^2	0.051
	30		17.06×10	0.0008

Energy—100 ergs/mm²/sec.

The higher acid yielding mutants were isolated by transferring colonies from the CM plates to CD (minimal) agar plates containing 0·2% Bromocresol green and subsequent selections were made on the basis of acid zones in the plates.

The selected mutants were further tested for their acid yielding capacity in 100 ml capacity Erlenmeyer flasks containing Doelger and Prescott's medium at 28° to 30°C, in still condition. Estimation of citric acid was done following the colorimetric method of Marrier and Boulet (1958). A few mutant strains gave high citric acid yield.

D.3.4. *Comparative study of the effects of gamma and UV irradiation in* Chaetomium aureum.

Repeated experiments on UV and gamma irradiations with *Chaetomium aureum* revealed that only a few types of mutants were obtained through these irradiations. In order to raise varied types of deficient mutants ascospores were irradiated with gamma and combined UV and gamma rays.

The ascospores of *Chaetomium aureum* were irradiated by kind courtesy of the Bhaba Atomic Research Centre at Trombay. The source of irradiation being 60Co emitting 6·141 Kr. energy per minutes. Dosages of gamma irradiation were 20, 30, 40, 50, 60 and 70 Kr. The samples of spore suspension meant for the different gamma treatments were taken in screw cap aluminium capsules and despatched in a double jacketed vacuum container filled up with ice maintaining the temperature between 0°–4°C so that the ascospores would not germinate.

For comparison of the efficiency of different mutagenic agents three types of irradiation experiments were performed.

i) Only with UV exposure
ii) Only with gamma exposure
iii) Combined gamma and UV exposure.

For the combined gamma and UV exposure 7 ml quantity of gamma irradiated ascospore suspension was taken out from the aluminium capsule and subjected to UV irradiation (2537 Å) for 30 minutes. The plates were so placed that the spores received an energy of 100 ergs/mm²/sec. Spores from all the treatments, were separately plated in sorbose complate medium. The colonies grown on the complete medium were then tested. Comparative survival percentage and mutation percentage at different dosages are given in Table 14.

TABLE 14

Showing survival percentage of ascospores of Chaetomium aureum *on irradiation with gamma rays and combined gamma and ultraviolet rays and the percentage of induced biochemical and morphological mutations*

Treatment		Average surviving ascospores/ml	Per cent survival	Total number			Per cent		
Gamma irr. in Kr.	UV irr.			Colonies Tested	Biochemical mutants	Morphological mutants	Biochemical mutations	Morphological mutations	Total mutations
Control untreated		105.80×10^5	100.00	1080	—	—	—	—	—
20	—	44.59×10^5	42.14	1080	—	5	—	0.46	0.46
30	—	15.02×10^5	2.14	1080	—	5	—	0.46	0.46
30	—	15.02×10^3	14.19	1080	15	5	1.38	0.46	1.84
40	—	6.96×10^5	6.57	1080	10	10	0.92	0.92	1.84
50	—	2.03×10^5	1.91	1080	25	5	2.31	0.46	2.77
60	—	1.65×10^5	1.55	1080	75	5	6.94	0.46	7.40
70	—	0.50×10^5	0.47	1080	20	—	1.85	—	1.85
—	+	2.22×10^5	2.09	1080	75	25	6.94	2.31	9.25
20	+	2.33×10^5	2.20	1080	50	30	4.62	2.77	5.39
30	+	0.68×10^5	0.64	1080	80	20	7.40	1.85	9.25
40	+	0.25×10^5	0.23	1080	35	20	3.24	1.85	5.09
50	+	0.09×10^5	0.085	1080	15	—	1.38	—	1.38
60	+	0.02×10^5	0.018	1080	45	20	4.26	1.85	6.01
70	+	0.018×10^5	0.0094	1080	70	10	6.48	0.92	7.40

Energy : Gamma 6·141 Kr./min.
Ultraviolet 100 ergs/mm²/sec.
Temperature of irradiation 25° to 30°C.

Legend : UV irr⁻ indicates no UV irradiation.
UV irr⁺ indicates ultraviolet irradiation given to 30 minutes at 2537A.
Gamma irr⁻ indicates no gamma irradiation.
— indicates no scoring of mutants.

From the table it is evident that the post treatments with UV after gamma irradiation gave a sharp decline in the survival percentage. Increase in dosages of gamma irradiation at 20, 30, 40, 50, 60 and 70 Kr. gave the survival percentage at 2·20, 0·64, 0·23, 0·085, 0·018, and 0·0094 respectively whereas irradiation only with UV for thirty minutes gave 2·22 as the survival percentage. A number of biochemical and morphological mutants (Table 14) were induced as a result of combined effect of irradiation.

Showing duration of treatment, number of mutants isolated, total number of colonies on minimal medium and percentage mutants in *Chaetomium aureum*.

Different types of mutants are classified according to their visible characters and broad nutritional requirements as given below :—

1. *Morphological mutants*—
   - i) Colonial types
   - ii) Pigmented mycelial types
   - iii) Apigmented mycelial types
   - iv) Spore colored and spore patterned types (only in UV-irradiation).

2. *Biochemical mutants*—

A classification of the biochemical mutants obtained is given in Table 15.

TABLE 15

Showing the range of Biochemical mutants in Chaetomium aureum *obtained on gamma and gamma+UV irradiation*

Nutritional requirements	No. of mutants obtained	
	Gamma	Gamma +UV
A. *Vitamins*		
i. Para-Aminobenzoic Acid (PABA)	2	
ii. PABA/Thiamine	1	
iii. Biotin	1	
iv. Nicotinic acid/PABA/Biotin/Ca-pantothenate/Pyridoxine-HCl	1	
v. Nicotinic acid/Biotin/Pyridoxine-HCl/Ca-pantothenate	1	1
vi. Ca-pantothenate/Biotin/Pyridoxine-HCl/Nicotinic acid/Folic acid/ Thiamine/PABA/Inositol	1	
vii. Ca-pantothenate-/pyridoxine-HCl/Nicotinic acid/Riboflavin/PABA/ Thiamine/Folic acid/Choline	1	
viii. Thiamine		1
ix. Insitol		2
x. Insitol/Thiamine		1
xi. Insitol/Nicotinic acid/Thiamine		1
xii. Thiamine/Insitol/Riboflavin		1
xiii. Riboflavin		1
xiv. Riboflavin/PABA		1
xv. Thiamine/Nicotinic acid		1
xvi. PABA/Inositol/Choline		1
xvii. Choline		2
xviii. Insotil/Riboflavin/Folic acid		1
xix. Thiamine/Nicotinic acid/Biotin/Ca-pantothenate/Pyridoxine-HCl/Choline		1
xx. Biotin/Ca-pantothenate/Pyridoxine-HCl/Folic aicid		1
B. *Amino acid*		
i. Lysine	1	2
ii. Leucine	2	
iii. Tryptophan	2	1
iv. Leucine/Tryptophan		1
v. Lycine/Leucine/Tryptophan		1

TABLE 15—Contd.

Nutritional requirements	No. of mutants obtained	
	Gamma	Gamma +UV
C. *Nucleic acid*		
i. Xanthine/Thymine/Hypoxanthine	1	
ii. Guanine/Adenine	1	1
iii. Xanthine/Hypoxanthine	1	
iv. Guanine/Cytosine		1
v. Cytosine/Hypoxanthine		1
vi. Adenine/Xanthine/Hypoxanthine		1
D. *Amino acid/Nucleic acid*		
i. Guanine/Adenine/Leucine	1	
ii. Xanthine/Hypoxanthine/Lysine-HCl	1	
iii. Guanine/Cytosine/Tryptophan		1
iv. Guanine/Adenine/Lysine-HCl		1
E. *Vitamin/Amino acid*		
i. Biotin/Lysine	1	
ii. PABA/Thiamine/Leucine	1	
iii. Biotin/PABA/Thiamine/Lysine/Leucine		1
iv. Ca-pantothenate/Pyridoxine-HCl/Nicotinic acid/Folic acid/Thiamine/ Tryptophan		1
v. Nicotinic acid/Tryptophan		1
vi. Folic acid/Leucine		1
F. *Vitamin/Nucleic acid*		
i. Riboflavin/Xanthine/Hypoxanthine		1
ii. Inositol/Thiamine/Hypoxanthine/Xanthine		1
iii. Thiamine/Thymine		1
G. *Vitamin/Amino acid/Nucleic acid*		
i. Guanine/Cytosine/Tryptophan/Thiamin/Biotin		1
ii. Cytosine/Hypoxanthine/Leucine/Biotin/PABA/Thiamine		1
H. *Nitrate Non-reducing*		1
I. *Vitamin/Nitrate non-reducing*		
i. Biotin/Ammonium sulphate		1
J. *Amino acid/Nitrate non-reducing*		
i. Tryptophan/Ammonium sulphate		1
K. *Nucleic acid/Nitrate non-reducing*		
i. Guanine/Cytosine/Ammonium sulphate		1
ii. Xanthine/Hypoxanthine/Ammonium sulphate		1
L. *Vitamin/Sulphate non-reducing*		
i. PABA/$Na_2S_2O_3$	1	
ii. PABA/Thiamine/$NIa_2S_2O_3$	1	
Total no. of mutants	22	42

D.3.5. *Effect of X-rays on* Penicillium javanicum.

Conidiospores of *Penicillium javanicum* were subjected to X-ray irradiation. Exposures of 1, 3, 6, 9, 15, 21, 27, 33 and 39 Kr were given by kind courtesy of the Biophysics Department of Saha Institute of Nuclear Physics, Calcutta. For irradiation thick suspension of conidia (12 days old) was prepared; 4 ml of such suspension were pipetted out in 5 cm diameter sterile Petridish for each dosage.

The spores were then diluted and plates on P-complete medium having 0·9% sorbose. It will be seen in Table 16 that at lower dosage i.e., 1 Kr the germination of spores increased to a great extent indicating that at this dosage X-ray stimulate spore germination. Beyond this dosage there is a gradual decrease in the survival of conidia. Total transfer of colonies were done on A_1 minimal medium which was devoid of any organic ingradients. Biochemical and morphological mutants which appeared in the treated population were isolated and characterized by following the standard technique of the laboratory.

TABLE 16

Percentage of biochemical and morphological mutants of P. Javanicum treated with X-irradiation

Treatment of X-ray irradiation	Percent survival	Number of colonies transferred to minimal plate $\times 10^3$	Percent	
			Biochemical mutants $\times 10^{-5}$	Morphological mutants $\times 10^{-5}$
Control	100.00	1.000	0	0
1	175.50	0.879	0	0
3	70.60	1.222	82	0
6	63.90	1.040	290	96
9	14.90	1.150	170	0
15	10.61	1.042	96	96
21	1.43	0.889	340	0
27	1.15	1.137	260	180
33	0.39	1.273	390	0
39	0.14	1.254	0	80

The percentage of biochemical and morphological mutants with X-ray irradiation, however, was found to be rather very low.

D.4. Molecular Genetics

D.4.1. *Modifying effect of thiourea on UV-irradiation*

D.4.1.1. *Mutation*

It has already been reported that thiourea is very much effective as a protective agent against killing of *Aspergillus terreus* conidia by UV rays. Here, we report that this chemical is especially effective in reducing the mutational damages also. The target molecule for the action of thiourea is the macromolecule most vital to the cell, is confirmed here,

Thiourea in 0·1 *M* soln. in sterile distilled water was used during irradiation, prior to irradiation and after irradiation with UV in the following manner.

D.4.1.2. *Pretreatment with the chemicals*

The spore suspension after centrifugation was treated with the chemical for 2 hours at 28°C. The suspension was centrifuged again and resuspended in minimal medium liquid (MML) and irradiated for different time periods, after which platings were done on Complete agar medium (CM).

D.4.1.3. *Post-treatment with the chemical*

The spore suspensions were irradiated for different time periods and then centrifuged and treated with the chemical for 2 hours at 28°C, centrifuged and resuspended in MML and platings were done as usual.

D.4.1.4. *Treatment with the chemical during irradiation*

The spore suspension after centrifugation was resuspended in the chemical and irradiated for different time periods. It was centrifuged again just after irradiation and resuspended in MML and plated in CM. For mutational studies mycelium from the irradiated and non-irradiated colonies were transferred on Minimal medium (MM) plates, in which 16 isolates were placed over each plate in a particular geometrical pattern. After 48 hours of incubation at 28°C, morphological mutants were sorted out by appearance like colour of the conidia, pigmentation, length of the conidiophores etc.

Biochemical mutants, on the other hand, do not grow on MM. Thus, the non-growing isolates of the MM plates were again transferred on CM, where it would grow normally. The results (Table 17) here indicate that when a gene becomes sensitive it remains sensitive both for lethality and mutation induction. Thus, thiourea when applied during irradiation, gives minimum no. of mutants (both morphological and biochemical) and maximum amount of survival.

TABLE 17

Modifying effect of Thiourea on UV-irradiation of A. terreus *condia* (100 *ergs/mm²/sec*) *at* 0·1*M conc.*

	Time of irradiation (in mins.)														
	0			2			4			6			8		
	% Sur.	% MM	% BM	% Sur.	% MM	% BM	% Sur.	% MM	% BM	% Sur.	% MM	% BM	% Sur.	% MM	% BM
Pretreatment	100	0	0	8.4	8.3	1.2	0.2	12.0	4.6	0.1	10.9	1.8	0.09	4.7	.04
Post-treatment	100	0	0	13.2	5.0	1.6	1.8	7.7	2.3	1.0	7.2	1.0	0.7	5.5	0.6
Treatment during irradiation.	100	0	0	100.0	0.6	0.3	91.6	0.7	0.7	83.3	0.7	0.6	81.7	0.5	0.5
Untreated	100	0	0	10.0	6.3	2.1	0.5	9.5	3.1	0.3	7.7	1.7	0.2	5.8	1.1

Sur.= Survival, MM = Morphological mutants, BM = Biochemical mutants.

D.4.2. *Isolation of Nucleic acid from conidia of* Aspergillus terreus

In order to find out the effect of thiourea at the molecular level i.e. whether the chemical has any role on the thymine dimerzation process, we tried to isolate the DNA from the conidia of *A. terrerus*. It is very difficult to obtain the nucleic acids from fungal conidia. This is because of hardness of the conidial walls, and lysozyme treatment is not helpful in cracking the external coating of the conidial walls. Also, direct digestion of the conidia with perchloric acid was found to be difficult. After several trials, rupture of conidial wall was found to be possible with the help of sonic oscillation. The detailed procedure, as has been stated below, is a little modification of Kogane and Yanagita. (Isolation and purification of DNA from *Aspergillus oryzae* conidia—(J. Gen. Appl. Microbiol, Vol. 40, No. 1, 1964).

D4.2.1. *Sonication of conidia* :

At first 820 ml. of thick conidial suspension was centrifuged out. After centrifugation wet weight of conidia was 14·9 gms with plate count of $8{\cdot}5\times10^8$/ml. Its dry wt. was (under vacuum drying) 0·9 gms. These conidia were taken and 17 ml of saline EDTA (0·15 *M* NaCl containing 0·1*M* ethylene diamine tetra acetate, *p*H 8·0), 1·2 ml of 25% Na-Lauryl sulphate and 17 gms. of glass beads were added, and the conidia sonicated under ultrasonic vibration (18,000 to 20,000 C.P.S.) for 60 mins at 0°–4°C with interval of 5 mins in the middle after 30 mins of first sonication. The suspension was separated from the glass beads by decantation.

D.4.2.2. *Isolation of Nucleic acids*

Water was added to the conidial suspension to make NaCl conc. 0·1 *M*. It was then shaken in a wrist action shaker for 60 mins for better dispersion of nucleic acids present within the conidia. Then this was shaken at 8°C for 10 mins with an equal vol. of 75% phenol. After centrifugation of the mixture upper aqueous phase was collected and phenol was eliminated by adding 5 portion of ether, which was removed finally from the soln. by air bubbling. NaCl was added to this soln. to make NaCl conc. equal to 1 *M*. 2 portion of 95% ethanol was then gently poured into the soln. Gentle mixing of the double layered solution for some period gave rise to white precipitates of nucleic acid along with some salts. The precipitates were immediately removed by centrifugation. Precipitates, collected in

TABLE 18

Bases Moles per cent from conidia of A. terreus

Bases	Moles per cent	$\frac{A+T}{G+C}$
Adenine	25.26	
Guanine	18.61	
Thymine	23.40	1.15
Cytosine	23.66	
Uracil	9.04	

this way did not contain fibrous nucleic acid possibly due to disruption of polynucleotide by sonication.

Amount of DNA collected from the dry conidia was found out by diphenylamine reaction. It was 2·6%, also, the moles percentage of bases were found out by two dimensional paper chromatography. Solvent systems used for 1st run was Isopropanol : HCl : H_2O (170 : 41 : 39) and for 2nd run it was *n*-butanol : Glacial acetic acid : H_2O (80 : 12 : 30).

D.4.3. *Action of Nitrous acid on H-13-3 and isolation of Penicillin-resistant strains.*

Freshly grown H-13-3 cells in exponential phases were treated with Nitrous acid (·017 *M*) at *p*H 4·5 for 0, 2, 4, 6, 10 and 12 mins respectively. The reaction was terminated by adding 1 ml of treated sample to 100 ml CM-medium in 250 ml flask at *p*H 7·0 and incubated for 24 hrs. Platings were done on CM-agar and CM agar containing 10 μg/ml of streptomycin (Str_{10}) and 200 I.U./ml of Penicillin (P_{200}). Results were taken after 24 hrs of incubation at 30°C.

It could be concluded from Table No. 19 that HNO_2 induced ten times increase in Penicillin resistant mutations with 2 mins. treatment with Nitrous acid as compared to untreated sample as well for samples treated for more than two minutes. No mutation occurred for Streptomycin resistance. This shows that Nitrous acid is incapable of producing alteration in the gene structure concerned with the determination of resistance to streptomycin. The 2 biochemical mutants which required organic nitrogen for their growth were unstable and reverted back to the wild type.

TABLE 19

Effect of Nitrous acid H-13-3

Conc. of Nitrous acid	Time of treatment in min.	% Survival	% Mutation		
			Str_{10}	P_{200}	Biochemical
.017*M*	0	100	Nil	2.1×10^{-5}	Nil
,,	2	0.1200	% ,,	2.7×10^{-4}	2
,,	4	0.0300	,,	2.9×10^{-5}	Nil
,,	6	0.0200	,,	2.6×10^{-5}	,,
,,	8	0.0010	,,	8.9×10^{-5}	,,
,,	10	0.0070	,,	% Nil	,,
,,	12	0.0050	,,	,,	,,

D.4.4. *Mutagenic action of N-methyl N'-nitro-N-nitrosoguanidine (NTG) on Rhizobium.*

The freshly grown H-13-3 cells on exponential phase were diluted (50 fold) and treated with different conc. of NTG and incubated for 18 hrs at 30°C. After removing the NTG by centrifugation and washing with normal saline, platings were done on CM-agar and CM-agar containing 10 μg/ml streptomycin, 50 I.U./ml Penicillin and 100 μg/ml chloromphenicol. Results were taken after 48 hrs of incubation at 30°C.

It could be summarised from the Table 20, that NTG treatment induced an overall ten times increase in the streptomycin resistant mutations as compared to untreated samples. Increase in Penicillin resistant and chloramphenicol resistant mutations were also observed but here the increase was very negligible.

TABLE 20

Percent survival and percent mutation in Rhizobium (strain H-133) at different concentrations of NTG.

Conc. of NTG μg/ml	Time of treatment in hrs.	% Survival	% Mutation		
			Str_{10}	P_{50}	Chl_{100}
0	18	100.0	1.7×10^{-6}	3.3×10^{-4}	1.2×10^{-4}
100	,,	98.2	1.4×10^{-5}	3.6×10^{-4}	2.2×10^{-4}
500	,,	96.2	6.0×10^{-5}	4.6×10^{-4}	3.3×10^{-4}
1000	,,	73.2	3.2×10^{-5}	5.9×10^{-4}	3.5×10^{-4}

D.4.5. *Effect of prolonged NTG treatment on Rhizobium*

The bacterial strain used was *R. lupini* H-13-3. The freshly grown cells of H-13-3 were incubated in a semisynthetic medium for 18 hrs at 30°C. After washing with normal saline, the platings were done on CM-agar plates as well as CM agar plates containing different concentrations of NTG. The plates were incubated for 48 hrs. at 30°C.

TABLE 21

Killing effect of prolonged NTG treatment

Conc. of NTG μg/ml	Time of treatment in hrs.	Total no. population plated	% survival	No. of colonies transferred to MM from CM	No. of Biochemical mutants isolated
0	48	5.1×10^{8}	100	150	Nil
1	,,	,,	100	150	Nil
5	,,	,,	100	150	Nil
10	,,	,,	16.9	150	1(a)
20	,,	,,	1.6	150	1(b)

(a) This mutant required proline for growth.

(b) This mutant required guanine for growth.

No killing was observed at 5 μg/ml of NTG. But as the concentration was increased to double, a sudden increase in killing was observed.

Two biochemical mutations were observed but these were unstable and reverted back to wild type after a few generations. In this experiment killing was observed at 10 μg/ml of NTG because some of the cells in solid agar were killed immediately, and only a few which formed colonies proliferated at their points of inoculum, as compared to liquid medium (Table 21) where even if, a few cells died immediately at 10 μg/ml concentration of NTG those that lived multiplied logarithemically after some time and thus were capable of forming innumerable colonies.

TABLE 22

Effect of continuous NTG treatment of H-13-3 (a strain of Rhizobium lupini)

No. of transfer	No. of cells/ml of 0 hrs. of incubation	No. of cells/ml after 20 hrs. of incubation		No. of colonies transferred to MM from CM plates		No. of Biochemical mutants isolated	
		In medium with NTG	In medium without NTG	From NTG containing medium	From medium not containing NTG	From NTG containing medium	From medium not containing NTG
1st	7.4×10^6	6.5×10^8	8.8×10^8	150	150	Nil	Nil
2nd	1.3×10^7	9.4×10^8	1.3×10^9	200	300	,,	,,
3rd	1.8×10^7	2.6×10^9	1.3×10^9	300	300	,,	1
4th	5.2×10^7	1.7×10^9	1.4×10^9	300	300	1+	Nil
5th	3.4×10^7	6.5×10^8	7.1×10^8	200	200	1	,,
6th	1.3×10^7	5.2×10^8	5.6×10^8	150	150	Nil	,,

+ Only one stable biochemical mutant was isolated which was designated as N_4B_4. Its growth requirement could be satisfied by either adenine, guanine, uracil, xanthine or hypoxanthine. The other 2 biochemical mutants were unstable and reverted back to the wild type.

D.4.6. *Effect of continuous NTG treatment on Rhizobium*

Freshly grown cells of H-13-3 in exponential phase were diluted 50 folds and incubated in semisynthetic medium containing 10 μg/ml of NTG for 20 hrs. After 20 hrs the treated sample was again diluted 50 folds in 2 sets. One set was incubated in semisynthetic medium containing 10 μg/ml of NTG and other set in ordinary semisynthetic medium without NTG. The treatment was continued likewise for six days. Platings were done after each treatment. Velvet replications from CM to MM were made to isolate biochemical mutations.

It is clear from Table 22, that 10 μg/ml of NTG did not effect much the growth of the strain concerned, though it seems that prolonged treatment with NTG may induce biochemical mutations.

A few streptomycin resistant mutants were isolated and used for transformation studies.

D.4.7. *Transformation with penicillin—resistant mutant strain M_2P_{50}*
(The strain resists 500 I. U/ml of pencillin)

Transformation experiments were carried out between H-13-3 and M_2P_{50} a penicillin resistant mutant of H-13-3 isolated in one of the earlier experiment. The former was used as recipient and the latter as donor strain. DNA was isolated from M_2P_{50} strain according to the method of Marmur (J. Mol. Biol., 1961).

The transformation mixture was prepared with freshly growing recipient cells (about 10^7 bacteria/ml) and 0·1 ml DNA of the donor strain. Contact between donor DNA and recipient cells was allowed for 18–20 hrs. at 30°C, without any disturbance. Control set was similarly made without the addition of DNA. Platings were done on CM agar as well as CM agar containing 50 I.U./ml of Penicillin. Results were taken after incuabation for 48 hrs. at 30°C. The transformants had the same replication pattern as M_2P_{50}.

It is clear from Table 23, that transformation had taken place. (Few penicillin resistant colonies were observed in DNA untreated samples but these were eighty times less than the DNA treated samples and were most probably spontaneous mutations. Eighty times more colonies in the DNA treated sample does signify that the DNA from resistant strain had a role in inducing resistance in the sensitive strain.

TABLE 23

Transfrormation of Penicillin sensitivity to Penicillin resistance

Recipient : H-13-3. Penicillin sensitive
Donor : M_2P_{50} . Penicillin resistant

	Cells plated /ml	DNA added μg/ml	Frequency of Penicillin resistant colonies
a)	7.8×10^8	12.0	8.4×10^{-7}
b)	9.8×10^8	0.0	1.0×10^{-8}

D.5. Petroleum Microbiology

There have been numerous reports on the microbiological contamination in aircraft fuel system. The air force and civil air lines in most countries are now fully aware of the microbial growth, specially from fungal spores, carried by the fuel stream. The spores get lodged onto the inner coating of fuel tanks and damage is done to the organic emulsion of the coating by fungal growth thus making the metallic surface liable to corrosion.

D.5.1. *Microbiological investigation of aviation fuel*

The investigations on the microbiological contamination of aviation fuel is done by collecting samples from oil lines under pressure. The samples are to be obtained free from extraneous contamination and for that adequate precautions are taken.

A sampler fitted with mill pore attachment with membrane filter is attached to the delivery line and 850 ml of aviation fuel is passed through the monitor. The membranes are then transferred onto agar plates in the laboratory and incubated for 96 hours, at 30°–32°C.

The development of the colonies on agar plates are examined and count is taken to ascertain the extent of contamination. The results obtained from a series of microbial tests are given in Table 24.

TABLE 24

Microbiological analysis of aviation fuel

Sample No.	Total count	Group Identification
1	0	—
2	0	—
3	2	A 2
4	6	A 5, P 1
5	0	—
6	12	A 8, P 2, U 1.
7	14	A 5, P 8, U 1.
8	16	A 10, P 6.
9	0	—
10	4	A 2, U 2.
11	5	A 3, U 3.
12	13	A 9, P 4.
13	5	A 3, U 3.
14	6	A 4, U 1.
15	0	—
16	1	A 1.
17	2	A 2.
18	0	—
19	0	—
20	3	P 2, U 1.
21	2	P 2.
22	0	—
23	1	A 1.
24	2	A 2.

0 —No. colonies developed.
— —None
A —*Aspergillus* spp.
P —*Penicillium* spp.
U —Unidentified spp.

D.5.2. *Microbial count of filter membrane*

The results indicate that there has been a seasonal variation of microbial contamination of aviation fuel as evidenced from the colonies appearing on culture plates. From the culture plates isolated colonies were tested and a tentative identification showed that there was predominance of *Aspergillus* spp. and *Penicillium* spp. There were some unidentified colonies as well. The appearance of high number e.g. 16 (sample 8) was an indication of recurring contamination and precautions were immediately taken by cleaning the tanks.

This microbial test enabled the aviation fuel suppliers to take immediate (precautions) against corrosion of fuel tanks which might produce disastrous effect on flying aircrafts.

D.6. Process Design of Fermentation equipment for citric acid production

D.6.1. *Design of glass fermenter.*

The design of glass fermenters are based on the construction of tall column-type vessels equipped with aerating system and stirrers of impeller-type. The vessels are made of tall 'corning' glass cylinders cap. 2 lit with thick rubber stoppers for housing air spargers, inlet and outlet tubes for spent gases and an impeller in the central position. The glass vessels are arranged in circular metal disc with all equipments to hold them in vertical position. The whole assembly was kept in a water bath at constant temperature for incubation. Filtered air at constant pressure could be made to pass through the liquid column, the bubble size being small to ensure greater absorption.

D.6.2. *Citric acid production with cane sugar and deionized molasses.*

The production of citric acid in media containing refined cane sugar and high grade molasses as carbon source was done successfully. Ammonum carbonate was found suitable as a nitrogen source and decationized water was added for keeping the substrate at an extremely low iron concentration. The glass fermenters were inoculated with pre-germinated spores at the rate of 4–6 percent of seed culture and pellet count per litre of the medium as $150–300 \times 10^3$ after 24 hours. The character of pellets was noted and their growth in aerated medium was followed upto 36–48 hours of fermentation. Antifoam solution was added during the early stage of fermentation and foam troubles were reduced by periodic addition of antifoam solution.

D.6.3. *Rate of citric acid production.*

The samples of the fermentation liquid were withdrawn at intervals of 12 and 24 hours. Total acid was estimated by titration of samples with $N/10$ NaOH standard solution and citric acid was determined colorimetrically. The percent sugar was estimated to determine the conversion ratio as follows :

$$\frac{\text{gm citric acid} \times 100}{\text{gm available sugar}}$$

As a check presence of oxalic acid in the fermented liquid was determined. The process of removing oxalic acid was adopted whenever required. The sugar concentration in the medium was 14 per cent and initial *p*H was 2·5. The results are given in Table 25. The fabricated apparatus had smooth operation and the process thus developed could be stepped upto Pilot scale. The elimination of Fe and Mn from the medium served very useful purpose in reducing the incubation time and increasing the conversion ratio, two cardinal factors of considerable interest in industry.

TABLE 25

Fermentation of citric acid with deionized cane molasses

Sugar : 12.5% Time : 120 hr
Volume : 1500 ml Sample : 2(*N*)

Fermenter % No.	Per cent			
	Final acid (Total)	Anhydrous citric acid	Residual sugar	Conversion—Available sugar to citric acid
1	10.2	8.2	1.84	65.6
2	19.5	8.5	2.00	68.0
3	10.0	8.1	1.92	64.8
4	10.2	8.2	2.00	65.6
5	9.6	7.7	2.00	61.6
6	10.0	8.1	1.90	64.8
7	10.4	8.4	1.80	67.2
8	10.6	8.6	1.41	68.8
9	9.8	7.9	2.40	63.2
10	9.8	7.9	2.30	63.2
11	10.5	8.5	1.30	68.0
12	10.6	8.6	1.25	68a8

Results are average of 2 runs under identical conditions.

D.7. Ecology of air microorganism

D.7.1. *Physiological studies on* Sordaria hypocoproides, Ceratocystis paradoxa *and* Stachybotry *sp. isolated from air.*

Soradaria hypocoproides, *Ceratocystis paradoxa* and *Stachybotrys* sp. showed negligible growth in a synthetic medium viz. Czapek Dox, while they showed normal growth when grown in organic medium with malt extract agar. The behaviour was attributed to some biochemical deficiency already existing among them. The growth factor requirements of the strains were therefore studied by the 'auxanographic' method.

A heavy spore suspension was prepared and distributed in a number of plates containing minimal CD medium which is a nutritionaly deficient agar medium. Among the possible required nutrients Vitamin B-Complex, Casein hydrolysate, Yeast extract and nucleic acids were used in the present study; the nutrients were spoteed on four loci of the agar plate. Only a single drop was sufficient to produce the required reaction. The plates were incubated at 35–37°C. After 7 days of incubation the plates were examined and the results were obtained as follows.

Sordaria hypocoproides showed good growth only on the locus where Vitamin B complex was added. *Sordaria hypocorpoides* was further studied with each of the constituents of Vitamin B-complex and a fair growth was obtained only on the spot where thiamine was added. Thus it was found that *Sordaria hypocoproides* was deficient in thiamine.

Ceratocystis paradoxa responded to casein hydrolysate and was seen on the spots where nutrient was added. However, growth was also noticed on nucleic acid and yeast extract loci. The growth of *Stachybotrys* sp. was stimulated with Vitamin B-complex.

D.8. Control of Fusarium wilt of pigeon pea by pure antibiotics and by antibiotic-producing microorganisms

D.8.1. *Effect of rootdip application of antibiotics on the control of wilt disease of pigeon pea.*

Rootdip application of antibiotics was followed for both therapeutic and protective purposes against the invasion of pigeon pea by *Fusarium udum* causing wilt disease. Two methods of treatment were followed :

Treatment of the seedlings with antibiotic was done 24 hours after inoculations. The treatment was therapeutic, and the treatment of the seedlings with antibiotics, prior to inoculation was administered as protective.

Seedlings were inoculated with spore suspension of *F. udum* containing 5×10^6 spores/ml, by immersing the roots of the seedlings into the suspension for 24 hrs. After the respective treatments (protective and therapeutic), the seedlings were examined regularly for wilting symptoms. The results are presented in tables 26 and 27.

TABLE 26

Protective effect of antibiotics on the wilt disease of pigeon pea

Antibiotic treatment (μg/ml)		Days of observation: Disease value 5	7	9	11	13	15		Days of observation: Percent control 5	7	9	11	13	15
Griseofulvin	0	0	0	3	9	10	18		—	—	—	—	—	—
	25	0	0	0	0	2	5	%	100	100	100	100	80	72.2
Boseimycin	0	3	9	15	%22	25	25	%	—	—	—	—	—	—
	25	0	6	17	22	25	25		100	33.3	0	0	0	0

TABLE 27

Therapeutic effect of antibiotics on the wilt disease of pigeon pea

Antibiotic treatment (μg/ml)		Days of observation: Disease value 5	7	9	11	13	15	Days of observation % : Percent control 5	7	9	11	13	15
Griseofulvin	0	7	8	14	20	20	20	—	—	—	—	—	—
	25	5	5	5	5	5	5	28.5	37.5	45.0	75.0	75.0	75.0
Boseimycin	0	11	14	15	16	19	20	—	—	—	—	—	—
	25	10	12	13	14	17	18	9.0	14.2	13.3	12.5	10.5	10.0

It appears from the results, that griseofulvin could be a good prorective as well as successful therapeutic agent. In protective rootdip treatment griseofulvin provided full

control of the disease upto 11 days. The antibiotic persisted within the plant for 21 days. Significant deterioration of the antibiotic was found to occur as treatment period continued resulting in increased disease intensity and thereby lowering the per cent control of the disease. As a therapeutic agent griseofulvin could provide better stand of the seedlings. In post inoculation treatment, only one seedlings was found dead on the 5th day, while the wilt symptom appeared on the 3rd day. Appearance of the wilt symptom was however delayed by the protective treatment upto 11 days as the effective concentration of the antibiotic was present within the host. In case of therapeutic treatment the antibiotic was not present initially within the host and as a result there was no delay in the expression of the wilt symptoms and the disease value was approximately equal to that of the untreated control at the beginning. With continued treatment the antibiotic was found to be translocated to the seedlings and the disease value remained more or less constant providing ultimately 75 per cent control of the disease which did not vary considerably from the protective treatment.

Boseimycin as a protective treatment, could not afford any protection of the seedlings against the invasion of *F. udum*, though initially some protection was given by the antibiotic ultimately the disease value became equal to that of the untreated control. As a therapeutic agent boseimycin was not significantly effective, the ultimate disease-control being only 10 per cent.

D.8.2. *Soil application of the antibiotics for the control of wilt disease of pigeon pea.*

Griseofulvin and fillipin were applied to soil as solution for controlling the wilt disease of pigeon pea. In 4 inch clay pots 400 gm of soil was sterilised and 100 g. inoculum of *F. udum* prepared in sand-maize meal medium was mixed with it and kept as such for 48 hours. Pigeon pea seedlings raised in soil were uprooted when they are 15-day old washed to remove soil particles and transplanted at the rate of 2 plants per pot. The antibiotics were treated at the rate of 50 ml on each day per pot, while the control treatments received equal quantity of water. The seedlings were observed regularly to note the appearance of wilting symptom and the results are presented in Table 28.

TABLE 28

Effect of antibotics as soil drench on the control of Fusarium wilt of pigeon pea

Antibiotic treatment (μg/ml)		Days of observation									
		8	10	12	14	16	8	10	12	14	16
		Disease value					Percent control				
Griseofulvin	0	4	9	34	54	70	—	—	—	—	—
	50	1	3	15	34	40	75.0	66.6	55.8	37.0	42.0
Filipin	0	11	15	16	22	25	—	—	—	—	—
	25	4	7	10	10	10	63.6	53.3	44.5	54.5	60.0
	50	8	14	23	25	25	27.2	16.6	0	0	0

In case of treatment with griseofulvin typical wilt symptom appeared in the control as well as in the treated pots on 8th day and the number of seedlings which succumbed to the disease was greater in the control compared to the plants receiving antibiotic treatment. The ultimate control of the disease by griseofulvin treatment was however 42 per cent. With filipin, expression of wilt symptom was delayed to some extent, especially with 25 μg/ml. Incidence of the disease was much lower in case of treatment with the above concentrations and 60 per cent control of the disease was attained. In case of 50 μg/ml of filipin, though initially some control was obtained, it was observed that the disease value became higher than the untreated control, and the higher percentage of mortality was due to phytotoxicity caused by the antibiotic.

D.8.3. *Biological control of wilt disease of pigeon pea caused by* Fusarium udum *through antagonism.*

Biological control of plant diseases may be defined as any condition under which, or practice whereby, survival or activity of a pathogen is reduced through the agency of any other living organism resulting in the reduction of the incidence of the disease caused by the pathogen. The antagonistic organisms selected for the investigation were *Penicilliium nigricans*, *Streptomyces filipinensis* and *Streptomyces* sp. Ac_6 569 producing the antibiotics—griseofulvin, filipin and boseimycin respectively. Inoculum of the organism were prepared in supplemented soil culture and that of the pathogen (*F. udum*) in sand maize meal medium. The soil in clay pots was preinoculated with the antagonists and incubated for 7 days after which the pathogen was added. After 15 days of incubation a lot of 15-day old pigeon pea seedlings were transplanted in the pots—2 in each pot. The growth of seedlings was examined at regular intervals—the results are presented in the Tables 29, 30 and 31.

TABLE 29

Control of Fusarium wilt of pigeon pea by soil inoculation with P. nigricans

Treatment	Days of observation											
	6	8	10	12	14	16	6	8	10	12	14	16
	Disease value						Percent control					
C	5	13	25	26	30	40	—	—	—	—	—	—
T	3	6	16	16	16	16	40.0	53.8	36.0	38.4	46.6	46.6

TABLE 30

Control of Fusarium wilt of pigeon pea by soil inoculation with Streptomyces sp. Ac_6 569

Treatment	Days of observation											
	6	8	10	12	14	16	6	8	10	12	14	16
	Disease value						Percent control					
C	1	9	17	21	25	25	—	—	—	—	—	—
T	0	2	8	15	17	25	100.0	77.7	52.9	28.5	28.0	0

TABLE 31

Control of Fusarium wilt of pigeon pea by soil inoculation with Streptomyces filipinensis

Treatment	Days of observation											
	6	8	10	12	15	16	6	8	10	12	14	16
	Disease value						Percent control					
C	2	6	16	24	30	30	—	—	—	—	—	—
T	2	6	15	24	28	30	0	0	6.2	0	6.6	0

It is evident from the results that *P. nigricans* had considerable effect in controlling the disease. Intensity of the disease was less as is expressed in the disease value presented in Table 29 and ultimately 46·6 per cent control of the disease was afforded by the treatment. In the treatment with *Streptomyces* sp. Ac_6569 development of the disease was delayed to some extent but ultimately no control of the disease was noted (Table 30). *S. filipinensis* was not found to be at all effective in the reducing the disease intensity (Table 31), wilting pattern and subsequent death of the seedlings were similar to that of the untreated control.

D.9. Mass-scale production of Rhizobium inoculant cultures

D.9.1. *Effect of carbon source on cell multiplication*

The production of Rhizobium inoculant culture was undertaken employing various Carbon sources in the fermentation medium. The response of the bacteria towards sucrose and mannitol was demonstrated in a composition containing yeast extract and a few essential minerals. In the basal composition of this medium mannitol was substituted with sucrose, molasses, malt extract (both in powder and liquid form) in equivalent proportions. The Rhizobial strains for groundnut (*Arachis hypogeae*) and soyabean (*Soy max*) were studied for determining the optimum conditions for growth and cell multiplication. The cultures were at first grown under shake culture condition in a rotary shaker in conical flasks (500 ml cap.) at 28°C for 6–7 days. The standard medium was yeast extract mannitol medium and the modifications are made as follows (Table 32). The count of viable cells were taken at the 7th day of incubation and the results are compared.

The results presented in Table 32 show that molasses could serve as a superior substrate for the growth of both the strains of Rhizobium. Liquid malt ext. could be ranked next in the list and the third one was medium having sucrose and mannitol in combination. However, after studying the storage condition of the liquid culture, it was noticed that in molasses medium the viability of cells diminishes quickly. The next best medium was malt ext. liquid the cost of which was prohibitive for large scale culture of bacterial cells. The medium with sucrose and mannitol in combination was finally selected on consideration of its moderate cost and suitability for the storage as the production of gum served as protective agent for live bacterial cells in liquid medium.

D.9.2. *Large-scale growth of Rhizobium in fermenters under aerated conditions.*

The fermenters were constructed with 4 flat bottom flasks cap. 10-litres each. Four such flasks were incubated in a tank waterbath fitted with all mechanical device to keep the flasks in vertical position and water temperature was maintained at 30±1°C employing a sunvic thermostat control system. Each unit of the battery of glass fermenters with 7·5 litre of the liquid medium were given sterile air by sparging at the rate 3–4 litre of air per minute per flask. The pressure of inflow air was 5–7 lbs/sq. inch. Frothing of the fermented liquid was a problem but addition of paraffin oil in small volumes was sufficient to control rising of foam in the flasks. The aeration was continued for 96–120 hours. In general the cell count was found to attain 2×10^9 within 96–100 hours provided the air flow was maintained in undistributed condition. The production schedule was 120 litre in 10 days commissioning 2 fermenters. Thus the monthly output was 360 litres of bacterial culture. The baceterial cultures of Rhizobium were quite thick and syrupy and each batch was pooled into 20 litre pyrex storing jars. The culture slurry was either distributed into 100 ml polythene bottles or mixed with soil-base for distribution to farmers.

TABLE 32

Growth of Rhizobium in liquid media under shake culture condition (Incubation period 144 hours)

Medium (Basal)	Carbon source	gm/lit.	Cell count × 10^8/ml	
			Rhizobium strain	
			Groundnut	Soybean
1. Yeast ext.+Minerals	Manntol	10	16.0	1.300
2. Yeast ext.+Minerals	Comml. sugar	10		
	Manntol	8	18.0	2.500
3. Molasses (cane)	Sucrose	10	120.0	27.000
4. Malt extract (liquid)	Maltose	10	61.0	1.400
5. Malt extract (dry)	Maltose	10	2.3	0 026

D.9.3. *Viability of Rhizobium in soil base medium and liquid culture*

The mass-scale culture production was followed by a study of viability of bacterial cells under conditions of storage of variable temperature. The cultures after production were stored at two different temperatures viz., (a) room temperature 25–35°C and cold room below 4–8°C. The shelf-life of cultures were thus determined several times upto 270 days.

D.9.3.1. *Soil-base culture and storage test.*

This soil base was prepared with rich garden soil 80 parts, sucrose—2 parts, green plant material 15 parts and $CaCO_3$ 3 parts. The mixture was allowed to decompose thus forming a good organic soil base and the mass was finally air-dried, crushed and seived. The seived material was heated in an oven at 120°C for 1 hour to kill mainly bacterial vegetative forms. The soil-base was finally mixed with the required culture liquid and packed in polythene bags

or in wide-mouthed bottled and kept in the storage. The viable counts of cells of Rhizobium were taken at intervals of 15, 30, 90, 180 and 270 days of storage. The results obtained are given in Table 33.

TABLE 33

Viability of Rhizobium cells during storage

Days of storage	Rhizobial culture			
	Liquid	Soil-base	Liquid	Soil-base
	Temperature 25–35°C		Temperature 4–8°C	
0	2.1×10^9	5.5×10^7	2.1×10^9	5.6×10^8
15	3.3×10^8	8.5×10^6	1.5×10^9	4.1×10^8
30	1.9×10^9	3.8×10^8	3.6×10^9	3.2×10^8
90	4.7×10^6	1.3×10^7	2.2×10^7	1.2×10^7
180	7.2×10^5	5.6×10^6	5.6×10^5	1.9×10^6
270	1.2×10^4	3.2×10^4	1.9×10^5	2.8×10^5

The cell count started to increase in enriched soil base medium at the room temperature during the first 15 days followed by a gradual reduction untill the end, the count being reduced from $5{\cdot}5\times10^7$ to $3{\cdot}2\times10^4$. The storage of culture at 4–8°C followed the same course of events except that there was stimulation of growth. The reduction in the cell count was slower and last count was $2{\cdot}8\times10^5$ after 270 days in the case of soil base culture.

D. 10. Microbial Decomposition of Organic matter in Indian soils under different climatic conditions.

D.10.1. *Physico-chemical and microbiological properties of soils of Orissa.*

Soils collected from 0–6″ depth from Maurbhanj, Bhubaneswar, Chakuli, Sambalpur, Cuttack, Berhampur and Balangir were analysed for the following physical and chemical properties :

(a) Maximum water holding Capacity—varies from a minimum of 22% in deltaic alluvial soil of Berhampore to 50% in black soil of Balangir.

(b) Soil reaction—*p*H of the soils varies from 5·30 in Bhubaneswar laterite to 7·65 in Balangir black soil.

(c) Cation exchange capacity—varies from 3·5 m.e./100 g. soil in Balangir black soil.

(d) Organic Carbon—varies from 0·693% in Maurbhamj red to 0·339 in Alluvial soils of Cuttack and Berhampore.

(e) Ammoniacal and Nitrate nitrogen—ammonium nitrogen varies from 5·25 ppm in Sambalpur latosol to 11·5 ppm in Balangir black and Chakuli laterite. Nitrate nitrogen ranges from 6·65 ppm in Chakuli to 15·80 ppm in Sambalpur.

(f) Available phosphorus—ranges from 2 phm in Balangir black to 10 ppm in Cuttack alluvium.

(g) Total nitrogen—varies from a minimum of 0·0304% in black soil of Balangir to 0·1340% in alluvial soil of Cuttack.

Soils from the above places were analysed for population of Bacteria, Fungi and Actinomycetes. There is wide range of variation in the total and individual population count, which is particularly pronounced with respect to the population of Bacteria and Actinomycetes. No significant generalisation about the relationship of population of microflora with either *p*H, organic mattter, or any other physico-chemical property of the soils could be obtained.

D.10.2. *A comparative study on the loss of native carbon from different soils of Orissa and West Bengal.*

The experiment was conducted to compare the rate of carbon loss from different soils of Orissa and West Bengal under optimum moisture and temperature conditions. The soils can be arranged in respect to the loss of native carbon as follows :

West Bengal : Alluvial soil of Mandauri > alluvial soil of Kalyani and forest soil of Kalimpong > deltaic alluvial of Kadkwip > deltaic alluvial of Canning > laterite soil of Sriniketan > red soil of Purulia.

Orissa : Laterite soil of Chakuli > black soil of Balangir > coastal alluvium of Cuttack > deltaic alluvium of Berhampore > laterite soil of Bhubaneswar > red soil of Baripada. No relationship is found between the organic carbon level of the soil and the rate of carbon loss, but *p*H of the soil is found to have an important role in determining this rate.

D.10.3. *Effect of inorganic fertilisers on the decomposition of native as well as added organic matter.*

The experiment was undertaken to study the effect of nitrogenous and phosphatic fertilisers alone and in combination on the decomposition of native soil organic matter as well as added organic matter (Rice husk). An alluvial soil of Kalyani University Farm was used for this purpose. There is no significant difference among the treatments with regard to CO_2 evolution and high dose of fertiliser application has no significant effect on the loss of native carbon as well as added carbon.

D.10.4. *Study on the decomposition of organic matter under incubated condition by carbon dioxide evolution method.*

Laterite soil of Bhubaneswar and 5 organic matters namely, Dhaincha, Rice straw, Rice husk, Mahua cake and F.Y.M. were used in the study. Organic matters were added @ 0·5 gm. carbon per 100 gm. soil.

The experiment was done under aerobic and waterlogged conditions at an incubation temperature of 30°C. After 142 days of incubation highest amount of CO_2 is liberated in

the treatment Straw and Mahua cake followed by Rice husk, Dhaincha, F.Y.M. and control. Under waterlogged condition the evolution of CO_2 is significantly lower.

D.10.5. *Mineralisation of nutrients during decomposition of organic matter.*

The present experiment was undertaken to study mineralisation pattern of nutrients under incubated condition with organic matters as above.

If organic matters are added on equal carbon basis it would appear that F.Y.M. is superior to Dhaincha, Husk and Straw in maintaining the organic carbon level of the soil. Change in Ammoniacal Nitrogen content of soil with increasing incubation period do not follow any definite trend. It is noteworthy that at later stages level of Ammonical Nitrogen increases in control over Dahincha and F.Y.M. treatments. At all stages upto 84 days F.Y.M. is superior to control, Dhaincha, Husk and Straw in releasing Nitrate Nitrogen. Throughout the period of investigation level of nitrate nitrogen with straw and husk is usually lower than in the rest, and the trend of increase or decrease is very erratic. Throughout the process of inucubation, level of available phosphorus in control is always lower than in original unincubated soil. Compared with all other treatments, F.Y.M. shows highest amount of available phosphorus.

D.10.6. *Changes in microfloral population associated with decomposition of organic matter in soil.*

Addition of organic matter into the soil breaks down the existent biological equilibrium. With the onset and progress of incubation there occurs a quantitative as well as qualitiatve change in microfloral population. It may be concluded that among the treatments microbiological equilibrium is restored first and at faster rate with F.Y.M. This shows that organic carbon reserve of soil may be maintained better by the use of F.Y.M., Dhaincha, on the other hand, will suffer a carbon loss at a very fast rate initially.

D.10.7. *Physico-chemical and microbiological properties of soils collected from 20 years permanent manurial trial plots, Chinsurah.*

Effect of long term application of organic and inorganic fertilisation at various doses on physical, chemical and microbiological characteristics of soil has been investigated under this part of study. Continued inorganic fertiliser application lower down the *p*H which is further decreased with addition of F.Y.M. as organic manure. Continued application of inorganic fertilisers reduces the C.E.C. of soils which is partially restored wih high organic manuring. Nitrogen content of soil is seen to increase both with inorganic and organic manuring, more with the latter. It is to be noted that exclusive high inorganic fertiliser application will be of less valne than moderate doses in maintaining organic carbon and total nitrogen at a satisfactory level. Application of organic manure increases the availability of phoshorus while decreases the level of exchangable Calcium.

Population of bacteria and actinomycetes in treatments with organic manure has increased significantly over the control as well as over the inorganic fertiliser treatments;

changes in the fungal population were not of high order. Effect on azotobacter is adversely reflected.

D.10.8. *Changes in nutrient status, organic fractions and microflora population in soil on treatments with various organic matters.*

An alluvial soil (Kalyani University Farm) with F.Y.M., Dhaincha and Rice straw was incubated for 170 days which provided the scope of studying the effect of a long standing decomposition of organic matters on important soil properties.

Straw and F.Y.M. as well as control shows a slight increase in *p*H whereas with Dhaincha it remains constant. Per cent increase of organic carbon over the original soil as a result of incubation was as follows :

F.Y.M.—0·185%, Dhaincha and Straw—0·054%. There was an increase of ammonical and nitrate nitrogen as follows :

Treatment	*Ammoniacal*	*Nitrate*
Control	1·5 times	12 times
F.Y.M.	2·5 ,,	28 ,,
Dhaincha	2·0 ,,	70 ,,
Straw	2·2 ,,	9 ,,

Available phosphorus content of the soils increased with all the treatments including control. With F.Y.M. there was a fivefold increase whereas with the other two treatments and in the control the increase was marginal over the original soil. Organic carbon build up though less with Dhaincha than F.Y.M., the performance of F.Y.M. and Dhaincha are quite comparable with respect to release of phosphorus and potassium. It is interesting to note that decomposition of native organic matter in control helped to increase available nitrogen and phosphorus status in soil.

On equal weight basis humic acid contents of the soils increased maximum with the F.Y.M. followed by Straw and Dhaincha. Carbon content of humic acids isolated from the soil with the different treatments did not differ from either the control or among themselves whereas percentage composition of nitrogen and phosphorus differ among the treatments. Altogether 13 aminoacids could be traced on the chromatograms of humic acids from the four treatmens. Humic acids from control and Dhaincha treated soils yielded seven amino acids and those from F.Y.M. and Straw ten and nine respectively.

The colonisation pattern by the 3 groups of flora is as follows :

Fungi	: Straw	Dhaincha	F.Y.M.
Bacteria	: Dhaincha	F.Y.M.	Straw
Actinomycetes	: Dhaincha	F.Y.M.	Straw

Difference in the colonisation pattern of the organic matters by the groups of flora reflects the nutritional requirements of the groups.

E. ANIMAL PHYSIOLOGY

E.1. Amphibian Physiology

E.1.1. *Studies on tadpoles*

E.1.1a. *Physiological consequence of thyroxine-protein binding in tadpoles*

The physiological consequence of the binding of thyroxine (T_4) with thyroglobulin antibody and albumin was studied on a system directly involved in the dynamic preparation of the tadpole for the transition from a water-living larval stage to a land-living adult form. That the thyroxine-protein interactions can greatly influence the biological effects of the thyroid hormone is supported by the present study, which demonstrated that antithyroglobulin, by its capacity to retain thyroxine in a physiologically unavailable complex form, inhibited the inducing action of this hormone on the operation of the urea cycle in tadpoles and thus inhibited the resulting increase in urea excretion.

A single injection of thyroxine (0·10 nanomoles/g of body weight) to tadpoles markedly increased the urea-N excretion, upto about 350 μg/g/24 hours. Slight resorption of the tail (5–6%) was found after the injection of this dose of thyroxine. The thyroglobulin antibody (antigammaglobulin), isolated by ammonium sulphate precipitation from thyroglobulin immunized rabbit sera, inhibited significantly the thyroxine-induced urea excretion as well as tail resorption. Similar results were found with bovine albumin. Normal gammaglobulin fraction (isolated from normal rabbit serum) and gammaglobulin, Fr. II, however, did not inhibit the action of T_4 on urea excretion or tail resorption. In treated and untreated controls the urea excretion rose slightly, but still markedly less than that of T_4 injected tadpoles. No difference in the urea excretion was noted in tadpoles injected with the previously incubated thyroxine-protein mixture as compared to that in tadpoles injected with thyroxine and protein separately at an interval of 10–15 minutes.

The failure of induction of the urea cycle by thyroxine in the presence of thyroglobulin antibody or albumin is evidently due to the inability of the tissues to absorb or assimilate T_4 in a bound form. The complete suppression of the action of thyroxine by antithyroglobulin or albumin indicates that these thyroxine-protein complexes are not split in the peritoneal cavity, thus liberating free and intact T_4 during the period of experiment. The electrophoretic studies revealed that the binding of T_4 to thyroglobulin antibody is strong and not easily affected by mild denaturation or even when frozen for two years and thawed 3 or 4 times during this period, although the antibody titre is slightly reduced. In view of such strong binding the question arises as to how the different systems in thyroglobulin immunized animals are affected, or whether such animals show symptoms of hypothyroidism when high thyroglobulin antibody titres and the abnormal binding of T_4 at gammaglobulin region exst in their sera even after 420 days have elapsed after immunization. The detailed studies of the physiological consequence of thyroglobulin immunization of animals may throw light on how antithyroglobulin-thyroxine binding affects the different systems.

Such knowledge may give further understanding on the effects of circulating autoantibodies which have been demonstrated in various forms of thyroid disease and even in normal subjects.

E.1.1b. *Further studies on the growth of tadpoles (Bufo melanostictus and Rana tigrina)*

In continuation of the previous works on the effect of thyroxine on the stimulation of growth of tadpoles further studies were made to examine any increase in protein content in tadpoles showing thyroxine-induced increase in body weight, tail length and body length.

It has been found that thyroxine at a physiological dose shows anabolic effect in tadpoles, as evidenced from the increase in body weight, body length and tail length. The anabolic effect of thyroxine is also supported by the fact that this hormone increases the total body protein content in comparison to the controls.

The growth studies were made also by the treatment of the tadpoles with para-amino benzoic acid (PABA). Preliminary results showed that PABA, a member of B-group vitamins, accelerated growth and metamorphosis of tadpoles. The mechanism of action of this vitamin in tadpoles and how it affects the metabolism of liver cells are under study. Preliminary experiments were also undertaken to study the physiological role of the tail of tadpole with special reference to its metabolic pattern at various stages of development. It was observed that surgical amputation, regression or regeneration by chemical agents have a marked effect on the overall body metabolism, so far as general growth, metamorphosis and liver metabolism are concerned. Detailed studies are being made.

E.1.1c. *Effect of thyroxine on melanin content in liver of toad (Bufo melanostictus)*

The physiological action of thyroid hormone in poikilotherms has not yet been established. Attempts are being made in this laboratory to evaluate the physiological role of this hormone in toad (*Bufo melanostictus*) as well as in lata fish (*Ophicephalus punctatus*). It was noticed during the studies on the cellular changes of toad liver that melanin was very sensitive to thyroxine (T_4) administration. This and the previous observations on the changes of melanin content by thyroxine in tadpole liver prompted further study on melanin metabolism in toad liver after thyroxine (T_4) treatment. Moreover, there is no evidence that thyroid hormone affects melanin metabolism in toad liver. Both toad sexes were used to show any sex difference in response to thyroxine (T_4) with regard to the melanin content in the liver.

A single injection of thyroxine (0·1 μg, 1·0 μg or 2·0 μg per gram of body weight) caused a profound increase in melanin contents in liver of male toads (Fig. 1). The increase in the amount of melanin was found from the first day and sustained up to the eleventh day of the experiment (the day of injection is taken as zero day). On further lowering the dose level of thyroxine (0·01 μg per g, single injection), the melanin content in liver was also found to be significantly greater from the first to the sixth day of experiment in comparison to the controls. This increase in melanin, however, was less compared to the results obtained with the higher doses (0·1–2·0 μg/g). A dose level of T_4 as low as 0·001 μg/g (single

injection) was also effective in increasing the melanin content in liver of male toads. This increase, of course, was found up to the third day of the experiment in comparison to the controls (Fig. 1).

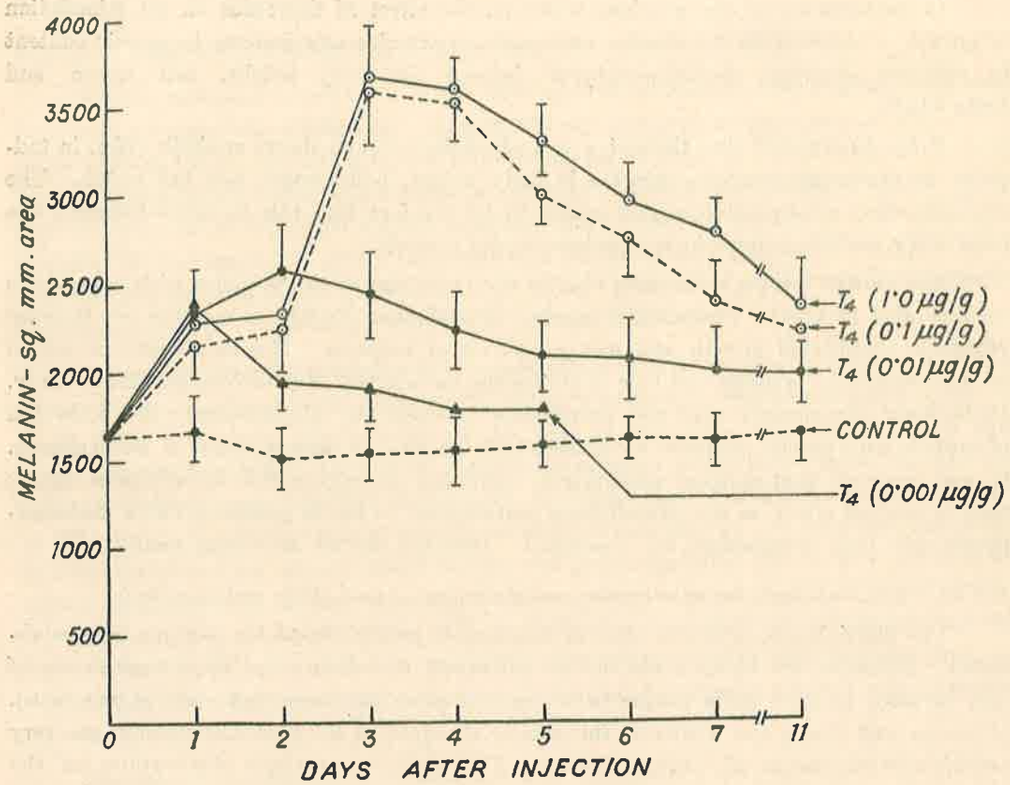

Fig. 1. Effect of various doses of thyroxine (T_4) on the melanin content of liver of male toad (*Bufo melanostictus*). A single injection of T_4 (0·001 µg, 0·01 µg, 0·1 µg or 1·0 µg) was given in the lymph sac at zero day. The sq. mm. area in the microscopic fields (magnified × 150) represents the amounts of melanin at different days of experiment. The toads were starved during the experimental period. A single injection of 2·0 µg of T_4 per g of body weight produced the same magnitude of response as obtained by a single injection of 1·0 µg/g.

The results of tyrosine are confusing and hence are not conclusive. After injection of thyroxine (1 µg/g) tyrosine in liver was found to increase slightly on the first day and thereafter no significant difference was observed in treated animals in comparison to the controls. With higher dose of T_4 (2 µg/g) this amino acid was found to decrease to a small extent on the first day after which no significant difference was noted in comparison to the controls.

The female toads did not show any increase in melanin content in their livers even after a single injection of 1µg or 2µg of thyroxine per g of body weight. With a higher dose level (4µg/g of body weight) twice the amount of melanin was found in liver of thyroxine treated female toads from the third to the sixth day.

Another peculiarity in response to thyroxine was found between the male and female toads. The male toads turned black and showed moulting within three days of thyroxine injection (1 µg/g). But the female toads did not show this response to T_4, even with a higher dose (4 µg/g). With lower doses of T_4 (less than 1 µg/g) the male toads did not show such moulting.

E.2. Piscine Physiology

E.2.1. *Thyroid hormone action in fish*

In view of the conflicting reports on the physiological action of thyroid hormones in poikilotherms (frog and fish) studies have been undertaken to show how lata fish (*Ophicephalus punctatus*) and toad (*Bufo melanostictus*) respond to the administration of exogenous thyroxine. The various aspects of the effects of treatments with thyroxine and thiourea on lata fish are under investigation.

It has been reported earlier from this laboratory that thiourea treatment produced well-marked goiter, characterized by hyperplasia and hypertrophy and complete depletion of colloid, in lata fish. It was also observed that thyroxine treatment caused a change in protein and RNA metabolism in liver of these animals. Liver weight also increased with thyroxine treatment.

E.2.1a. *Effect of thyroxine, triiodothyronine and tetraiodothyroacetic acid on NH_3-N and urea-N excretion in lata fish at different temperatures*

The lata fishes, purchased from the local suppliers at different seasons of the year, showed a variation of NH_3-N excretion, ranging from about 150 to 400 µg/g/24 hrs. at 25°C. Similarly urea-N excretion ranged from about 15 to 40 µg/g/24 hrs. A single injection of thyroxine at a dose level of 1 µg/g produced no alteration in NH_3-N as well as urea-N excretion at 25°C. With higher doses of T_4 (2 µg/g or 4 µg/g) mixed results (increase, decrease and no change in both NH_3-N and urea-N excretion) were obtained in different groups. When all the data with a particular dose of the hormone (2 µg or 4 µg/g) were pooled, no difference in nitrogen excretion was noted between the control and T_4 treated groups of animals.

By carefully scrutinizing of all the individual data at different days of the experiment, it was revealed that with a single injection of 2 µg of T_4 per g. of body weight about 53% of the experimental animals (lata fish) showed increased NH_3-N excretion and the rest 47% of the animals showed reduced NH_3-N excretion at 25°C. In case of urea-N, 21% and 54% of the animals showed increased and decreased excretion respectively. The rest 25% did not respond with respect to urea-N excretion with this dose of the hormone. With

higher dose of T_4 (4 μg/g, single injection) increased NH_3-N excretion was noted in 64% of the animals, decreased excretion in 23% and no response in 13% at 25°C. With respect to urea-N excretion with this dose catabolic action was found in 38% of the animals, anabolic action 43% and no change in 19%.

It is interesting to note that the animals excreting around 200 μg of NH_3-N per g of body weight in 24 hours before injection showed increased NH_3-N excretion after injection of 2 μg or 4 μg of T_4 per g of body weight at 25°C, while those excreting around 400 μg/g/24 hrs. before injection showed decreased NH_3-N excretion after injection of T_4 at this temperature. But a single injection of T_3 (4 μg/g) or TETRAC (4 μg/g) caused increased NH_3-N and urea-N excretion in all animals irrespective of the nitrogen excretory level before hormone treatment.

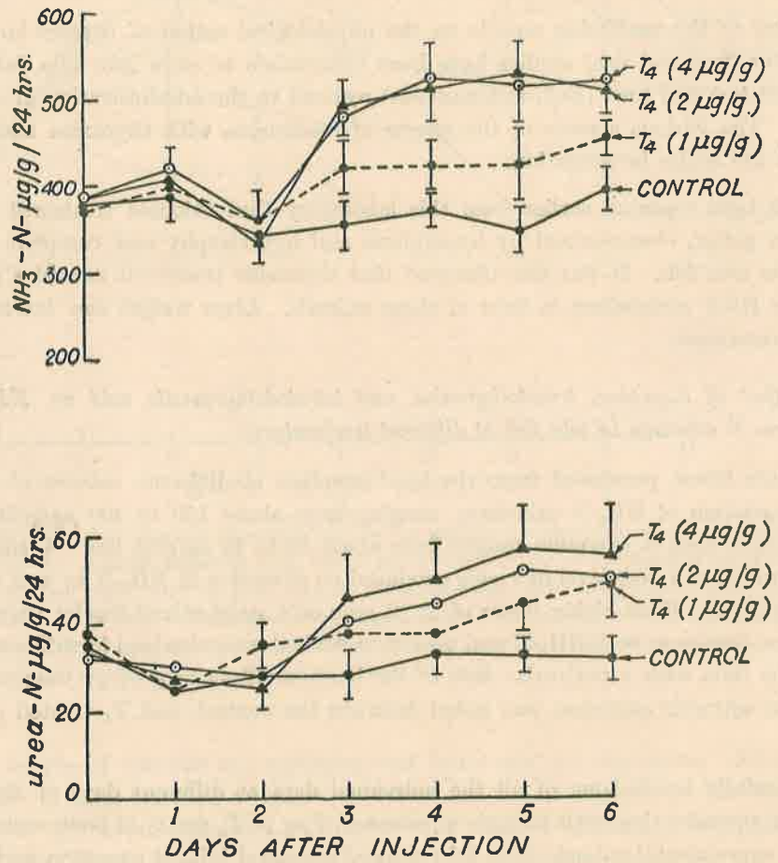

Fig. 2. Effects of various doses of thyroxine (T_4) on the NH_3-N and urea-N excretion in lata fish (*Ophi cephalus punctatus*) at 30°C. A single intra-peritoneal injection of T_4 was given at zero day. The animals were starved during the period of experiment.

The anomalous results, as mentioned above, were not observed when the lata fishes were kept at 30°C. Compared to the controls, increased NH_3-N and urea-N excretions were found with a single injection of 1–4 μg of T_4 per g of body weight in all animals at 30°C (Fig. 2). In normal animals rise of environmental temperature from 25°C to 30°C caused a significant increase of both urea-N and NH_3-N excretion. At lower temperatures (10°–20°C) nitrogen excretion significantly reduced (Fig. 3). It may be mentioned here that whenever

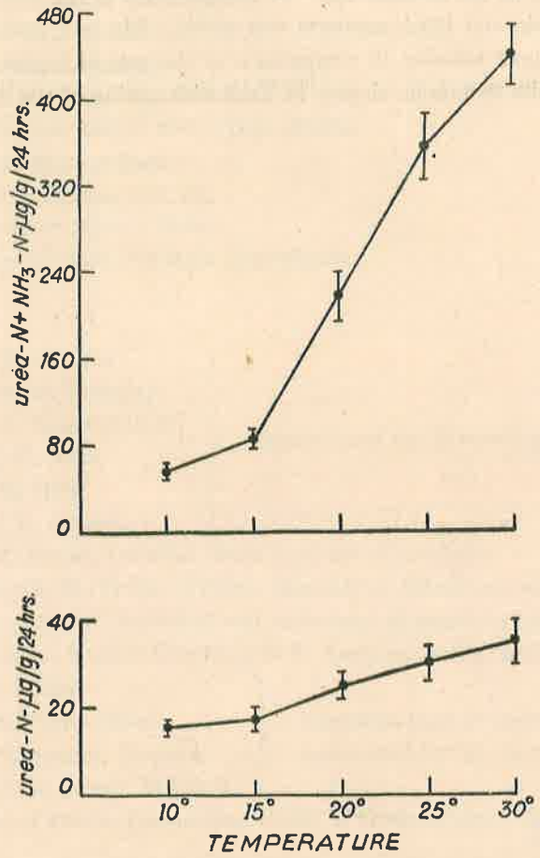

Fig. 3. Effect of variation of temperature on urea-N and urea-N+NH_3-N excretions in lata fish (*Ophicephalus punctatus*). The animals were starved during the period of experiment.

any increase or decrease in NH_3-N excretion, caused by hormone injection or by variation of temperature, the difference in NH_3-N excretion in comparison to the controls was found at the level of around 100 μg/g/24 hrs., which is statistically significant. When the animals (lata fish) were transferred from room temperature to 15°C, more than 50% reduction in NH_3-N excretion occurred.

E.3. Gerontology

E.3.1. *Studies on the nucleo-cytoplasmic interactions in liver of rats at different age groups*

The experiments have been undertaken to study the cell area and nuclear area in liver of rats at different age groups, 5 days, 15 days, 30 days, 60 days, 120 days, 1 year and 2½ years. Attempts are being made also to correlate the size of the cells and their nuclei with the protein and RNA content. Preliminary results indicate that the cell area in liver of rats gradually increases as the animals age. A definite ratio of the cell area to nuclear area with a change in protein and RNA contents was noted. The cell walls of the liver of rats of 5 days old showed poor staining in comparison to the rats of higher age groups. These results indicate a definite metabolic change in liver with ageing of the animals.

APPENDIX A

I. Governing Body :

1. Dr. Debendra Mohan Bose
3. Dr. Sunando Bose
3. Shri Saibal Kumar Gupta I.C.S. (Retd.)
4. Prof. Satis Ranjan Khastgir
5. Shri Sudhir Kumar Mandal
6. Dr. Sushil Kumar Mukherji
7. Shri Amiya Nath Mitra
8. Dr. Sivatosh Mukherji
9. Dr. Basanti Dulal Nagchowdhuri
10. Prof. Bhupati Mohon Sen, I.E.S. (Retd.)
11. Prof. Ajit Kumar Saha
12. Prof. Arun Kumar Sharma
13. Dr. Sourindra Mahon Sircar, Director, Bose Institute (Ex-officio).

II. The Council :

1. Shri S. K. Mandal
2. Dr. Sivatosh Mukherji
3. Dr. B. D. Nagchowdhuri
4. Prof. A. K. Saha
5. Dr. D. M. Bose
6. Prof. A. K. Sharma

} Nominees of the Governing Body

7. Dr. S. M. Sircar, Director, Bose Institute (Ex-officio)
8. Secretary to the Govt. of India, Ministry of Education and Youth Services.
9. Director-General, Scientific and Industrial Research or his nominee.
10. Shri Krishna Kumar Chatterji, M.P. Representative of the Lok Sabha.
11. Prof. P. Parija, Utkal University.
12. Dr. R. Ramanna, Director Physics Group, B.A.R.C.

} Scientists from outside West Bengal nominated by the Govt. of India.

13. Director of Public Instruction, Govt. of West Bengal. Representative of the Govt. of West Bengal.
14. Accountant General, West Bengal.
15. Representative of the Calcutta Corporation.

III. Finance Committee.

1. Prof. S. Mukherji (Representative of the Council)
2. Accountant General, West Bengal.
3. Dr. D. M. Bose (Representative of the Governing Body)
4. Dr. S. M. Sircar, Director (Ex-officio).

APPENDIX B

Director : Dr. S. M. Sircar, Ph.D.(Lond.), D.I.C., F.N.A.
Registrar : Shri A. K. Ghosh, B.Sc.
Emeritus Scientist : Dr. D. M. Bose, F.N.A.
Dr. P. K. Bose, F.N.A.

Department of Physics

Head of the Dept. : Dr. A. M. Ghose, D.Sc.
Readers : Dr. T. C. Bhadra, M.Sc. D.Phil
Dr. Bimalendu Mitra, M.Sc. D.Phil (from 1.12.70)
Research Fellows : Dr. Radha Kanta Mandal, M.Sc., D.Phil
Dr. Arun K. Chatterji, M.Sc., D.Phil (from 1.1.71)
Research Assistant : Shri Satyanarayan Chongdar, M.Sc.
Research Scholars : Sm. Biva Das, M.Sc.
Sm. Manjushri Mozumder, M.Sc.
Govt. of India Research Training Scholar : Sm. Minakshi Basu, M.Sc.
DAE Senior Research Fellow: Shri Suprakash Roy, M.Sc.

Department of Chemistry

Head of the Dept. : Dr. A. Sen, Ph.D. (Leeds)
Readers : Dr. A. K. Barua, M.Sc., D.Phil.
Dr. D. P. Chakraborty, D.Phil., D.Sc.
Dr. Sudhamoy Ghosh, M.Sc. D.Phil.
Shri Jyotirmoy Datta, M.Sc.
Research Fellows : Dr. N. K. Sinha, M.Sc., D.Phil
Dr. (Miss) P. Chakraborty, M.Sc., D.Phil
Research Assistants : Shri Amitava Ghosh, M.Sc.,
Shri H. C. Chakraborty, B.Sc.
Research Scholars: Shri Swapan K. Ghosh, M.Sc.
Shri Achinta K. Sanyal, M.Sc.
Shri Gautam Sen, M.Sc.
Sm. Gopa Das, M.Sc.
Shri Shambhu Nath Chakraborty, M.Sc
Shri Kashi Nath Nag, M.Sc.
Govt. of India Research Training Scholar : Shri Sandip Saha, M.Sc.
Shri Samir K. Gayen, M.Sc.
Sm. Jasodhara Roy, M.Sc.

Mary & Richard Keating Scholar Shri Manash Roychowdhuri, M.Sc.
C.S.I.R. Ad-hoc Fellows : Dr. Mina Rani Mukherji, M.Sc., D.Phil.
Dr. Saktipada Basak, M.Sc. D.Phil.
Shri Ajoy Basak, M.Sc.
DAE Fellows : Dr. Rama Chatterji, M.Sc., D.Phil
(Sir J. C. Bose Fellow Calcutta University) Shri Pranab Kr. Chatterji, M.Sc.
C.S.I.R. Pool Officer : Dr. Mominul Haque, M.Sc. D.Phil.

Department of Botany

Head of the Dept. : Dr. Sunando Bose, Fil. Doct. (Lund)
Actg. Head of the Dept.: Dr. B. B. Biswas, M.Sc., D.Phil (form 10.8.70)
Readers : Dr. B. B. Biswas, M.Sc., D.Phil
Dr. R. K. Basu, M.Sc., D.Sc.
Dr. V. N. Gadgil, M.Sc., D.Phil (from 1.12.70)
Research Fellows : Dr. Balaram Mozumdar, M.Sc., D.Phil
Dr. Sunirmal Chanda, Dr. Sc. Nat (Gottingen)
Dr. Asoke Das, M.Sc., D.Phil.
Dr. (Mrs.) Susweta Biswas, M.Sc., D.Phil (irom 1.12.70).
Statistician : Dr. A. K. Roychowdhuri, M.Sc., D.Phil
Research Assistants : Shri A. K. Adhikary, M.Sc.
Dr. Sukumar Gupta, M.Sc., D.Phil (from 1.1.71)
Shri Hrishikesh Mandal, M.Sc. (from 3.3.71)
Research Scholars : Sm. Subha Mitra, M.Sc.
Shri Dilip Kumar Biswas, M.Sc.
Sm. Juthika Dasgupta, M.Sc.,
Sm. Aloka Devi, M.Sc.
Govt. of India Training Scholar : Shri Kalyan Mukherjie, M.Sc.
Sm. Shalini Sahay, M.Sc.
Sm. Krishna Mitra, M.Sc.
Sm. Oendrila Chandra, M.Sc. (upto 9.11.70).

Department of Microbiology

Head of the Dept. : Dr. P. N. Nandi, Ph.D. (Lond), D.I.C.
Readers : Shri K. L. Chowdhuri, M.Sc.
Dr. A. K. Mishra, M.Sc., D.Phil.
Dr. Sankar Mitra, Ph.D. (Wisconsin)
Research Fellows : Dr. Ashalata Chandra, M.Sc., D.Phil.
Dr. S. L. Chakraborty, M.Sc., D.Phil.
Dr. R. K. Sinha, M.Sc., D.Phil.

Research Assistants : Dr. Gita Nanda, M.Sc., D.Phil.
Sm. Kalyani Sen, M.Sc.

Research Scholars : Sm. Sima Sinha, M.Sc.
Shri Ranjit K. Dasgupta, M.Sc.
Shri K. K. Bhattacherji, M.Sc.
Shri Tapan K. Mishra, M.Sc.

Govt. of India Research Training Scholar : Sm. Mina Rani Bhattacherji, M.Sc.
Shri Brojen Sen, M.Sc.
Shri Monoj Kanti Roy, M.Sc.
Sm. Suparna Roy, M.Sc.

DAE Senior Research Fellow : Shri Aurobindo Roy, M.Sc.

C.S.I.R. Pool Officer : Dr. Rama Chakraborty, M.Sc., D.Phil.

Department of Animal Physiology

Research Fellow : Dr. A. K. Medda, M.Sc., D.Phil.

Research Assistant : Dr. Rama Ghosh, M.Sc., D.Phil

Govt. of India Research Training Scholar : Shri Arun Kumar Ray, M.Sc.

Guest Worker : Shri G. C. Bhattacherjee

Superintendent-General: Shri M. L. Chatterji

-do- Electrical and Refigeration: Shri S. R. Ghosh

Librarian : Shri P. K. Chakraborty

Asstt. Librarian : Sm. Ila Biswas, B.A., Dip. Lib. (Cal.)

Library Assistant: Sm. Pratima Chakraborty, B.A., Dip. Lib. (Cal.)

Medical Officer : Dr. S. R. Ghoshal, M.B.B.S., D.C.H. (Cal.)

STAFF APPOINTED UNDER GRANT-IN-AID SCHEMES :

A. Department of Atomic Energy :

Scheme No. 1. Investigations in Nuclear Physics using Neutron and Photons.

Investigator-in-Charge : Dr. A. M. Ghose, D.Sc.

Scheme No. 2. Biosynthesis of histone and its applications on the metabolism of nucleic acids.

Investigator-in-Charge : Dr. B. B. Biswas, D.Phil.

Scheme No. 3. Biochemical investigations on the the nature of replication DNA in bacteriaphage.

Investigator-in-charge : Dr. Sankar Mitra, Ph.D.

Junior Research Fellow: Sri Pranab Kumar Chatterjee, M.Sc.

B. Indian Council of Agricultural Research :

Scheme on Microbiological decomposition on the organic matter in Indian soil'.

Investigator-in-Charge : Dr. P. Nandi, Ph.D.

Senior Research Scientist : Dr. N. C. Debnath, M.Sc., Ph.D.

Asstt. Microbiologist : Dr. Subhendu Chandhuri, M.Sc. Ph.D.

Senior Research Assistant : Sri Dodul Som., M.Sc.

Asstt. Soil Chemist : Shri Jitendra Nath Hazra, M.Sc.

C. International Atomic Energy Agency :

Scheme on 'Use of radiation-induced mutants of Aspergillus Sps. etc.

Investigator-in-charge : Shri K. L. Chowdhuri, M.Sc.

Junior Research Fellow: Shri T. K. Abraham, M.Sc.

D. Council of Scientific & Industrial Research

1. Biochemical mechanisms of genetic recombination

Investigator-in-charge : Dr. Sankar Mitra, Ph.D.

Junior Research Fellow : Sri Ranjit Dasgupta, M.Sc.

2. Synthesis of some potential antifertility agents

Investigator-in-charge : Dr. A. K. Barua, D.Phil

Junior-Research Fellow : Shri Sunil K. Paul, M.Sc.

3. Studies on Proteolytic enzymes etc.

Investigator-in-charge : Dr. N. K. Sinha, D.Phil

Junior Research Fellow : Shri Susanta K. Das, M.Sc.

4. Chemical investigations of Swietenia mahogani etc.

Investigator-in-charge: Dr. D. P. Chakraborty, D.Sc.

Junior Research Fellow : Shri Azizul Islam, M.Sc. upto 12.5.70
Shri Phanindra Nath Majhi, M.Sc.

5. Aeropalynological Survey of Eastern India, etc.

Investigator-in-charge : Dr. Sunirmal Chanda, Dr. Sc. Nat.

Junior Research Fellow : Shri Nimai Chandra Nandi, M.Sc. (upto 23.9.70)

6. Quantitative estimation of DDT resistance mosquitoes in local area.

Retired Scientist : Dr. B. C. Basu, F.N.A.

E. Sir J. C. Bose Research Unit :

Investigator-in-charge : Dr. D. M. Bose, F.N.A.

Research Assistants : Shri B. K. Datta
Shri A. T. Guhathakurtha

F. PL—480 Projects :

1. Investigations on gamma rays with matters.

Investigator-in-charge : Dr. A. M. Ghose, D.Sc.

Research Scholar : Shri Suprokash Roy, M.Sc.

2. Regulatinn of RNA and protein synthesis.

Investigator-in-charge : Dr. B. B. Biswas, Ph.D.

Research Fellows : Shri Netai Chandra Mondal, M.Sc.
Shri Hrishikesh Mandal, M.Sc.
Shri Asoke Ganguli, M.Sc.
Shri Asis K. Das, M.Sc.
Sm. Sujata Burman, M.Sc.

3. Isolation and characterization of plant hormones.

Investigator-in-charge : Dr. S. M. Sircar, Ph.D.

Plant Physiologists : Dr. Bharati De, M.Sc., D.Phil
Dr. P. K. Sircar, M.Sc., D.Phil

Chemist : Dr. Subhendu K. Ganguli, M.Sc., D.Phil

Scholar : Sm. Tapati Ganguli, M.Sc.

APPENDIX C

LIST OF PUBLICATIONS

Title of papers	Authors	Where published	Reference : Vol., page and year
1. Physicochemical properties and amino acid composition of goat trypsin.	S. K. Das N. K. Sinha	Jt. Convention of Chemists, Abst. Madras, IIT.	1970
2. Immunochemical comparison of α-lactalbumins	P. K. Sarkar, J. Dutta & A. Sen and Nancy I. Phillips and Robert Jenness	*J. Diary Sc.*	54, 120, 1971
3. Physico-chemical investigations on buffalo β-lactoglobulin.	S. K. Ghosh, Shila Chaudhuri, Jasodhara Roy, N. K. Sinha and A. Sen	*Arch. Biochem. Biophys.*	144, 6, 1971
4. A new imide from Butea frondosa.	A. K. Barua, P. Chakrabarti, K. G. Das, M. S. B. Nair	*Chem. & Ind.* (Lond.)	1376, 1970
5. Tiliacoridine — a new alkaloid from Tiliacora racemosa.	A. K. Barua, P. Chakrabarti, A. S. Dutta Gupta	*J. Ind. Chem. Soc.*	47, 9, 1970
6. Studies on the structure of careyagenic acid—a new triterpene from Careya arborea.	A. K. Barua, P. Chakravarti, A. S. Dutta Gupta, K. Basu, K. G. Das	Abstract, Convention of Chemists, Madras.	P. 5, 1970
7. The constitution of mollugogenol E—a new sapogenin from Mollugo hirta.	P. Chakrabarti A. K. Sanyal	*Ind. J. Chem.*	8, (11), 1942, 1970.
8. Structure of murrayacine	D. P. Chakraborty, K. C. Das and B. K. Chowdhury	*J. Org. Chem.*	36, 725 (1971)
9. Synthesis of girinimbine.	D. P. Chakraborty A. Islam	*J. Ind. Chem. Soc.*	48, 91 (1971)
10. Synthesis of 2-hydroxy 6-methyl (2′ : 2′ dimethyl 5′ : 6′) 1 : 2 pyrano carbazole.	D. P. Chakraborty, D. K. Chowdhury, D. Chatterjee	*J. Ind. Chem. Soc.*	48, 226 (1971)

Title of papers	Authors	Where published	Reference : Vol., page and year
11. Cyclomahogenol, a new teracyclic triterpene from *Swietenia mahogoni.*	D. P. Chakraborty, S. P. Basak B. C. Das and R. Beugelmans	*Phytochemistry*	10, 1367, (1971)
12. Mukoic acid, the first carbazole carboxylic acid.	B. K. Chowdhury D. P. Chakraborty	*Phytochemistry*	10, 1967-70, (1971).
13. Detection & estimation of trace fatty acids in sesame oil by gas-liquid chromatography.	J. Dutta and Amitabha Ghosh	*Indian J. Appl. Chem.*	33, 254 (1970)
14. Fatty acid composition of Swietenia mahagoni seeds oil by gas-liquid chromatography.	Sandip Saha, Amitabha Ghosh J. Dutta.	*Fette Scifen Austrichmittel.*	—
15. Regulatory Role of Adenosine Triphosphate on Hog Kidney N-Acetyl-D-glucosamine 2-Epimerase.	A. Datta	*Biochemistry*	9, 3363 (1970)
16. Studies on hog spleen N-acetyl glucosamine kinase I. Purification and properties of N-acetyl-glucosamine kinase.	A. Datta	*B. B. Acta*	220, 51 (1970)
17. Roll of Allosteric Enzymes in the Regulation of N-Acetylglucosamine metabolism.	S. Ghosh, A. Dutta	*Trans. Bose Res. Institute*	30, 167 (1967)
18. RNA polymerases and factors from Eukaryotic cell.	B. B. Biswas H. Mondal R. K. Mandal	Chemical Convention CRC (CSIR) held at Madras.	1970.
19. Factors and rifampicin influenceing RNA polymerase isolated from chromation of eukaryotic cell.	H. Mondal R. K. Mandal B. B. Biswas	Biochem. Biophys. Res. Commun.	40, 1194, (1970).
20. Factors influencing RNA polymerases isolated from chromatin of eukaryotic cell.	H. Mondal R. K. Mandal B. B. Biswas	Second International convention of Biochemists (Baroda).	p. 13, 1970.

	Title of papers	Authors	Where published	Reference : Vol., page and year
21.	Dissimilarity of plant and animal histone revealed by electrophoretic and immunological studies.	R. K. Mandal H. Mondal S. Som B. B. Biswas.	*Indian J. Biochem, Biophys.*	8, 50 (1971)
22.	Pollen plants and their significance.	S. Chanda	*Sci. Cult.*	36, 11 (1970)
23.	Some effects of X-irradiation on the pollen of *Trigonella.*	S. Chanda Shalini Sahay	*Grana,*	10, 2 (1970)
24.	The dark side of green revolution.	S. Chandra A. Bose	*Sci. Cult.*	37, 2 (1971)
25.	A preliminary report on the Aeropalynology of greater Calcutta.	S. Chanda N. C. Nandi	Proc. 5th Conv. of I. C. A. I.	1971.
26.	Hutchinson's system of flowering plant classification.	S. Chanda H. Chatterjee	*Sci. Cult.*	37, 9 (1971)
27.	Potentiality and problems of Quaternary pollen analysis of India.	S. Chanda	Proc. Seminar Palaeopalynol Ind. Stratigr.	1971.
28.	A note on palynotaxonomy and phylogeny of Phrymaceae.	J. Mukherjee	*J. Palynol.*	7, 1 (1971)
29.	Pollen analysis of a few Quaternary deposits of lower Bengal basis.	B. B. Mukherjee	Proc. Seminar Palaeopalynol Ind. Stratigr.	1971.
30.	Effect of prolactin, growth harmone and ACTH on the urea excretion of bull frog tadpoles during normal and induced metamorphosis.	A. K. Medda E. Frieden	*Endocrinology*	87, 356, 1970
31.	Effect of antigammaglobulin-thyroxine comlex on the urea excretion of tadpoles.	A. K. Medda	*Acta Endocrinologica*	(In press)
32.	Boseimycin-Antimicrobial activity *in vitro* and *in vivo.*	R. K. Sinha P. Nandi	*Ind. J. Pharmacy*	32, 4.

	Title of papers	Authors	Where published	Reference : Vol., page and year
33.	Modifying effect of Pre- and post-treatment with nitrogen mustard on the effect of UV-irradiation on *Aspergillus chevalieri.*	G. Nanda P. Nandi A. K. Mishra	*Ind. J. Expt. Biol.*	8, 319
34.	A-435, a new antifungal agent.	A. Pal P. Nandi	*Ind. J. Expt. Bio.*	8, 321.
35.	Boseimycin–A new streptothricin like antibiotic.	R. K. Sinha	*J. Antibiotics*	23, 360
36.	Increased fragity of *Escherichia coli* after infection with bacterio-phage M-13.	A. Roy S. Mitra	*J. virol.,*	6, 333.
37.	Denaturation of DNA after X-ray irradiation *in vitro.*	R. Dasgupta	*Biochem. Biophys. Res. Comm.*	40, 793.
38.	A new actinomycin like antibiotic produced by a mutant strain of *Streptomyces indicus.*	S. L. Chakraborty P. Nandi	*Experientia*	27, 595
39.	Transformation of actidione resistance in *Aspergillus niger* Van Tieghen.	K. Sen A. K. Mishra	*Sci. & Cult.*	37, 52.
40.	Mechanism of action of Boseimycin.	T. K. Mishra R. K. Sinha	*Experientia.*	27, 642.
41.	Comparative studies on the effect of Boseimycin and Streptomycin in *Bacillus aubtilis.*	T. K. Mishra	*Curr. Sci.,*	40, 440.
42.	Studies on the control of brown spot disease of rice with a new antibiotic Boseimycin.	S. Chakraborty P. Nandi	*Sci. & Cult.*	36, 669.