

Annual Report of the Haffkine Institute

1979 – 1980



HAFKINE INSTITUTE
PAREL, BOMBAY-400 012
INDIA

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CHAPTER I

INTRODUCTION

In the year 1979, Haffkine Institute had the privilege to have Dr. K. D. Sharma, a well known research scholar and reputed pathologist as its new Director. The institute continued its training, testing and research activities under his able leadership. The scientists and scholars actively carried out this task with the able guidance of the Director and various advisory bodies like Research Advisory Council, Academic Council and Teacher's Council.

Biochemistry

Previously undernutrition during prenatal and postnatal periods in rat population had shown depressed acetylcholinesterase activity during weaning period which was subsequently elevated, but in the present studies rate of incorporation of radioactive acetate and choline into brain acetylcholine was not altered in the same condition.

Vitamin D affects the lipid metabolism through its active metabolites. This was confirmed by investigating the activity of lipogenic enzymes in nephrectomised rats which had received different active metabolites of Vitamin D.

Chemotherapy

The attempt for using newly synthesized organic compounds and extracts of indigenous plants against various infectious agents was continued. This led to the synthesis of several organic compounds which showed promising antimalarial, antituberculosis, amoebic and fungal effects *in vitro* or *vivo* or both systems. Further extracts of *H. suaveolens* and *P. tuberosa* plants have exhibited hypoglycaemic effects equivalent to tolbutamide and another plant extract of *I. cylindrica* has shown curare-like neuromuscular blocking activity in animal experiments. These extracts are being purified and chemically characterized for future animal experiments. Diet and nourishment affects absorption and metabolism of drugs. In malnourished rats absorption and metabolism of aspirin were depressed as compared to nourished ones. A similar type of study is being conducted in human beings with respect to antibiotics given by oral route.

Immunology

Adhesion of bacteria to the epithelial lining of the intestines and their entero-pathogenicity or toxicity are interlinked phenomena. This interesting problem is being studied in *V. cholerae*, *E. coli*, *Salmonella* and *Shigella* spp. and has proved true for the former organism (78-79).

Whether triple vaccine provides adequate titre of antitetanus antibody in sera of immunized child? This is being investigated by employing I^{125} tagged tetanus toxin by radioimmunoassay technique.

Parasitology

A purified protein (P_8) isolated from cobra venom when added in small quantities in culture, inhibited the motility and viability of *L. donovani*. It also affected the intracellular amastigotes in animal experiments. These findings suggest the possibility of therapeutic use of venom in kalazar.

In order to identify the schistosoma species responsible for few cases of haematuria in village Gimvi, bifurcated cercaria liberated from *F. tenuis* and *P. obesa* snails were injected into hamsters and mice to produce adults in these animals.

Vaccine

A polyvalent pseudomonas vaccine comprising of LPS of eight serotypes of *P. aeruginosa* is being tested for potency and toxicity in animals. A similar trial is being conducted for ICRC bacilli as a leprosy vaccine.

Virology

HIBOMA-HSP (human hepatitis B virus) has been successfully passaged in livers of langurs (*Presbytis entellus*). This could be confirmed by immunofluorescent, histopathological and biochemical evidence. Efforts are being made to grow the virus in tissue culture systems. Similar efforts are being made to grow rabies virus in primary cultures of Baby Hamster Kidney and Human Diploid cells.

Sponsoring Research Bodies

Many of the research activities are being financially aided by ICMR, DST and Central Council of Research in Ayurvedic and Siddha System of India. The institute gratefully acknowledges their support for the various research activities.

Service to the public

The Blood Transfusion Service, Immunization Clinic and Typhoid Unit of the institute provide valuable service to the citizens of Bombay. The former two units provide blood transfusion and immunization service to the public at nominal cost while Typhoid Unit provides free service by collecting blood samples from cases of suspected enteric fever on information by registered practising physicians. Rabies Research Unit screens brains of dead human patients while keeping in view the research ideologies of the institute.

Academic Council

This body met twice this year to consider the progress made by various training and post-graduate courses. The members were happy to note the good progress made by M.Sc. (by paper) courses in microbiology and biochemistry. They recommended the introduction of a single course in Clinical Pathology (3 months duration) and in Drug Analysis (one year duration) during 1980-81.

Teachers' Council

All the post-graduate teachers are the members of the Council. The teachers provide guidance and review the research progress of the post-graduate students. The Council met six times, and approved synopses of 23 post-graduate students and its subcommittees met twice to consider the progress reports of 42 students.

Critics' Club

A total of twenty meetings were held during this year out of which seven meetings were addressed by guest speakers. These meetings provide a valuable experience for the post-graduate students to create a good speaking habit and for improving their knowledge as regards advancement made in biological sciences.

Oration and other Academic Activities

The sixth oration of Major General Sahib Singh Sokhey took place in the month of November 1979. On this occasion, Professor A. B. Chowdhary of School of Tropical Medicine, Calcutta spoke on the subject "Conquest of Tropical Diseases". The oration was well attended by the scientists of the institute and invitees of other post-graduate medical and bio-medical institutes of the city.

It was heartening to know that the first batch of M.Sc. (by paper) students in Microbiology and Biochemistry fared remarkably well in the final examinations of Bombay University held in June 1979. There was hundred per cent success and four out of ten students got first class.

More young scientists are actively taking part in the academic and research activities of the institute. This year they presented a number of good papers in the Annual Conference of All India Medical Microbiologist Congress and Regional Chapter Meeting of Indian Association of Pathologist and Microbiologist held in Bombay and Nagpur during the months of November and March 1979. The list of papers and of speakers is shown in Appendix XX.

In order to improve the quality and catalyse research activities, the Director allowed training and participation of several scientists in various

workshops organized at Calcutta, Chandigarh and Delhi. The subjects of training were Statistical Technique (Shri A. S. Arekar), Immunodiagnosis in Parasitology (Dr. M. K. Bhopale), Identification of insects and mites (Shri B. D. Sathe) and Malaria Serology and Epidemiology (Dr. S. Mukerji).

Honours and Awards

Shri D. S. Chitnis, Senior Scientific Officer (Class I) of Department of Clinical Pathology was awarded the First Research Prize of Haffkine Institute. His subject of research was on "The role of somatic antigens in the pathogenesis of cholera".

Participation in National and International Conferences

Dr. K. D. Sharma, Director, Haffkine Institute, was elected as President of Indian Association of Pathologists and Microbiologists in the year 1980.

Dr. M. V. N. Shirodkar, Head of the Department of Virology chaired the session on "Viral Hepatitis" and "Viral Diseases" at the conferences of Indian Society of Gastroenterology and All India Medical Microbiologists Congress held in October at Pune and in November in Bombay during 1979. He was nominated by Association of Microbiologists of India to represent India at the Sectional Council (Virology) in October 1979 for the International Association of Microbiologist Societies held in Paris. He has been nominated as a member of Editorial Board of Indian Journal of Microbiology from March 1980.

Dr. Gadre, Head of Department of Bacteriology attended and took part in the Seminar and Workshop on "Micro-organisms concerned with treatment of Industrial Wastes" at Pune in March 1980.

Dr. M. B. Bhide, Head of Department of Pharmacology participated in the First joint conference of Indian College of Allergy and Immunology and Indian Immunology Society held at Institute of Medical Sciences, Srinagar in November 1979.

DEPARTMENTS

Bacteriology

The department is mostly involved in research and teaching of Microbiology leading to M.Sc. and Ph.D. degree of Bombay University. The first batch of M.Sc. (by paper) students admitted during 1977-78 fared well in the final examination of 1979 and dissertation titles of three students working under Dr. S. V. Gadre were: (1) Purification of cholera toxin; (2) 11 alpha-hydroxylation of progesterone, and (3) Bacterial deterioration of water based emulsion paints.

Biochemistry

The training programmes of DMLT and the modified Short term courses in Clinical Pathology were continued. The department admits five students every year for M.Sc. (by papers) course in Biochemistry. The thoroughness in practical training imparted to the post-graduate students was commended at the final examinations of 1979. The details of routine biochemical tests carried out by the department are shown in Appendix III.

Chemotherapy

The department continued to follow the programme of synthesising newer compounds of possible antihelmentic, antimalarial, antituberculosis, antiamoebic and antifungal activities. The new compounds were screened for the above and antibacterial activity. A number of synthesised compounds referred by other research laboratories were also screened for antituberculosis activity. An additional batch of Dapsone (injection) was prepared for use in Acworth Leprosy Hospital, Bombay. The Micro-analytical Section carried out a total of 1941 estimations for organic compounds out of which 610 and 721 were Carbon, Hydrogen and Nitrogen respectively. Screening Section screened 380 and 60 compounds originating from departmental and outside sources respectively.

Clinical Pathology

The department undertook investigations from various hospitals and agencies and the services offered are shown in Appendix IV. The services included — (1) Cytology of various body fluids; (b) Routine haematological examinations; (c) Diagnostic Serology, Bacteriology and Mycology. The staff of the department participated in DMLT, Short Term Training in Clinical Pathology courses and in comprehensive teaching programme for M.Sc. (Microbiology), Ph.D. (Microbiology), M.D. (Pathology) and M.D. (Microbiology) students.

Human Pharmacology and Bioavailability

Microbiological assays of new antibiotics (Cephalexin, Cloxacillin and Amoxicillin) were standardized to assess their level in periodic blood samples. Besides these other antibiotics were tested for blood absorption studies. These are being shown in Appendix V. Cross over studies for two oral preparations of dirutics, Kinex and Lasix (Unique and Hoechst Pharmaceuticals) were also carried out.

Immunology

The department continued its research work on mycobacterial antigens as regards their role in cellular immunity. This scheme is also preparing anti-human reagents against various immunoglobulins and their light and heavy chains.

Pharmacology

The department undertakes training of students for degrees in Pharmacology (M. Pharm, M.D. and Ph. D.), Applied Biology (M.Sc. and Ph.D.) and for Drug Analysis Course. The department regularly conducts journal clubs, seminars and research progress meetings. In addition, some eminent professors from different institutes are invited to deliver lectures for the post-graduate students.

Phytochemistry

This section collects indigenous plants from different parts of Maharashtra for studying possibility of their extracts having larvicidal or bactericidal or snake repellent action with the help of Zoonosis Department. Plant extracts showing the above activity or activities are afterwards purified to isolate the active principle and know their chemical nature. Some of the extracts are also tested for medicinal use.

Radiation Biology

Uptake of I^{131} radioactive isotope in patients suffering from various thyroid disorders constitutes the major activity of the Section (Appendix VII). Besides serological titration of antitetanus antibody and radiometric assays of chemical preservatives of various products are also being tested.

Rabies Research

This section maintains stocks of viruses in mice, hamsters and rabbits and also prepares hyperimmune sera for various purposes. For diagnosis of rabies, a total of ten postmortem brain specimens were received. Their particulars and results of the tests carried out are shown in Appendix VI.

Testing Unit

This unit is engaged in testing of samples received from Prohibition and Excise Departments of the state and of commercial samples of drug manufacturers. These have been detailed in Appendix VIII.

Toxicology

The department is actively engaged in toxicological evaluation of different types of pesticides, drugs, chemicals and plastics. Appendix IX shows detail information regarding this subject. Short term and Drug Analysis students are also trained in this department.

Virology

Department activities continued with respect to studies in host-virus interaction of HIBOMA, Rous sarcoma (RSV) and influenza viruses. In particular;

active efforts continued, in species heterologous to man, towards the development of vaccine against HIBOMA hepatitis (HBV) viruses.

During 1979-80, two M.Sc. (Microbiology by Papers) students completed their dissertations under the guidance of the Head of this Department. They were entitled: "*Studies on the Action of Inflenza Virus-induced Interferon*" and "*Preliminary Characterization of the Antiviral Compound Plasma Factor-Mediated RV-RSV Blockade: Further studies on Interference with Herpes, Inflenza and Vaccinia Viruses.*"

The latter work has been of great interest, since plasma factor (PF) does not, apparently, behave like classical interferon, since it has been shown to inhibit two RNA viruses (RSV and influenza), but not two DNA viruses (vaccinia and herpes simplex type 2). This lends further support to our earlier hypothesis that PF may be an entirely new antiviral agent, since its physicochemical properties also differ from those of classical chick interferon (which is pH- and heat-stable).

The postgraduate training activities of the Department continued, the number of Ph.D. students in Microbiology and Applied Biology being seven and those working for the M.D. degree in Microbiology, three, as in the past. A complete course of lectures and practicals/demonstrations in Virology was conducted for the M.Sc. (Microbiology by Papers), Ph.D. M.D. and D.M.L.T. students of the University of Bombay, during February-March 1980.

The Clinical Virology Section of this Department continued its valuable Immunization Clinic and Diagnostic Virology service for the staff of our Institute, for those from the general and private hospitals of Bombay and for the public, at large. The total number of patients undergoing immunisation was 692, the figure including those administered oral polio, antirabic, triple, TT, DT, anticholera and antityphoid vaccines.

The Diagnostic Virology Unit of the Clinical Virology Section of the Department of Virology conducted tests on virus isolation/identification on a total of 212 specimens from patients with suspected viral aetiology. Fifty-nine out of 136 sera tested by haemagglutination inhibition for anti-rubella antibody had titres less than 1:10 or higher. Of 12 urine samples observed after Giemsa staining, for the possible presence of cytomegalovirus inclusions in the renal epithelial cells, none was found positive. Out of a total of 8 specimens accepted for testing for presence of herpes simplex virus, four were blood samples from patients with skin eruptions. None of these was found to contain herpesvirus; of two corneal specimens inoculated to the CAM of chick embryos, a herpes simplex-like virus was isolated from one; material from a swab taken from the *cervix uteri* was positive, whereas a single CSF specimen from a case of suspected herpes encephalitis was negative. One blood and a CSF sample from a patient suffering from myalgia were inoculated intracerebrally into mice for possible presence of Coxsackie viruses and were found negative. Fifty sera from patients with viral hepatitis

were tested for HBsAg presence using the CIEP/RPHA tests — ten of these were found positive.

Zoonosis

The department collaborates with the Department of Chemotherapy in screening of their synthesized drugs against malarial parasites, intestinal and tissue nematodes and cestodes. The same help is extended to the Department of Phytochemistry to test their plant extracts for larvicidal, insecticidal and bactericidal activities.

Experimental immunological studies of *N. americanus* are being continued. Colonies of vectors of medical importance, strains of *L. carinii*, *A. ceylanicum*, *H. nana* and *P. berghei* are being maintained for various testing and research purposes. Various poisonous and nonpoisonous snakes are also being maintained, and venom of the former is extracted and lyophilized (Appendix-XI).

Rats of different species are being collected and maintained for bacteriological and serological studies on plague. For the latter, haemagglutination test is being employed using F 1 antigen of *Y. pestis*.

CHAPTER II

DEVELOPMENT IN TEACHING

The institute runs various types of training and post-graduate courses. (Appendix XII), Short term course (3 months) in Clinical Pathology, DMLT (Diploma in Medical Laboratory Technology of Bombay University) and Drug Analysis Course belong to the former group. While the latter included post-graduate qualifications by papers (M.Sc. in Biochemistry and Microbiology) and by research (M.Sc. and Ph.D.). This task is accomplished by well qualified teachers (Appendix XII) and a biostatistician provides valuable statistical advice in the formulation of various research schemes. WHO sends fellows from S.E. Asia for training in various specialities provided by the Institute. This year, one fellow from Bangla Desh and Nepal received training in Microbiology and Medical Laboratory Technology respectively.

It is the one and only institute in Bombay which provides training in the subject of Virology and training and teaching facilities to post-graduate students in Microbiology of Bombay University. Every year lecture, demonstration and practical programme of a month's duration is provided by the institute for the M.Sc. and M.D. (Microbiology) students.

DMLT

This year fifteen students were admitted for the course and twelve of them passed in the University examination.

M.Sc. (by papers)

This year the 3rd batch of the course was admitted for Microbiology and Biochemistry. The previous two batches have done well in the final examinations of the courses.

Post-graduate Courses (by research)

Appendix (XII, XIV, XVI) provides information regarding the courses, number of students admitted, their research problems and their success in obtaining of degrees.

CHAPTER III

RESEARCH PROGRESS

BACTERIOLOGY

Microbiological transformation of steroids using fungal spores with special reference to 11-hydroxylation of progesterone using fungal spores :

Insertion of an oxygen function at C₁₁ in the steroid nucleus is a crucial step in the synthesis of corticosteroids. This microbiological process can be carried out in almost nonsterile conditions by using fungal spores as suspension in buffer solution.

Fermentation conditions were standardized to obtain transformation of progesterone into its 11-hydroxy derivative by *Aspergillus ochraceus* NRRL 05 conidia. The product (*Compound I*) formed (in more than 90% yield) as crystallized and the melting point and the IR spectra data established the identity of this compound with the authentic sample of 11-hydroxyprogesterone.

On studying the effect of various factors such as concentration and particle size of the substrate, inoculum size, pH of the buffer solution and phosphate concentration, it was observed that in addition to 11-hydroxy progesterone and 6B, 11-dihydroxyprogesterone (reported earlier), a new compound was obtained. This new compound (*compound III*) was crystallized as fine needles, white in colour, with M.P. 214°C-216°C. (same as that of the authentic sample of Epicortisol). The polarity of the compound was very close to Epicortisol thus suggesting it to be a trihydroxy derivative. This was further supported by the IR spectra data.

The effects of ultra-violet light and N-methyl-N-nitro-N-nitrosoguanidine were also studied. The results suggest that these could be used for strain improvement as well as for studying the enzyme system.

Development of polyvalent pseudomonas vaccine

Studies on the antigenic response to the lipopolysaccharide (LPS) preparations from eight serotypes of *Ps. aeruginosa* are in progress. Of the three serotypes tested serotype V evoked an antibody response in a dilution of 1:320 while serotype I and II were effective in a dilution of 1:1280.

Before proceeding to human trials, we are now confirming the results of the potency and toxicity studies in mice.

In addition to studies with LPS, we have now begun the extraction of slime layers from various serotypes by the method of Bartel (1970). These extracts will also be used in the protection studies.

Studies on vaginal flora in Indian women of low socio-economic status.

In the last two years, studies were concentrated on age group 20-35 years. During this year, our studies were extended to age group 40 and above. Twenty-eight clinically asymptomatic females with normal pelvic examination were studied for vaginal flora and out of these cases only 5 females showed regular ovulatory cycles. The pattern of results was comparable with our previous recordings, minimum aerobic flora on the 6th day and maximum on the 28th day. Anaerobic flora did not show such variations. Out of the remaining cases, 3 were menopausal, 14 premenopausal, and 6 were hysterectomised cases. These did not show a significant rise or fall in the aerobic and anaerobic flora on any particular day in a month.

BIOCHEMISTRY

Biochemical studies on *Bordetella pertussis* cell extracts

In this work it is planned to isolate, purify and conduct physiochemical studies on the blood sugar lowering (hypoglycemia causing) factor from *B. pertussis* extracts. Initial studies indicated that DEAE cellulose column chromatography of preparations from crude alkaline saline extracts yielded fractions with significantly higher hypoglycemic effect in mice. However, no more ready cells were made available to continue the studies. After receiving a grant-in-aid from the ICMR, *B. pertussis* (strain 25525) cell cultivation was begun in May 1979. Several lots of acetone dried cells, both live and formalin treated, have been prepared separately and initial comparative studies on the hypoglycemic actions of these two cell preparations are in progress.

Studies on cholinergic mechanism of brain in malnourished animals

Earlier studies in this laboratory showed that undernutrition during pre-natal and postnatal periods had differential effects on rat brain cholinergic system. The acetylcholinesterase activity was found to be decreased in the former case whereas marked elevation was found in the latter. However choline-acetylase activity remained lowered in both cases. Thiamine deficiency at all stages of brain development although reduced the levels of brain acetylcholine concentration, the enzyme activities however remained unaffected. Further studies with radioactive acetate and choline revealed that there was no substantial difference in the rates of incorporation of these precursors into brain acetylcholine.

Isolation and purification of trypsin from bovine pancreas

The earlier studies contributed to the development of a suitable method for purification of grade II trypsin from bovine, porcine and sheep pancreas collected from Deonar abattoir. The procedure was further scaled upto lots of 6-8 kg batch size. This scaling-up was found to incur losses in the activity of the preparation. Affinity chromatography method was employed to obtain

grade I trypsin with an activity of 8000-10000 BAEE units per mg protein. This method has yielded encouraging results.

Studies on Rickets

Our studies have shown that vitamin D alters the hepatic lipid parameters by affecting the lipogenesis rather than the lipolysis. Further, this effect of vitamin D was found to be dependent on calcium status of the animals, suggesting that metabolites of vitamin D, rather than vitamin D itself, may be causative factor(s). Studies of lipid metabolism in the nephrectomised rats showed that nephrectomy has caused changes in lipid profiles, which resemble to Type IV lipoproteinemia, along with the higher activities of the hepatic lipogenic enzymes. This suggested that nephrectomy has stimulated the lipogenesis. The higher incorporation of 1-¹⁴C acetate into liver lipids confirmed the above contention.

To ascertain the role of different metabolites of vitamin D, preliminary studies with 1,25 (OH)₂D₃ were carried out in the nephrectomised rats. Electrophoretic pattern of the serum lipoprotein had shown that when 1, 25 (OH)₂D₃ was administered, the rise in the pre-B-lipoprotein caused by nephrectomy could be reversed to certain extent, but the administration of 24,25 (OH)₂D₃ did not show such effect. The lipogenic enzymes viz., G6PD, ME and CCE were inhibited by 1,25 (OH)₂D₃ whereas 24,25 (OH)₂D₃ administration caused further increase in the activities of all these enzymes in the nephrectomised animals, suggesting that these two metabolites have different effects on the hepatic lipogenic enzymes.

Our earlier work had shown that Vitamin D elevates cholesterol concentration by the enhanced cholesterol genesis in the liver. It could also be possible that the degradation of cholesterol could have been affected simultaneously. Therefore, to investigate the degradative aspects, studies with ¹⁴C-cholesterol were carried out in the animals reared on different dietary vitamin D regimen. It was found that after 24 hrs of the administration of radioactive cholesterol, nearly the same amount of radioactivity was recovered in the non-saponifiable lipid fraction of the liver of the animals maintained on either low, normal or high dietary vitamin D. These findings have indicated that the degradation or the excretion of the cholesterol did not seem to be affected by different doses of vitamin D in the diet.

The purification of hepatitis B surface antigen and its use in the reversed passive haemagglutination test (RPHA)

The reversed passive haemagglutination test (RPHA) for the detection of hepatitis B surface antigen (HBsAg) in human serum was standardized. The test was found to be about 800 times more sensitive than counter-immuno electrophoresis (CIEP) on the basis of the HBsAg titers observed by these two methods. The reagents were found to be stable for at least ten months when stored at 10°C.

A novel method was standardized for the purification of HBsAg using colloidal silicic acid (Aerosil) with the yield of 12%. In the purified HBsAg, antigen titer was 1:2 (CIEP) for the corresponding protein concentration of 110/ $\mu\text{g/ml}$. The antiserum (anti HBs) was obtained by the immunization of guinea pigs and rabbits. As judged by immunoelectrophoresis, this antiserum also contained antibodies against some components of normal human serum. The nonspecific antibodies can be removed from this antiserum by absorption with normal human serum.

Effect of purified protein (P6) of cobra venom on *Leishmania donovani*

Parasitic diseases are in the forefront of biomedical research. Pertinence of this topic to India is obvious and needs no elaboration. The newer approaches in this area are directed more and more to various aspects of cell biology of agents of infection. Modern probes and techniques developed for the study of mammalian cell and the great store of knowledge acquired in the study of cancer cells have been widely applied with successful results to the study of parasites.

In keeping with the objective of investigating the action of certain proteins from cobra venom on properties of parasitic organisms, experiments reported deal with the action of a purified protein P6 from Cobra Venom on the growth and viability of the causative organism of Indian Kala Azar, *Leishmania donovani*.

The experimental plan and results obtained are discussed in the following sections.

Note on P6

Cytotoxin P6 is a small molecular weight protein (M.Wt. 11,000) purified from cobra venom as per methods and properties described by Braganca *et al*. P6 was of interest as it is preferentially toxic to certain cells and not others. It inhibited the growth of some animal tumours in rats and mice as well as human tumour cells in tissue culture, in doses much less than toxic dose. More detailed studies have shown the P6 can selectively block specific groups in the plasma membrane of susceptible cells.

Note on *Leishmania donovani*

Experimental work using *Leishmania donovani* was carried out at NICD in collaboration with Dr. Topa and Dr. Hussain, Deputy Director and Head of Biochemistry Department.

Leishmania was grown and maintained in Brain Heart infusion medium under conditions routinely followed in the department.

Leishmania grown in non living media occur in promastigote (leptomonad) forms, the optimum temperature for growth is 21-30°C. In naturally occurring form or in experimentally injected animals the parasite exists in amastigote state.

Experimental studies: Before exploring the action of cytotoxin P6 in culture, the direct effect of P 6 on the motility of L.D. was tested at 25° and 37° and on incubation at different times. This was done by direct counting of motile organisms in the microscope using a haemocytometer. In a system containing 10^5 L.D. it was seen that 72 μg P6 produced 50% inhibition of motility at 37°. Incubation time was 30 min. Incubation for different periods showed that 30 min. and 45 min, were suitable and did not affect the controls either at 25° or 37°.

Viability tests in which L.D. was treated with 144 ng/ml 72 $\mu\text{g/ml}$, 36 ng/ml , 18 ng/ml showed absence of growth after 7 days of culture in B.H.M. This indicates that the organisms were either dead or not enough to produce countable growth. However, on concentration of the parasites by centrifuging there was also no growth detected. Whereas control tubes treated in identical manner showed profuse proliferation of the parasites.

It is apparent that 18 $\mu\text{g/ml}$ P6 can immobilise 10^5 L.D. Even the organisms which remain motile after treatment appear to be affected and are non viable.

Action of P6 on growing cultures of *Leishmania*

Following the results of experiments described above a systematic study of the inactivating action of P6 on growing cultures of L.D. was carried out.

The experiments were planned to obtain information with regard to quantitative effects of P6 on L.D. and conditions for maximum inactivation with minimum amounts of P6. The two series of experiments consisted of (i) effects produced by varying amounts of P6 given daily and (ii) with doses of different concentrations given under different schedules of time.

Addition of three different concentrations of P6 (18,36 and 72 $\mu\text{g/ml}$) to culture tubes in five daily doses and assay of growth after seven days showed that higher the concentration of each of the above doses the greater was the inactivating action as would be expected. Five doses of 72 $\mu\text{g/ml}$ P6 give daily decreased the growth from 1.3×10^7 in controls to 5×10^5 L.D. in experimental tubes. To study the conditions for optimum inhibition with minimum concentrations of P6 the following type of experimental studies were carried out.

The growth curve for controls in which no P6 was added was a typical S shaped (sigmoid) curve. There was some initial depression in growth after 24 hours, this was followed by log growth phase (48 to 96 hours)

which flattened out sharply without significant growth increases between 96 to 168 hours. In the controls the initial inoculum of 1.15×10^6 rose to 1.6×10^7 . Results of experiments in which concentrations of P6 were added at different times during the 7 days were briefly as follows.

P6 100 $\mu\text{g/ml}$ when given in two doses. The 1st dose being given initially and 2nd after 72 hours produced highest inactivation and the growth declined from 1.6×10^7 to 1.2×10^4 . Whereas 72 $\mu\text{g/ml}$ P6 given in six daily doses brought down the growth from 1.6×10^7 to 1.6×10^5 . The curve reflecting the effects of single dose of 72 $\mu\text{g/ml}$ P6 given initially does not differ from the curve showing the effects of P6 72 μg given in two doses added to the culture, 1st dose initially and 2nd after twenty four hours. These experiments bring out the fact that P6 is not equally toxic to all stages of growth of *Leishmania donovani*. Thus 72 $\mu\text{g/ml}$ given in six doses making a total of 432 μg produced significant less inactivation than two doses of 100 $\mu\text{g/ml}$ making a total of 200 μg . These latter doses were given initially and during the log phase of growth. It appears that L.D. in the stage of log phase of growth are more susceptible to the inhibitory effects of P6.

The above experiments have demonstrated that minute amounts of P6 can inactivate the growth of promastigote from of L.D.

To determine whether P6 can attack the *amastigotes*, the form in which the leishmania are present in macrophages and deep viscera, the following experiments were tried.

The spleen and liver of mice and hamsters previously infected with L.D. were individually homogenised. Part of emulsion served as control and similar volumes were treated with P6 for 30 min. at 25° and inoculated in B.H.M. The untreated controls were also inoculated under identical conditions. After seven and fourteen days the treated cultures did not show growth of L.D. But controls showed profused proliferation of parasites. These experiments suggested that P6 can also the attack amastigote form. However, more experiments are needed to establish the point as the inhibitions observed may reflect the action produced after transformation of amastigotes into promastigotes during growth in culture.

CHEMOTHERAPY

Anthelmintic Agents

Pyridothiazole derivatives

The importance of the benzoyl group in the benzimidazole derivatives is well known. Attempts were made to introduce that group in the pyridothiazoles. Thus 2-[4' (p-substituted benzoyl) phenylamino-pyrido, (3, 2-d)-thiazoles were prepared and screened against *N. dubius* in mice. Of these

only 2-[4'-(p-nitrobenzoyl) phenylamino] pyrido (3, 2-d) thiazole exhibited significant anthelmintic activity at 200 mg/kg dose level.

Attempts were then made to incorporate halogens and ether linkage in the lead molecule. Thus the series of 2-(3', 5'-dihalogeno-4-alkoxy phenylamino) pyrido (3, 2-d) thiazoles were synthesised and screened against *H. nana*. Among these only 2-(3', 5'-dichloro-4'-ethoxyphenylamino) pyrido (3, 2-d) thiazole had shown very good activity against *H. nana* in mice at 200 mg/kg dose.

Synthesis and study of 2-substituted-aryl-6 (and 7-) nitroimidazo (2, 1-b) thiazolo (5, 4-b) pyridine and 2-substituted aryl-6-nitroimidazo (1, 2-a) pyridines :

Bicyclic and tricyclic heterocycles with nitro and without nitro are well known for their anthelmintic activity. Hence, 2-substituted aryl-6-nitroimidazo (2, 1-b) thiazolo (5, 4-b) pyridine derivatives and 2-substituted aryl-6-nitro-imidazo (1, 2-a) pyridines were synthesised and submitted for screening as anthelmintic agent. Results are awaited.

Synthesis and study of 5-(6)-substituted-2-substituted benzylthio (and corresponding sulfones) as well as 2-substituted heterocyclic thio benzimidazoles

The well known broad spectrum anthelmintics like thiabendazole, mebendazole, flubendazole etc. prompted the synthesis of the new benzimidazole derivatives. The majority of the compounds synthesised were 5(6)-substituted-2-benzylthiobenzimidazoles and their corresponding sulfones. In continuation of this work some 5 (6)-substituted-2-heterocyclic thio-benzimidazoles were also prepared and tested for their anthelmintic activity. However, none of them exhibited good activity except one i.e. 5 (6)-chloro-2-pyrimidylthio benzimidazole against *H. nana* in mice. The standard drug taken was niclosamide.

Synthesis of 3-substituted-phenyl-6-phenyl-5, 6-dihydroimidazo (2, 1-b) thiazoles.

A large number of variations in tetramisole molecule have been investigated but substitution in the thiazole part are rare. It was therefore decided to introduce aryl substitution on the thiazole part to obtain new 3-substituted phenyl-6-phenyl-5, 6-dihydroimidazo (2, 1-b) thiazoles. These were prepared by the action of various phenacylchlorides on 2-mercapto-5-phenylimidazoline.

These compounds were screened at 25 mg/kg dose against *H. nana* and *N. dubius* in mice. However, none showed activity higher than 60% of that of tetramisole.

ANTIMALARIAL AGENTS

Curd and Rose have reported 2-arylamino pyrimidine derivatives carrying dialkylaminoalkylamino group at 4-position to possess strong therapeutic

effects against *P-gallinaceum* in chicks. Our attempts were to extend this observation of Curd and Rose to get new pyrimidine derivatives. Thus

- (i) 2-(3', 5'-dihalo-4'-alkoxyanilino)-4-[(dialkylamino) alkyl amino]-6-methylpyrimidines;
- (ii) 2-(3', 5'-dihalo-4'-alkoxyanilino)-4-[3'-(-diethylamino methylene)-4'-hydroxyphenyl]-6-methylpyrimidines;
- (iii) 2-substituted-4-methyl 5-(-hydrox-6-hydroxyethyl) pyrimidines; and
- (iv) 2-substituted-4-methyl-5, 6-dihydrofuro (2, 3-d) pyrimidines were synthesised.

About 50% of compounds prepared are tested for their *in vivo* antimalarial activity against *P. yoelii* in mice by Rane's blood schizonticidal test. A few compounds in these series showed activity at dosages ranging from 20 to 160 mg/kg. Three compounds gave cures at 160 mg/kg dose.

Cycloguanil compounds: Cycloguanil, a symmetrical triazine derivative is the active metabolite of antimalarial paludrine. The work on synthesising new cycloguanils was undertaken starting from 3, 5-dihalo-4-alkoxy anilines and 4-aminophenylthiazoles. These anilines and thiazoles have given compounds active in antimicrobial and antiparasitic screening.

Some of these cycloguanils were screened for their activity against *Plasmodium yoelii* in mice and found active.

ANTITUBERCULOSIS AGENTS

Our attention was attracted to the highly effective but most unpleasant ethionamide. Similarly, strict medical supervision necessary for administering another highly potent drug 'pyrazinamide also drew our attention.

(i) New thioamides analogous to ethionamide were prepared and subsequently converted them to the new thiazoles. Thus a number of 2-aryloxy-thioisonicotinamides analogous to ethionamide and the thiazoles derived therefrom, namely 2-(2'-aryloxy-4-pyridyl)-4-arylthiazoles were prepared.

The screening results of these new compounds show that the thiazoles were active, some of them being equal to thioacetazone.

Pyrazinamide derivatives

The new pyrazinamide derivatives namely, N-(3', 5'-dihalo-4'-alkoxy) phenyl pyrazinamide were synthesised and screened for antituberculosis activity. A few of them possessed activity superior to pyrazinamide under identical conditions of *in vitro* testing.

Bis-(thiazolyl) methanes

Interesting biological activity has been observed with many butter-fly structures i.e. bis-compounds. We have got very good antituberculosis activity with a number of thiazole derivatives and so it was planned to synthesise bis-(substituted thiazolyl) methanes as antituberculosis agents. About 30 new compounds have been synthesised and submitted for their antituberculosis activity *in vitro*. Activity at a concentration of 2- μ g/ml which will be studied in further details.

ANTIAMOEBIIC AGENTS

4-[p-N-Methyl-N-dichloroacetyl-amino) phenyl]-thiazoles, semicarbazones and thiosemicarbazones of p-(N-methyl-dichloroacetyl-amino) acetophenone

Diloxanide fuoroate and Chlorophenoxamide are effective cystocidal and luminal amoebicidal drugs used today in clinics. The active moiety in these drugs is the dichloro acetamide group. Secondly, good antituberculosis and anti-parasitic activity is reported to have been exhibited by 2-substituted-4-(o-aminophenyl)-thiazoles as well as N⁴-substituted phenyl thiosemicarbazone.

Taking lead from these two observative work was undertaken to introduce dichloroacetamido grouping in our 2-substituted, 4-(p-aminophenyl) thiazoles and N⁴-substituted phenyl thiosemicarbazones.

Thus 4-[4'-(N-Methyl-N-dichloroacetamido) phenyl]-2-substituted thiazoles, and 4'-(N-methyl-N-dichloroacetamido) acetophenone, N⁴-substituted thiosemicarbazones were synthesised and screened. The screening results show that many of these compounds are active. Two compounds are as active as metronidazole and three are twice as active. Larger quantities of these three compounds are being prepared for detailed study.

ANTIFUNGAL AGENTS

Miconazole, an imidazole derivative, a broad spectrum fungicidal agent, is highly effective against candida, dermatophytes, aspergilli and other pathogenic fungi. Besides, it provides effective coverage against gram positive bacteria.

In the attempts to synthesise compounds parallel to miconazole, the ether function of the miconazole has been replaced by the thio ether function. Secondly, oxadiazole and thiadiazole moieties have been introduced in such compounds. Thus 1-[phenyl-2-(5''-substituted aryl-1, 3', 4' oxadiazolylthio) ethyl]-1-H-imidazoles (series I) and 1-[2-p-chlorophenyl-2-(5'-substitute aryl-1', 3', 4', oxadiazolyl thio) ethyl]-1-H-imidazoles were synthesised.

About 30 compounds were prepared and the screening results for antifungal activity of 11 compounds of series (I) were received. Five compounds have shown activity at 100 $\mu\text{g/ml}$. concentration. The results of the second series are awaited.

CLINICAL PATHOLOGY

Preparation and utility of an antigen specific for mycelial phase of *C. albicans* in the serodiagnosis of candidiasis

Earlier it was shown that neither the normal individuals nor the individuals suffering from superficial candidiasis had antibodies against purified mycelial antigen when tested by passive haemagglutination test. (Details in the previous annual report)

Now an animal model resembling human systemic candidiasis has been established. Five rabbits were given deep intramuscular injection of 5×10^9 live *C. albicans* in yeast phase. All the rabbits developed a large abscess at the site of injection. At the end of 3-4 weeks the rabbits developed focal lesions of necrosis and abscess formation in the kidney (5/5), brain (5/5), heart (1/5) and liver (2/5). Mycelial forms of *C. albicans* could be demonstrated in them. The sera of these rabbits were tested for antibodies against mycelial forms by passive haemagglutination test and 3/5 were reactive with a titre of 1:8 to 1:16. It was felt that the test was not sensitive and hence it is proposed to standardise a much more sensitive technique such as ELISA.

Studies on Pathogenesis of Cholera

Employing the following two techniques the role of the various bacterial surface molecules in the adhesion of *V. cholerae* to the mucus membrane and in the pathogenesis of cholera was investigated.

1. Adult rabbit ileal loop technique for passive protection against challenge with live *V. cholerae* or its exotoxin.

2. In-vitro adhesion of *V. cholerae* to the adult rabbit intestinal loop.

The following significant conclusions could be drawn.

1. *V. cholerae* achieve adhesion to the mucus membrane primarily through interactions between the somatic antigens and the intestinal epithelial cells.

2. Adhesion is an important step in the pathogenesis of cholera, because the adherent vibrios can induce fluid accumulation by amounts of exotoxin which are at least 10 fold less than the amount that would be required had exotoxin been secreted by non adherent vibrios lying free in the lumen of the intestine.

We have now achieved our first objective, namely to identify *V. cholerae* surface antigens responsible for protective immunity. (see 1978-79 annual report)

Studies on the mechanism of immunity against *Salmonella*

The following significant information has been generated during the last year.

1. Immunisation with formalised vaccine enables mice to withstand up to 100 LD 50 dose challenge with live *S. enteritidis*.
2. Formalised vaccines stimulate antibody response, but cell mediated immunity is not induced.
3. Sublethal doses of live *S. enteritidis* employed as a vaccine stimulate low levels of antibody response, but induce excellent cell mediated immunity as tested by mouse footpad swelling and activated macrophage reaction in response to soluble antigenic challenge with protein antigens of *S. enteritidis*.

Studies on Enterotoxigenic *E. coli* (E T E C)

This new project aims to identify surface antigens of E T E C responsible for colonization.

Studies were conducted on cases of sporadic diarrhoea and 121 isolates of *E. coli* from cases of diarrhoea in infants and children were subjected to serotyping and typing for Colonisation Factor Antigen (C F A — accepted as E T E C surface molecule responsible for colonisation of the intestinal mucus membrane). Toxigenicity tests were performed by adult rabbit ileal loop technique. Following were significant findings.

1. As many as 50% of the toxigenic strains did not bear C F A meaning there by they were pathogenic to rabbit intestine even without C F A. Since these were isolated from cases of diarrhoea and since they were toxigenic for rabbit ileum it was concluded that these were strains of human E T E C capable of causing disease without C F A.
2. The frequency of C F A positivity in various serotypes of E T E C was not the same. Certain serotypes had a higher frequency of C F A.

The data have been interpreted to be in favour of the following conclusions.

1. C F A are not absolutely essential for E T E C to be pathogenic to man.
2. There is a possibility of dual interaction between the mucus mem-

brane and the E T E C. The two bacterial molecules participating in the interactions are likely to be somatic antigens and C F A.

Studies on invasive enteric pathogens

The aims and objectives of this new project will be (1) to standardise an *in vitro* assay for identification of invasive enteric pathogens, (2) to study nature of interaction between bacteria and the mucus membrane prior to invasion, and (3) to study sporadic cases of diarrhoea and dysentery to identify invasive enteric pathogens other than *Shigella* and *Salmonella*. An assay for bacterial proliferation in Hela cell monolayers is being standardised. Ability of the strains to penetrate Hela cells is being compared with standard Sereny test. Uptil now 4 strains have been tested in two systems and the results are comparable.

DIRECTOR'S RESEARCH CELL

The possibility of using *Semicarpus anacardium* extracts (supplied by the National Chemical Laboratories) as anti-carcinogenic agent is being studied. Till now seven extracts from nut of *S. anacardium* have been received and they have been put through various tests to study their pharmacological effects.

IMMUNOLOGY

Study of Mycobacterial antigens

This new project is financed by ICMR. The aims and objectives of this study will be — (1) to isolate and identify mycobacterial antigens responsible for cellular immunity, (2) to study cross reactivity pattern of common mycobacterial antigens, (3) to utilize some of the antigens so isolated for setting up diagnostic tests of clinical utility.

Earlier report (78-79 annual report) had shown that antigens of *M. tuberculosis* H₃₇Rv tend to form clumps in aqueous medium and they cannot be easily separated by standard techniques such as gel filtration and ion exchange chromatography. However it was observed that the antigenic mixture separated into multiple fractions both in polyacrylamide "disc" electrophoresis and agarose gel electrophoresis. Hence it was decided to attempt preparatory polyacrylamide "disc" electrophoresis. A large cylinder of polyacrylamide gel measuring 10.0 cm in length and 2.5 cm in diameter was prepared and the sample was electrophoresed on such a gel. After electrophoresis the gel was cut into 3.0 mm fractions. Each fraction was ground with 5.0 ml of phosphate buffered saline (PBS) to elute proteins. The protein concentration of each fraction was estimated by O.D. reading at 280. One could get 10 distinct peaks. The first three fraction near the cathode could elicit DH in patients suffering from pulmonary tuberculosis. Each of these fractions is being characterized.

Preparation of reagents for assay of immunoglobulins in serum and body fluids

The aims and objectives of this study will be (1) to prepare anti-human reagents against light chains, heavy chains, alpha, gamma and mu (2) to standardise Manson's test for estimation of immunoglobulins.

Till now purified anti-IgG, a crude anti-IgM contaminated with anti-IgG and anti-light chains and partially purified IgA from human colostrum have been prepared.

PARASITIC DISEASES AND ZONOSIS

Malaria

A study of host parasite relationship and effect of chemotherapeutic agents in rodent malaria are the principle objectives of the project. Synthetic compounds supplied by Chemotherapy department are screened and evaluated by both Peters' 4 day test and 'Rane's method' for anti-malarial properties. Out of 20 compounds, only 5 compounds are found to be active, but that too at high doses.

Schistosomiasis

During the schistosomiasis survey at Gimvi village in Ratnagiri (Maharashtra State) the snail population, density, ecology and other related environmental conditions were studied. When dissected the snails either showed bipolar or unipolar or both types of cercariae in these vectors. Further investigation for the identification of all types of cercaria is going on. Attempts to develop the adults in the experimental animals are in progress.

Hookworm infection

Human hookworm (*Necator americanus*) strain is being established very well in rabbit to use this model for various studies. Counterimmunoelectrophoresis (CIEP) test was standardised to use as an immunodiagnostic tool at various duration of infection. Result indicated that out of 45 rabbit positive sera samples only 4 samples were positive with larval antigen and 5 with adult antigen prepared from the rabbit source. Similarly with 7 positive human sample, only 2 were positive with adult antigen. This investigation suggests that this tool has limited use in the diagnosis of hookworm infection. Our second aim is to use this rabbit model in Chemotherapeutic evaluation of the synthetic compounds. In vitro testing was performed with known compound like mebendazole, betoscanate, alcopar and Levamisole against *N. americanus* larvae (RSG-3) 0.1 mg/ml, 0.2 mg/ml and 1.0 mg/ml

concentrations. 100% efficacy of all the compounds was found at the concentration of 1.0 mg/ml. within 24 hours at the cost of zero mortality in control. Further standardization and evaluation of unknown compounds are under progress for the *in vivo* test.

Cotton rat Filariasis

Litomosoides carinili infection was maintained in cotton rats. Out of 64 infected animals 26 were found microfilaria positive. The infected rats were supplied to Pharmacology Department for the chemotherapeutic evaluation of synthetic compounds. Besides cotton rat filariasis, we maintained the *Ancylostoma ceylanicum*, *Nematospiroides dubius* and *Hymenolepis nana* strains for various research purposes including drug evaluation and immunological studies.

Larvicidal activity of indigenous plants

Forty indigenous plant extracts supplied by Phytochemistry cell were screened against the 4th stage larvae of *Culex P. fatigans* mosquito in the laboratory. Out of forty, only two were found effective.

Plague :

Three species of rodents, *Rattus rattus*, *Rattus norvegicus* and *Bandicota bengalensis* were collected from different wards of Greater Bombay. Blood samples were collected by cardiac puncture. Haemagglutination test was used to determine the plague antibody using F₁ antigen fraction of *Y. pestis* supplied by W.H.O.

Snake Research

(A) Rearing of young ones of snakes in captivity :

Attempts are being made to rear young ones of snakes in the captivity. In the month of July 1979, 45 young ones of *R. viper* were born in the laboratory. These young ones were fed with young ones of mice once in a week. Out of these 45 young ones only one young one survived. This study is continued.

(B) A study of parasites collected from snakes :

12 *R. vipers*, 12 cobras, 10 kraits and 1 *Echis* were examined for parasites. It was observed that *R. vipers* were heavily infected with endoparasites. Endoparasites primarily helminths, collected from G.I. tract are being identified.

(c) *Snake Repellent study* :

A scheme for screening snake repellent activity of various Indian plants was started in collaboration with Phytochemistry Department. It was proposed and decided that Phytochemistry department will collect plants bearing essential oils for screening.

Terminalia bellarice and *Bambusa arundancea*, plants collected from Mahabaleshwar area and reported to have snake repellent activity, were taken up for this study. Various extracts of these plants were prepared by Phytochemistry department and were screened by this department. Preliminary tests show these plants do not possess any snake repelling activity.

PHARMACOLOGY

Indigenous Drug Research

The Indian traditional medicine is a rich source of drugs for different ailments particularly where the modern drug falls short due to toxicity or ineffectiveness. A study of Indian Traditional Medicine at the Dept. is supported by grants from Central Council for Research in Ayurveda and Siddha system of Medicine; and from Arex Laboratories Pvt. Ltd., Bombay.

(a) *Neuromuscular blocking activity* :

(1) Active principle from *Imperata cylindrica* plant is isolated in pure crystalline form and its structural elucidation is undertaken with the help of National Chemical Laboratory. The compound showed antidepolarising type of activity *in vivo* and *in vitro*.

Dioscorea hispida :

(2) Alkaloids were separated from the plants and one of them was shown to be a new one, 4 epidiascorine. Further work to separate the alkaloid exhibiting depolarising activity is in progress.

(3) A neuromuscular blocking activity of mixed type is detected in plant coded as CBIF. The work is under progress to isolate the pure compound.

(b) *Hypoglycemic activity* :

Plant Jitsai (*Hamiltonia suavealens*) was shown to possess powerful hypoglycemic activity. Methanolic extract was dried and redissolved in water and extracted successively with pet. ether, ether, chloroform and ethyl acetate. The ethyl acetate extract shows 38% fall in blood sugar in normal rat when given in a dose of 50 mg/kg while tolbutamide in 50 mg/kg dose caused 32% fall in blood sugar of rats. The extract is as effective as tolbutamide in monkeys. The flavonoids are being separated by using column chromatography.

(c) *Antiamoebic activity:*

The chloroform extract of *Oroxylum indicum* exhibited antiamoebic activity *in vitro* at the concentration of 500 µg/ml while metronidazole exhibited the MIC at 10 µg/ml. The drug was of no use in hepatic amoebiasis in hamsters at the dose of 400 mg/kg. Further work to isolate the pure compound and testing its activity in caecal infection in rats is in progress.

Screening of synthetic compounds

The department continued to screen synthetic compounds received from chemotherapy department. The screens used were: (i) antifilaria activity against *L. carini* in cotton rats, (ii) Antiamoebic *in vitro* and *in vivo* in hepatic amoebiasis in hamsters and caecal infection in rats, (iii) *N. dubius* and *H. nana* infestation in mice. Out of the compounds tested for antiamoebic activity 2 compounds of diloxanide series showed more activity than diloxanide *in vitro*.

Only 18 compounds were tested against filariasis but did not exhibit any activity. The compounds synthesised for activity against *N. dubius* and *H. nana* could not be tested this year.

It was decided to lay more stress on antitubercular and antileprosy compounds during the period under report.

Antileprosy drugs

DDS does not penetrate the cell of *M. leprae*. So it was thought to prepare conjugates of DDS and amino acid and/or peptides. At present the following compounds are prepared and are under test:—(1) DDS-methyl dopa (ii) DDS-dopa.

Antileprosy vaccine

In our earlier report it was shown that dead ICRC bacilli protect mouse against *M. leprae* infection by inducing cell mediated immunity. The work was further extended by using live bacilli injected at the interval of 10 days by increasing the dose from 1,000 to 10,000. Later on the mice were challenged with 7500 *M. leprae* but no protection could be seen. It is shown that live vaccine induces better cell immunity than dead. Probably the number of live organisms used was very low as compared to the dead organisms and so it failed to induce immunity.

Antibodies to Tik-20

The concept of using hapten specific antibodies to reverse the toxic effects of the hapten has been advanced only recently, and the toxic effects of digoxin have been successfully reversed in a human patient by Smith *et al.*

Fenitrothion is an organophosphorous compound used very widely today as an insecticide. Unfortunately, it is also misused widely for suicidal purposes. At present the poisoning due to this agent is treated with atropine and oximes. However, oximes are not available in our country and still the treatment is inadequate.

Antibodies were raised to fenitrothion, its active metabolite Fenitro-oxon and another pesticide of the same class, methyl-parathion, with a view to treat the poisoning by these agents. The aromatic *nitrogroup* on the pesticide molecule was reduced by using Raney Nickel, Diazotized and then coupled with bovine serum albumin. These conjugates were purified by extensive dialysis against saline, lyophilised and stored.

The analysis of the conjugates was done by washing the conjugate at 580-66c and measuring the phosphorous content of the ash by the method of Fiske and Subbarao.

Immunization of rabbits was done in the following manner. A solution of the conjugates in saline was emulsified with an equal quantity of Freund's complete adjuvant. The backs of rabbits were shaved and 0.2 ml of the emulsion was injected at five different sites. The immunizing dose was 5 mg per rabbit. Booster doses were given intramuscularly each month, 1 mg of the conjugate per rabbit.

The rabbits were bled from ear vein to check the presence of antibodies. Blood was allowed to clot, the serum expressed and tested for the presence of antibodies by reacting it against the respective conjugates using gel diffusion method.

The rabbits showed good antibody response to the conjugates. To obtain the antibodies in large amounts, the rabbits were bled, the plasma separated by centrifugation and the globulin fractions isolated by 33% saturation with ammonium sulphate. The fractions were dialysed against saline to remove the residual ammonium sulphate.

The anti-hapten antibody was isolated by adsorbing the anticarrier (anti bovine albumin) antibody on bovine serum albumin, and removing the resulting precipitates by centrifugation. The supernatants showed a precipitin reaction against the respective conjugates, but no reaction against the native carrier, thus showing the presence of the antihapten antibody.

The active metabolite of fenitrothion was prepared by oxidizing fenitrothion with conc., fuming nitric acid and extracting the oxidized product—fenitro oxon—by diethyl ether. This product inhibited serum cholinesterase and also potentiated the response to acetylcholine by the frog rectus abdominis muscle preparation.

However this potentiation could not be blocked by the anti-fenitro-oxon antibody.

A method for estimation of cholinesterase activity was standardized for mouse whole blood. Mice were fed the pesticide orally as a suspension and cholinesterase levels were shown to be depressed. To another group of mice, the poison was fed orally and simultaneously the antibody to the poison was given intravenously. However the antibody failed to prevent the depression of the enzyme levels showing that there was no protection.

Human Pharmacology and Bioavailability

Sojourn of tetracycline 250 vs 333 mg was given in repeated doses to human volunteers. These two preparations were assessed in cross over studies and the blood samples at 2, 4, 6, 8 and 10 hrs along with repeated doses were assessed for blood levels of antibiotics. The preparation contained 333 mg of tetracycline can be conveniently given three times a day and at 8 hourly schedule which maintains the blood levels adequately above mean inhibitory concentration.

(2) Effect of dietic factors on absorption of antibiotics was studied by giving most of the currently marketed preparations of antibiotics orally after meals instead on empty stomach to assess the differential absorption in a number of individuals. This study is being done with different types of diets.

Phytochemistry

Larvicidal and Snake Repellent Activities of Indian Plants Collected from Different Parts of Maharashtra

The following plants were collected from Ratnagiri, Kolaba, Mahabaleshwar and Panchgani by the section for testing various activities.

Plants	District	Activity
1. <i>Duranta plumeri</i>	Ratnagiri, Panchgani	Insecticidal
2. <i>Vitex negundo</i>	Ratnagiri	-do-
3. <i>Cymbopogon martini</i>	-do-	-do-
4. <i>Anacardium occidentale</i>	-do-	-do-
5. <i>Cuscuta reflexa</i>	-do-	-do-
6. <i>Ocimum basilicum</i>	Colaba, Mahabaleshwar Ratnagiri, Mahabaleshwar	-do-
7. <i>Agave americana</i>	Colaba	-do-
8. <i>Euphorbia antiuorum</i>	-do-	-do-
9. <i>Sepindus laurifolium</i>	-do-	-do-
10. <i>Bryophyllum Pinnafum</i>	-do-	-do-
11. <i>Euphorbia thymifolia</i>	-do-	-do-
12. <i>Mudlucca indica</i>	-do-	-do-

Plants	District	Activity
13. <i>Pteris quadrifolia</i>	Colaba	Insecticidal
14. <i>Vernania cinerea</i>	-do-	-do-
15. <i>Pogostemon legseuna</i>	Panchgani	-do-
16. <i>Axisema tortuosum</i>	-do-	-do-
17. <i>Nicandra physalodes</i>	-do-	-do-
18. <i>Nicotiana tabacum</i>	Mahabaleshwar	-do-

Plants for Snake Repellent Activity

1. *Clerodendron Serratum*
2. *Cassia fistula*
3. *Calotropis gigantia*
4. *Bambusa arundinacea*
5. *Alternanthera sessilis*
6. *Azanthus spinosus*
7. *Ipomea biloba*
8. *Terminalia belerica*

Other Medicinal Plants	District	Activity
1. <i>Swertia decussata</i>	Panchgani, Mahabaleshwar	Pharmacology
2. <i>Randia brandizil</i>	Mahabaleshwar	-do-

The Plants were used to screen various extracts using different solvents for insecticidal activities in collaboration with Zoonosis Department and to study and purify the active principle and elucidate its structure.

Azadirachta indica (Nim)

Further work was done on *A. indica* (Nim). It has been already reported that isolated pure compound which is hydrocarbon showed 100% mortality against mosquito larvae after 24 hours and also showed 5 days residual activity at 10 ppm and 8 days at 100 ppm.

Extraction, isolation, purification and crystallization of the same compound from *A. indica* (Nim) was done in order to conduct further insecticidal studies. Two per cent solution of compound was tested on mosquitoes, house-flies, bed-bugs and cockroaches. The results are as follows:

Mosquitoes	—	78% mortality
House-flies	—	86% -do-
Bed-bugs	—	60% -do-
Cockroaches	—	35% -do-

Ocimum basilicum

Petroleum ether extract of *O. basilicum* showed promising larvicidal activity. Therefore, it was subjected to the alumina column chromatography and eluted with petroleum ether, benzene, benzene-methanol (1:1) and methanol. Petroleum ether elute was crystallized and gave -ve test for nitrogen, sulfur and halogens. UV, IR NMR of the same compound were taken up. This compound seems to be a hydrocarbon. When tested for larvicidal activity, it was observed that 100% mortality was obtained at 100 ppm concentration within 24 hours.

Another fraction i.e. benzene methanol (1:1) elute showed 100% mortality at 10 ppm concentration at the end of 24 hours. When subjected to further purification using alumina column, several fractions were collected. All these fractions were subjected to TLC (silica gel G) using Benzene: Chloroform (7:3) solvent system. Fractions showing equal R_f value were pooled together. Eight such fractions were obtained. The fraction having R_f value 0.2 showed larvicidal activity (100% mortality at 100 ppm and 50 ppm). From Co-TLC and other test, it was concluded that active compound was β -sitosterol.

Duranta Plumeri

Powder of berries of *Duranta plumeri* was serially extracted with petroleum ether and methanol. Both the extracts showed promising activity. From petroleum ether extract ursolic acid and β -sitosterol were isolated. Methanolic extract was processed for total alkaloids by digesting in 5% HCL and by filtering and extracting with chloroform to remove non-alkaloidal chloroform soluble material. It was basified by liquor ammonia and extracted with chloroform till it showed -ve test for Mayer's reagent (alkaloids).

The total alkaloids were chromatographed on silica gel column and eluted with chloroform, chloroform +methanol. Two bands were observed which were collected separately. The work of screening these two fractions for mosquito larvicidal activity and antimicrobial activity will be taken.

Studies of Indian Medicinal Plants: Antidiabetic Study of Pueraria tuberosa

Pueraria tuberosa was collected from Goa. Dried powder of the tubers was extracted with various organic solvents. Methanolic extract showed promising hypoglycemic activity, therefore, it was taken for the isolation of active principle.

A flavanoid pure compound was isolated by using column chromatographic technique. Purity of the compound was confirmed by thin layer and paper chromatography. Hypoglycemic activity was studied with various dose levels and compared with tolbutamide. It was observed that drug produced 35-40 mg% reduction in blood sugar of normal rats which was more or less similar to that of tolbutamide.

Following biochemical parameters were taken up which are generally studied for antidiabetic drug. The methods have been standardized in order to find out the action of the drug on carbohydrate and fat metabolism.

1. Cholesterol
2. Triglyceride
3. Free fatty acids
4. Glycogen
5. Pyruvic acid
6. Total lipids.

RADIATION BIOLOGY

Radio-immunoassay of Antitetanus antibodies

The study was aimed at assessing the level of antibodies following immunization with tetanus or triple antigen in a group of selected individuals. In the past, detection methods like mouse protection test or passive haemagglutination tests were carried out for the same purpose. Radioimmunoassay, which is highly sensitive techniques for detection of low concentration of antibodies was employed.

(A) *Radioimmunoassay.*

A sandwich technique was employed. Purified tetanus toxin (antigen) coated polystyrene beads as a solid phase was allowed to react with unknown serum containing antitetanus antibodies in a buffered medium at 37°C. The antigen antibody complex with excess of antibodies was washed and to it finally labelled purified tetanus toxin was added. Subsequent washings removed excess of labelled antigen and the one which was attached to the beads was counted in a gamma counter.

A standard curve was prepared using dilutions of human Tetanus Immunoglobulins of known concentration (500 I.U./ml) and by this method it was possible to detect antibody concentration upto .0001 I.U./ml. The comparative assay using all the above conventional methods is being done, using sera from children immunized with triple antigen as well as from individuals hitherto unimmunized with any kind of tetanus antigen in earlier life.

(B) *Radioimmuno-electrodiffusion*

Instead of solid phase assay with polystyrene beads, an attempt was made to assess unknown antibody levels in a state of antigen antibody complex precipitated as a band in agarose medium. In an electric field opposite movement of antigen antibody complex can be cut and counted for the radioactivity embedded in it. Once again I^{25} labelled purified tetanus toxin was used for the purpose. This method is still in an experimental stage.

Radiometric assay for chemical preservatives

All the growing microorganism require glucose for energy metabolism. If radioactive glucose is substituted in the medium its uptake by the organism at certain fixed time was measured.

Chemical preservation arrests the division of micro-organisms and thus maintains the sterility of products. This if added to medium interferes with the uptake of radiolabelled glucose at periodic intervals.

TOXICOLOGY

Acute toxicity studies of commonly used pesticides

Complete acute oral toxicity in rats of 2-4D amine salt, chlorocholine chloride, chlorocholine chloride 50%, Folithion (Fenitrothion), Accothion 50% EC, Cycocel, Ethion (Tech), Quinalphos Tech, Quinalphos 50%, Ethion 50% EC, Dimethioate 40 and 30 EC and PMA was investigated. 2-4D amine salt and Cycocel were less toxic than others ((LD₅₀ 1559 mg/kg and 1305 mg/kg) in rats by oral route. Quinalphos 70% was most toxic by this route (LD₅₀ 25.1 mg/kg).

Acute dermal toxicity studies of Quinalphos Tech, Ethephon, Ethion 50% EC, and Ethion Tech in rabbits were done. Quinalphos Tech was more toxic (LD₅₀, 118.7 mg/kg) and ethephon less toxic (LD₅₀ 2100 mg/kg) by dermal route in rabbits (Appendix X shows the name of the pesticide, species, route, LD₅₀, fiducial limits and symptoms).

Protein deficiency and drug action

Protein malnutrition was created in young rats weighing between 60 to 70 gms by feeding 3% protein isocaloric diet for a period of 4 weeks while for comparison 15% protein isocaloric diet was fed to control animals for the said period. Only those animals which showed 20% or more reduction in their initial body weight were selected for the study from the 3% protein fed animals. LD₅₀ and ED₅₀ studies were completed earlier. Blood absorption studies were conducted this year. The result indicates—

(a) The absorption of aspirin and salicylic acid is slower in the animals fed on 3% protein diet than the animals receiving 15% protein diet.

(b) The metabolism of aspirin is faster in the rats fed on 15% protein diet than the animals fed 3% protein diet.

Salicylate level in body tissues like kidneys and liver are in progress. Also determination of Aspirin metabolites in urine in normal and protein mal-nourished animals is in progress.

VIROLOGY

*The In Vivo Development of Vaccines Against Viral Hepatitis Type B Using the Newborn Oryctolagus cuniculus Model System, Phase II. Development of a Non-human Primate Model**Newborn and Foetal Rabbit Model Systems*

As recorded in the *Annual Report* (1978-1979) and the *Consolidated Report* (1976-1979), results accruing from studies involving very substantial numbers of newborn and foetal rabbits confirmed the earlier findings concerning highly significant weight loss in animals inoculated intrahepatically, at birth, or intravenously (pregnant female inoculation), with HIBOMA-I (5) (the lapinized human HBV), or HIBOMA-HSP (human HBV consisting of a serum pool from 24 HBsAg-positive patients prepared at this Institute) and HIBOMA-HSP (RPI) the first rabbit liver passage of HIBOMA-HSP. It is believed that this is the *first* such demonstration in any non-human mammal, the work on this *Oryctolagus cuniculus* model having been initiated in 1974-1975 and being continued even at present. Biochemical evidence (SGPT elevation as a marker of acute, or chronic hepatitis), following intrahepatic inoculation at birth with HIBOMA-I (5) virus, was provided, in the above mentioned documents in a total of 96 experimental and 38 control animals (acute hepatitis) and in 71 experimental and 55 control animals (chronic hepatitis).

Concerning the development of a non-human primate model, it will be recalled that the langur system experiments were initiated when a single langur inoculated intravenously with HIBOMA-I (5), the lapinized HBV, became progressively emaciated and was then inoculated intravenously with HIBOMA-HSP, the *human virus*. Thirty four weeks following the HIBOMA-I (5) injection, the liver of this animal revealed a well-marked portal triaditis, of the type seen in human chronic persistent hepatitis. During 1978-1979, when a liver homogenate from this animal was inoculated intravenously into two fresh langur, one developed the same emaciation, significantly elevated SGPT levels and histopathological evidence of liver damage, whereas the second was clinically healthy, but had liver lesions. Thus, it became clear that HIBOMA-I (5), or HIBOMA-HSP (or, possibly, a mixture of both) could be passed into the langur, thus satisfying one of the most important criteria of demonstrable viral aetiology and suggesting that, probably, a linking experiment had been performed, tying up the rabbit and a langur HIBOMA/HBV systems.

Non-human Primate Model Development — The Langur System

Virological Studies: A crucial step in the development of new animal model systems and live attenuated/inactivated vaccines in virology is the demonstration of replication of the human viruses in the new, heterologous hosts, leading to the phenomenon of adaptation. It is, furthermore, essential

to demonstrate that the animal-adapted strain of the original human agent is present intracellularly in the target organ.

In our earlier *Annual Reports* (see Report for 1978-1979), as well as in the present one, it has been stated that, in the newborn rabbit inoculated with HIBOMA/HBV intrahepatically, or in newborn animals delivered from females inoculated intravenously in the fourth gestational week, the originally clinically observed signs of weight loss and emaciation could be objectively confirmed by actually demonstrating highly significant loss of weight in such animals. In langur intravenously inoculated with the same viruses, clinical observation had shown that these animals, too, exhibited anorexia and emaciation, in addition to a number of other clinical signs of infection (see *Consolidated Report, 1976-1979*). Thus, the first such clinical signs were recorded in a non-human mammalian and a non-human primate species. In the langur model, since these signs were observed not only with HIBOMA-HSP, the human hepatitis B virus, but with its liver passages, as well, it was believed that successful serial passage had been accomplished in this species. In addition, the biochemical and morphological (liver histopathology) parameters of infection with HIBOMA/HBV had also been studied and encouraging results had accrued from such experiments.

For demonstration of the intracytoplasmic presence of HIBOMA/HB viruses and their langur liver passages, the direct immunofluorescence test, utilizing anti-HBs raised in sheep in this laboratory, was utilized. Liver sections were obtained from the animals at open liver biopsies carried out pre-inoculation and post-inoculation (at six week intervals).

The total number of langur investigated for the presence of *infection with HIBOMA-HSP* was 23. Of these, 16 animals were examined for specific intracytoplasmic direct immunofluorescence, out of which six were positive.

It has been pointed out that adaptation of HIBOMA-HSP to the langur liver had been accomplished by serial passage in this heterologous host system. It was further necessary to demonstrate that the simianized virus shared some antigenic determinants with the parent human strain, this being a significant step towards the ultimate development of vaccine strains. Hence, further serial passage experiments were carried out, along with immunofluorescence studies on liver sections from animals infected with such passaged virus. Thus, HIBOMA-MLP (liver passage 1 from 6 monkeys receiving HIBOMA-HSP) was inoculated into 4 langur. One of these animals (No. 26) which was positive for elevated SGPT and histopathological lesions of viral hepatitis, showed positive direct immunofluorescence, as well. This demonstration of antigen reacting with anti-HBs in liver sections established the transmission of HIBOMA-HSP, the human virus, through 2 serial passages. In a further serial passage experiment, two animals were injected intravenously with a 10% liver homogenate (designated HIBOMA-MLP (26/2) from langur No. 26. Intense immunofluorescence was demonstrated in all three open liver biopsy specimens taken at 7, 8½ and 10 weeks post-inoculation in both animals.

Thus, the presence of HIBOMA viruses was demonstrated, through 3 serial passages, in langur inoculated initially with human HBV (HIBOMA-HSP)—this presence had been anticipated on the basis of virological, biochemical and histopathological findings.

Biochemical Studies: It was decided to test whether a significantly elevated SGPT level, a classical marker of the hepatocellular necrosis of viral hepatitis, could also be established as one of the parameters of HIBOMA/HBV infection in the langur, as demonstrated in the rabbit model. Here, again, it was necessary to obtain baseline data from an adequate number of serum samples from *freshly caught, normal* langur, prior to any virus inoculation, or any other treatment, after arrival at our laboratory. For this purpose, the SGPT levels of 48 sera from langur (2.5–6.0 kg) were determined, using the Reitman-Frankel method, the data being subjected to statistical analysis. The 3-sigma range for normal langur was found to be 3-45 IU.

In the establishment of an elevated SGPT level (greater than 45 IU), as being a criterion of hepatocellular necrosis in the langur, a total of 32 langur was involved, in four of the following experiments:

In the first experiment, which consisted of nine animals, the pre-inoculation sera were tested for SGPT levels, as were the post-inoculation weekly samples, the observational period of the experiment being 28 weeks. The virus inoculated intravenously was HIBOMA-HSP, the human agent. The results clearly showed that 8/9 langur exhibited elevated SGPT levels post-inoculation, the majority showing recurring peaks. All preinoculation sera were within the normal (3-45 IU) range.

The second experiment involved 2 langur and the virus inoculated intravenously was HIBOMA-LILS (the first langur passage consisting of HIBOMA-I (5), the lapinized virus, alone, or a mixture of this virus with HIBOMA-HSP the human HBV). Here, again, weekly-drawn post-inoculation serum samples from both animals showed elevated SGPT levels, whereas neither of the pre-inoculation specimens showed any such elevation. One of the two langur showed recurring peaks of SGPT elevation.

In the third experiment, comprising 4 langur, the virus inoculated was HIBOMA-MLP (a first liver passage pooled from 6 monkeys receiving HIBOMA-HSP and showing histopathological evidence of viral hepatitis, acute or chronic). Here, all four monkeys showed elevated SGPT levels post-inoculation, whereas none of their pre-inoculation sera exhibited any elevation. One of the monkeys showed a high frequency of recurring peaks.

The fourth experiment was of a more sophisticated design, intended to provide an unequivocal answer as to whether, or not a raised SGPT level was a definite criterion of hepatocellular necrosis for the langur model and involved a total of 13 animals: a group of 7 experimental monkeys was inoculated intravenously with the same human virus, HIBOMA-HSP, as used in the first experiment, while a control group of 6 langur was inoculated

intravenously with a normal human serum pool from 24 subjects, shown to be free of HBsAg by RPHA.

The SGPT levels of all animals were determined prior to inoculation and, subsequently, during a twenty-eight week observational period, at weekly intervals. The results of this experiment were unequivocal: six out of the 7 experimental animals showed significantly elevated SGPT levels, 5/6 showing recurring peaks and 4/6 levels between 95 and greater than 100 IU.

Thus, the above four experiments clearly established that an elevation of the SGPT level in the langur, beyond the upper limit of the 3-sigma range (3-45 IU), is a definite marker of hepatocellular necrosis produced by viral hepatitis, following HIBOMA/HBV intravenous inoculation.

Cell Culture Studies: Human HBV/HIBOMA viruses have not, to date, been cultivated in non-transformed cells *in vitro*. Hence, in accordance with one of the objectives set forth in the original SERC Project on attempting the development of vaccines *in vitro*, it was decided to test whether trypsinized cells from kidneys from langur infected with HIBOMA viruses could be cultivated *in vitro*.

Hence, kidneys from infected and control (uninfected) langur were aseptically excised (at necropsy, as and when these were carried out during the course of *in vivo* experiments described in this Report) and the cells explanted following trypsinization. The time required for the completion of growth (formation of incomplete or complete monolayers in the culture tubes) was noted for the experimental and control kidneys. When the maximal growth possible had been attained, the cultures were stained with haematoxylin and eosin and cytopathology noted.

In an experiment, a total of 15 langur kidneys was available. The animals had been either infected *in vivo* with HIBOMA-HSP (Group 1) HIBOMA-LILS (Group 2) or HIBOMA-MLP (Group 3), or were uninfected controls (Group 4). Table 1 summarizes the data obtained from this experiment:

TABLE 1

Incidence of and Time Period for Maximal Growth of Kidney Cultures from Langur Infected with Different HIBOMA Viruses

Group	Infecting Virus	Maximal Growth Period (Days)	Mean (Days)	Kidneys Showing Growth (Per Cent)
1	HIBOMA-HSP ¹	10	9.7	66.6
2	HIBOMA-LILS ²	12	12	50.6
3	HIBOMA-MLP ³	18	18	33.3
4	Control	5	5	100.00

1. Human HBV serum pool from 24 HBsAg-positive patients.
2. Langur passage 1 consisting of HIBOMA-I (5), the lapinized human HBV, or its mixture with HIBOMA-HSP.
3. Langur passage 1 pooled from 6 monkeys receiving HIBOMA-HSP.

The preliminary results indicated that, whereas 100% of the control langur cultures attained maximal growth with the formation of complete monolayers, in a mean period of only 5 days, monkeys inoculated with the human HBV yielded 66.6% growth at 12 days, those with the lapinized virus (+ human HBV) 50.0% growth at 18 days, the poorest growth (33.3%) and the longest growth-period (18 days) being seen with HIBOMA-MLP, the first langur passage of the human virus. It might be speculated from the above data, that the rabbit—and langur-adapted strains of human HBV produced greater kidney-damage in the langur, yielding poorer growth.

When Leighton tube coverslip preparations of the above cultures were examined by light microscopy, several types of CPE were seen: intracytoplasmic and intranuclear eosinophilic inclusions, cytoplasmic vacuolation and granulation and its formation of multinucleate giant cells.

Serological Studies on Langur Infected with Different HIBOMA Viruses

During the *in vivo* virological studies already described, a number of langur sera became available for serological studies, in particular for testing for the presence of viraemia, as an indicator of replication of virus, in this newly-developed non-human primate model.

For this important purpose, 401 langur pre-and post-inoculation sera from animals inoculated with HIBOMA-HSP, or HIBOMA-MLP were tested for the presence of HBsAg, using a locally modified RPHA technique. Initially, 75 serum samples (reference sera) from the National Institutes of Health, Bethesda, U.S.A./U.S.B. were utilized for the standardization of the method. Forty human sera from voluntary blood donors were also included in the experiment. Table 2 presents the results:

TABLE 2

Presence of HBsAg in Sera Tested by RPHA from Langur Inoculated with Different HIBOMA Viruses

Group	No. Sera Tested	No. Positive	No. Positive After Specific Neutralization	No. Negative
Pre-inoculation	75	Nil	—	75
Post-inoculation	401	23	7	394
Voluntary Donor (Human)	40	2	1	39

From the above results it was clear that, although 23/401 sera were initially found positive for the presence of HBsAg with the modified RPHA test, 7/23 were definitely positive after a specific neutralization test had been performed to eliminate false-positives. Nevertheless, it was clear that the presence of HBsAg had been definitely demonstrated in 7/401 sera from langur inoculated with HIBOMA viruses. Further work is in progress in this laboratory.

RABIES RESEARCH UNIT

I. Project on the Development of a Tissue culture Vaccine against Rabies

Work on this project is sponsored by the Department of Science and Technology, Govt. of India, New Delhi.

A. Adaptation of Rabies Virus in Primary Cultures of Baby Hamster Kidney (pBHK):

Further experiments were put up to study the viral kinetics in pBHK. The results of the experiments suggested that the replicated virus appeared in the fluid phase in cycles. The first cycle of maximal yield appeared to be around 3rd or 4th post infection day (PIDay). The next cycle was noticed on 8th to 9th PIDay. The exact timing seemed to depend on the amount of the seed virus employed. Nonetheless, the titres of the progeny virus were extremely low ($10^{0.5-1.5}$ LD₅₀ in mice). Subpassage of the low titered medium failed to infect fresh set of cultures. It is therefore proposed to mix fresh seed virus along with the progeny virus for propagation in serial subcultures. Alternatively, the harvested culture medium might be subjected to ultrafiltration to concentrate the progeny virus before further passages.

B. Growth and Standardisation of Human Diploid Cells

Employing a continuous cell line of human diploid cultures offers some acknowledged advantages for adaptation of a virus for the purpose of vaccine development for human use. As the liquid nitrogen storage facility has not yet arrived, a suitable cell line certified for vaccine work could not be procured. However, preliminary work has already been started on a cell line of human skin fibroblast cultures obtained from Cancer Research Institute, Bombay.

More recently, arrangements were made with a neighbouring hospital to collect human foetuses after abortion. After a good deal of standardisation work, we have succeeded in growing foetal lung tissue *in vitro* in two instances: in one of these, we have been able to establish a cell line. Work is now underway to screen the cell line for suitability for its vaccine developmental work, e.g. sterility, genetic stability etc. Its potential as a viral diagnostic tool can also be assessed after its susceptibility to different viruses is known.

C. *Detection of Rabies Virus Antigen/Antibody (RVAg/RVAb)*

During the course of adaptation of rabies virus to cell cultures, it was necessary to detect and quantify the virus in the culture medium. The conventional method requires inoculation of laboratory mice and observation for a period of 2 to 3 weeks. This is not only time consuming but expensive as well. Apart from the tissue culture medium, it was also intended to detect the virus antigen from animal and human brains. The following two methods were attempted for the rapid detection of the virus.

(1) *Immunofluorescence*

(a) *Preparation and tagging of hyperimmune serum*: Rabbits were immunised with four doses of killed vaccine given at weekly intervals followed by 3 doses of live virus. The killed vaccine comprised of 1 ml of an imported rabies tissue culture vaccine from human diploid cells mixed with 1 ml of Freund's complete adjuvant and given intraperitoneally. The live doses comprised of 1 ml of a virus-infected mouse brain suspension diluted to contain about 100 MLD₅₀ of the virus as determined in mice. Serum samples were obtained from the rabbits pre-immunisation and at the end of 4th, 5th, 6th, 7th dose and 10 days after the last live virus dose. The last sample was used for the immunodiffusion tests as well as for labelling with fluorescein.

The serum was labelled with fluorescein isothiocyanate (FITC) employing standard techniques. Briefly, this involved precipitation of immune globulins, dialysis, fluorescein tagging and gel filtration. The material obtained finally was used to detect RVAg by fluorescence microscopy.

(b) *Test Antigens*: The antigens tested included known positive and known negative materials in addition to the test materials from animal brains and tissue culture fluids.

(c) *Results and Conclusions*: While many of the known positive test samples gave positive results, some of the negative materials also gave similar results. This non-specificity was traced to the presence of albumin in the antigen which was used for rabbit immunisation. A more purified antigen is therefore being prepared for fresh immunisation.

(2) *Radial Agar-gel-Immunodiffusion (R-AGID) and Counter-immune electrophoresis (CIEP)*:

The test antigens and the hyperimmune serum employed in these tests has been described earlier under immunofluorescence. The tests were performed as follows:

R-AGID: Three ml. of 1% Noble Agar at 50 C was allowed to set on each of the many standard microscope slides at 4 C. Cavities were punched

out in agar according to a template suited for the purpose. The immune serum was placed in the central cavity and the test materials in the peripheral cavities. Suitable controls were included. Results were read after incubation of the slides at room temperature for 5-6 days.

CIEP: Slides were prepared in the same way as mentioned above, but pattern of cavities was varied to suit the requirements of the test.

After loading the slides with serum in appropriate cavities, they were subjected to a current of 150 V for 30 mts. The slides were then loaded with antigens to be tested and subjected to a current of 50 V for 3 mts. The polarity of the current and other methodology of the test was maintained as per the current literature. The slides were then removed from the electrophoretic chamber and washed and dried at 37 °C. Finally they were stained and observed for precipitin lines between the antibody and antigen cavities.

Results and Conclusions: As the results for the two tests are similar, they are presented here together.

Results of the R-AGID and CIEP Tests

Antiserum	Antigen & Source	Results
Rabbit Hyperimmune globulin	A R Vaccine	+
	T C Vaccine	+
	Rabies Virus ex rabbit brain suspension	+
	Infected Human brain suspension	+
	Normal Human brain suspension in saline	+
	Normal Human brain suspension in horse serum saline	++
	Normal brain suspension in saline from rabbit, hamster, mice	-
	Normal sera from rabbits, hamster and mice	-

+ One line

++ Two lines

- No line

The results showed that normal human sera as well as the normal human brain suspensions gave non-specific positive lines with the hyperimmune serum employed. Similar non-specific reactions were also seen in the immunofluorescence tests described earlier. Further work is being continued on preparation of a more purified antigen for the tests.

II *Non-Project (Departmental) Research*

A: Intramuscular (IM) Adaptation of rabies Virus

The present day potency tests on rabies vaccines (Habel and NIH tests) employ an intracerebral (IC) route of challenge virus for the vaccinated mice. It was therefore thought desirable to adapt the challenge virus to the intramuscular (IM) route which could be employed to provide a more natural route of challenge. Such adapted strain could also be advantageously utilised for testing any drug or method employed to prevent rabies in the infected animals.

The CVS rabies virus was adapted by the IM route in adult laboratory mice. Initially the deaths were noted to occur irregularly and at late intervals after inoculation. By about 5th passage, there were regular deaths occurring on 7th-8th day. The virus has now undergone 22 passages in mice, and a titre of $10^{2.5}$ LD₅₀ has been achieved in adult mice.

(1) *Potency Tests on AR Vaccine*: Mice Immunised with standard A.R. Vaccine were challenged by the 2 different routes. Initial results indicate that mice can withstand a higher dose of virus given IM than by IC route. Further work is continuing.

(2) *Drug Therapy for rabies prevention*: This work was done in collaboration with the Department of Pharmacology, which provided 2 drugs for testing. Each of the drugs was tested by oral administration to the mice for 5 days after administering about 20 LD₅₀ of the IM-adapted CVS rabies virus. The mortalities between this group and the controls given virus alone were similar. Thus, the drugs were not found to be protective.

CHAPTER IV

GENERAL ACTIVITIES

Administration :

The Government has sanctioned funds for the development of departments of Parasitology and Epidemiology in the sixth five year plan. This will meet the long felt need of expansion of these units which were already existing in rudimentary form in the Institute and is expected to provide incentive to research in these fields.

However, the financial position of the Institute continued to be critical because the annual Government Grant sanctioned during 1975-76 remained static while expenditure due to increase in pay, dearness allowances and cost of chemicals, glasswares and equipments continued to rise. This fact was brought to the notice of the Government but till now this has not brought any relief.

Artist section

This section prepares slides, charts, photographs required by staff, research scholars for teaching, thesis, conferences and also for exhibitions arranged by the Institute from time to time.

Library

During the year, six meetings of the Library Committee were held and the library matters were discussed. The Library continued to participate in the International Exchange Programme sponsored by the World Health Organization.

The CIBA Geigy of India and the World Health Organization presented 31 books to the Library.

The work of collecting reprints on communicable diseases from 1968 onwards has been continued and 70 more reprints have been added to the collection.

The inter-library loan facility has been continued and extended to two more libraries. The extension of working hours of the library from 5.30 p.m. to 7.00 p.m. from Monday to Friday and the practice of keeping the library open on Saturday from 9.00 a.m. to 5.00 p.m. has been also continued.

APPENDIX I

Activities of Artist Section

<i>Work</i>					<i>Number</i>
Preparation of Charts, graphs etc.	...	...	...	...	400
Preparation of slides	...	...	...	...	400
Preparation of Prints	...	...	...	...	200
Exposed microphotograph	...	...	...	...	

APPENDIX II

Bacteriology Department

<i>Tests</i>					<i>Number</i>
Lyophilization of bacterial cultures	...	...	...	...	40
Bacteriological analysis of water samples	...	...	...	...	4
Bacteriological analysis of papain samples	...	...	...	...	4
Bacteriological analysis of paint samples	...	...	...	...	3
Bacteriological analysis of silicon samples	...	...	...	...	2
Testing of candle filters	...	...	...	...	9
Testing of Biocides	...	...	...	...	6

APPENDIX III

Biochemistry Department

<i>Tests</i>					<i>Number</i>
Blood	...	...	...	...	879
Urine	...	...	...	...	109
CSF & other body fluids	...	...	...	...	004
Special tests	...	...	...	...	561
Total				...	1553

APPENDIX IV

Clinical Pathology Department

The routine investigations carried out during the year are enumerated below.

					<i>No. of samples</i>
A. Histopathology	...	...	...	...	541
B. Hematology	...	...	...	...	828
V. Cytology for malignant cells	...	...	...	...	22
D. Clinical Pathology	...	...	...	...	
Urine for routine examination	...	...	...	...	200
Stool for routine examination	...	...	...	...	127
Semen examination	...	...	...	...	16
Immunological Pregnancy Tests	...	...	...	...	1163
Biological (Rat Test) Pregnancy Tests	...	...	...	...	132

	No. of samples
E. Bacteriology	
Culture for Tubercle Bacilli ...	136
Animal Inoculation for Tubercle Bacilli ...	62
Smear & Culture for <i>C. Diphtheriae</i> ...	55
Urine Culture ...	381
C.S.F. Culture ...	4
Stool Culture ...	23
Blood Culture ...	31
Sputum Culture ...	21
Miscellaneous Body Fluids & Exudates Culture ...	66
Antibiotic Sensitivity Tests ...	419
Stool Culture (Cholera) ...	5
Stool Culture (Typhoid) ...	16
Urine Culture (Typhoid) ...	14
Blood Culture (Typhoid) ...	11
Pus Culture ...	82
Enteropathogenecity Test ...	69
Sputum for Gram staining ...	1
Culture of Theatre Swabs for Anaerobes ...	236
F. Serology	
Widal Test ...	270
Weil Felix Test ...	78
Brucellosis Test ...	81
V.D.R.L. Test ...	69
Kahn Test ...	1
Paul Bunnel Test ...	181
Casoni's Test ...	7
R. A. Test ...	6
Cold Agglutinins ...	4
G. Mycology	
Isolation and identification of Pathogenic Fungi from Clinical Material	10

APPENDIX V

Human Pharmacology and Bioavailability

Name of the Company	:	Antibiotics Tested
Martin & Harris	:	Tetracycline Ampicillin
Lyka Labs	:	Tetracycline Ampicillin
Hindustan Antibiotics	:	Erythromycin Cephalexin Cloxacillin Amoxicillin
Unique Pharmaceuticals	:	Kinex Icicillin Amoxicillin Iifimycin Injection
Symphony Pharmaceuticals	:	Symphactin
Citadel	:	Prestet D
Pfizer	:	Coded Sera
Gufic	[:]	Ampicillin
IPCA	:	Erythromycin
Indo Continental	:	Reformin Cefocin

APPENDIX VIA

Hospitals and Agencies making use of the services of Clinical Pathology Department

Bombay Area		From Maharashtra
Cama and Albless Hospital	...	Civil Hospital, Thana.
St. George's Hospital	...	Civil Hospital, Alibag, Kolaba.
Jagjivanram Hospital	...	General Hospital, Sawantwadi.
Central Railway Hospital	...	I.S.P. Hospital, Nasik.
B.Y.L. Nair Charitable Hospital	...	Public Health Centre, Buldhana.
K.E.M. Hospital	...	Civil Surgeon, Sangli.
M.G.M. Hospital	...	District Hospital for Women, Akola.
L.T.M.G. Hospital	...	Civil Hospital, Akola.
E.S.I.S. Hospitals	...	Primary Health Centre, Jalgaon.
K.B. Bhabha Hospital	...	General Hospital, Osmanabad.
Kasturba Hospital	...	Civil Hospital, Dhulia.
G.T. Hospital	...	Civil Hospital Nasik.
J.J. Group of Hospitals	...	Hindustan Aeronautics Ltd., Nasik.
Police Hospitals	...	
M.A. Podar Hospital	...	
Greater Bombay Municipal Dispensaries	...	

APPENDIX VI

Rabies Research Unit

Materials	No. of Samples	Negribody Present	Nos. positive for Biological Test
Human brain	6	Nil	1
Human CSF	1	Not done	Nil
Dog brain	2	Nil	2
Total	9		3

APPENDIX VII

Radiation Biology Unit

Thyroid Uptake studies						Nos.
Hyperthyroid	...	...	...	...	...	15
Toxic Goitre	...	...	...	...	...	9
Non-toxic Goitre	...	...	...	...	...	11
Myxoedema	...	...	...	...	...	4
Thyroxine suppression test Adv.	...	...	...	...	...	13
Euthyroids	...	...	...	...	...	50
Total	...	...	...	...	...	102

APPENDIX VIII
Testing Department

Nature of samples				No received	No tested
(A) Prohibition & Excise					
IMFL	...	...		1132	1127
Beer	...	...		887	959
Toddy	...	...		268	297
Bhang	...	...		5	5
Ganja	...	...		31	31
Opium	...	...		9	9
Charas	...	...		2	2
(B) Drug samples					
				No received	No tests done
Infra-red and polymorph	...	...		108	108
Complete analysis	...	...		200	978
Pyrogen & Sterility	...	...		284	284
Microbiological assays	...	...		78	78
Adrenaline	...	...		34	34
Depressor substances	...	...		174	174
R.W.C. & S.A.	...	...		38	51
Insulin	...	...		38	38
Miscellaneous	...	...		90	90
Total					4265

APPENDIX IX
Samples tested for toxicity

Sr. No.	Classification of the samples		No. of samples tested		Toxicity Parameters
1.	Drug	...	...	4 4 5 151	Acute oral toxicity Acute intravenous toxicity Undue toxicity Depressor substance test
2.	Chemical	...	...	5	Acute oral toxicity
3.	Cosmetics	...	...	3	Primary skin irritation
4.	Pesticides	...	...	13 5 4	Acute oral toxicity Acute dermal toxicity Primary skin irritation
5.	Packaging material	...	...	1	Acute oral toxicity.

APPENDIX X

Acute oral toxicity of some pesticides

Sr. No.	Name of the compound	Species	LD ₅₀ value	Fiducial limits	Symptoms
1.	2:4-D amine salt	... Rats	1559 mg/kg	1334-1821 mg/kg	Excitation, respiratory distress and death
2.	Chlorocholine chloride (Tech)	Rats	440 mg/kg	411.2-470.8 mg/kg	Excitation, respiratory distress and death.
3.	Chlorocholine chloride 50%	Rats	1031 mg/kg	824.9-1289 mg/kg	Excitation, respiratory distress and death.
4.	Folithion	... Rats	445 mg/kg	356-556 mg/kg	Excitation, convulsions, respiratory distress and death.
5.	Accothion 50% EC	... Rats	550 mg/kg	363-824 mg/kg	Tremors, animals appear sluggish & death.
6.	Cycocel	... Rats	1305 mg/kg	1088-1566 mg/kg	Tremors, excitation, respiratory disturbances and death.
7.	Ethion 50% EC	... Rats	60.9 mg/kg	43-85 mg/kg	Excitation respiratory distress and death.
8.	Ethion Tech		33.8 mg/kg	27.3-44.2 mg/kg	Excitation followed by depression, respiratory disturbances and death.
9.	Quinalphos (Tech) 70%	... Rats	25.1 mg/kg	21.1-29.9 mg/kg	Excitation, respiratory distress and death.
10.	Quinalphos (Tech) 50%	... Rats	40 mg/kg	33-48 mg/kg	Excitation, respiratory distress and death.
11.	Quinalphos 50%	... Rats	36 mg/kg	26-50 mg/kg	Excitation, respiratory distress and death.
12.	Dimethoate 40 E	... Rats	675 mg/kg	613.4-743 mg/kg	Excitation, respiratory disturbances and death.
13.	Dimethoate 30 E	... Rats	750.4 mg/kg	641.7-880 mg/kg	Excitation, respiratory distress and death.
14.	P M A	... Rats	36 mg/kg	27.7-46.8 mg/kg	Excitation, respiratory distress and death.
15.	2:4-D Sodium Salt	... Mice	410 mg/kg	359.65-467.4 mg/kg	Excitation, respiratory distress and death.

APPENDIX X (Contd.)

Acute dermal toxicity studies

Sr. No.	Names of the Compound	Species	LD ₅₀ value	Fiducial limits	Symptoms
1.	Ethephon	... Rabbits	2100 mg/kg	1990-2310 mg/kg	Excitation, salivation, irritation to skin, diarrhoea, loss of appetite & death.
2.	Ethion (Tech)	... Rabbits	195 mg/kg	88.6-429 mg/kg	Excitation followed by depression, respiratory distress and death.
3.	Ethon 50% EC	... Rabbits	210 mg/kg	115.5-378 mg/kg	Excitation followed by depression, respiratory distress and death.
4.	P.M.A.	... Rabbits	450 mg/kg	173-1170 mg/kg	Excitation followed by depression, respiratory distress and death.
5.	Quinalphos (Tech)	... Rabbits	118.7 mg/kg	107.4-135.6 mg/kg	Excitation, salivation, diarrhoea, motor incoordinational loss of appetite, respiratory distress and death.

APPENDIX XI
Snakes, Scorpions and Venom

Type of Work					Number
(A) Venom extraction demonstration					7740
(B) Animals purchased					
Cobra	...	...	...	...	209
Viper	...	...	...	...	212
Krait	...	...	...	...	472
Echis	...	...	...	...	31
Red scorpion	...	...	...	...	4378
Black scorpion	...	...	...	...	298
(C) Venom produced (in g.)					
Cobra	...	...	...	...	103.54
Viper	...	...	...	...	53.20
					33.70
Echis	...	...	...	...	Nil
Red scorpion	...	...	...	...	6.58
Black scorpion	...	...	...	...	0.36
(D) Venom supplied (in g.)					
Cobra	...	...	...	...	78.37
Viper	...	...	...	...	83.74
Krait	...	...	...	...	22.18
Echis	...	...	...	...	44.73
Red Scorpion	...	...	...	...	5.28
Black Scorpion	...	...	...	...	1.50

APPENDIX XII

*Post-graduate Courses**Degrees**By Research.*

M.Sc.	...	...	Microbiology, Biochemistry Zoology, Organic chemistry, Applied Biology.
Ph.D.	...	...	Microbiology, Biochemistry, Zoology, Organic chemistry, Applied Biology and Pharmacology.
M.Pharm	...	...	Pharmacology and Pharmaceutical Chemistry.

By Papers

M.D.	...	...	Pathology, Pharmacology and Microbiology.
M.Sc.	...	...	Microbiology, Biochemistry.

Diploma.

D.M.L.T.	...	...	Diploma in Medical Laboratory Technology.
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Certificates.

...	...	...	Course in Drug Analysis. (Chemical)
...	...	...	Course in Drug Analysis. (Biological & Microbiological Methods)
...	...	...	Short-Term Training Course.

APPENDIX XIII

Post-Graduate Teachers

The following post-graduates teachers are recognised by the University of Bombay:

Name		Qualifications
Dr. M. B. Bhide	...	M.D.
Dr. R. S. Kamat	...	M.D.
Dr. F. L. Saldanha	...	M.Sc., Ph.D., FIC (Cal.)
Dr. A. J. Baxi	...	M.Sc., Ph.D.
Dr. D. M. Renapurkar	...	M.Sc., Ph.D.
Dr. M. H. Shah	...	M.Sc., Ph.D.
Dr. K. H. Dave	...	M.D., DPB.
Dr. M. V. N. Shirodkar	...	M.B.B.S., AB., Sc.D., PBK., F.M.A.S.
Dr. S. Mukherji	...	M.Sc., Ph.D.
Dr. P. Ramakrishnan	...	M.Sc., Ph.D.
Dr. S. V. Gadre	...	M.Sc., Ph.D.
Dr. D. T. Chaudhari	...	M.Sc., Ph.D.
Dr. B. G. Khadse	...	M.Sc., Ph.D.
Dr. V. R. Rao	...	M. V. Sc. Ph.D.
Dr. M. K. Bhopale	...	M.Sc., Ph.D.

APPENDIX XIV

Statistics Relating to Post-graduate students

Degree				Subjects				No. of Students
M.D.	...	...	...	Pharmacology	...	...	...	1
				Microbiology	...	...	...	2
Ph.D.	...	...	...	Applied Biology	...	...	...	4
				Biochemistry	...	...	...	2
M.Sc.	...	...	...	Applied Biology	...	...	...	1
				Microbiology	...	...	...	4
				Organic Chemistry	...	...	...	1
M.Pharm	...	...	...	Pharmacology	...	...	...	3
								18

APPENDIX XV

Short-Term Training Courses

Forty-four students attended short-term training courses during the year. Details of subjects offered and the number of students attended these courses are recorded here.

Clinical Pathology	...	...	...	...	24
Blood Banking	...	...	...	...	4
Biochemistry	...	...	...	...	8
Preparation of Vaccine and Parenteral solution (Bacteriology and Media)	...	...	...	...	2
Entomology and Parasitology	...	...	...	...	4
Testing	...	...	...	...	2
Grand Total					44

APPENDIX XVI

Degree Awarded and Research Topics

Name of the Candidate	Degree Awarded	Topics
1. Shri K. K. Vora ...	Ph.D. (Applied Biology)	The development of an indigenous isolated liver perfusic system and its application to drug metabolism studies.
2. Kum. R. V. Kolluri ...	Ph.D. (Applied Biology)	"Genetic studies in Down syndrome".
3. Shri Sami Y. Guirges	Ph.D. (Microbiology)	Studies on the pathogenicity of <i>Entamoeba histolytica</i> (Schaudinn, 1903) in laboratory animal. The <i>Entamoeba</i> -hepatitis B Virus interaction in infant rabbits and the serological diagnosis of amoebiasis.
4. Shri P. B. Deshmukh	Ph.D. (Zoology)	Larvicidal, insecticidal and insect repellent properties of some indigenous plants.
5. Shri S. V. Athlekar ...	M.Sc., (Applied Biology)	Studies on <i>Candida</i> species in cases of leucorrhoea.
6. Kum. Anita K. Dave	M.Sc. (Microbiology)	"Natural immunity against tetanus among the adivasis of Virar".
7. Shri V. M. Rangnekar	M.Sc. (Microbiology)	Studies on transferable drug resistance in <i>Enterobacteriaceae</i> .
8. Kum. S. V. Utgi ...	M.Sc. (Microbiology)	Development of a polyvalent <i>Pseudomonas</i> vaccine.
9. Shri V. L. Khairnar ...	M.Sc., (Org. Chemistry)	Synthesis of organic compounds of possible therapeutic value.
10. Shri P. P. Chaudhari	M.Sc., (Org. Chemistry)	Synthesis and study of substituted Aromatic isothiocyanates and pyridothiazoles as anthelmintic agents.
11. Shri S. L. Pendalwar	M.Sc., (Org. Chemistry)	Synthesis of organic compounds of possible therapeutic value.

APPENDIX XVII

Trainees, Research Fellowships and Scholarships

Trainee admitted:

Diploma in Medical Laboratory Technology (D.M.L.T.)	...	...	16
Course in Drug Analysis (Chemical)	...	...	08
Course in Drug Analysis (Biological & Microbiological Methods)	...	...	03
Short Term Training Course	...	...	44

Number of Research Fellowships and Scholarship:

Senior Research Fellowships (Haffkine Institute)	...	...	04
Junior Research Fellowships (Haffkine Institute)	...	...	02
Senior Research Scholarships (Haffkine Institute)	...	...	01
Junior Research Scholarships (Haffkine Institute)	...	...	05
Senior Research Fellowships (Platinum Jubilee)	...	...	02

APPENDIX XVIII

Critics Club lectures

Speaker	Department	Subject
<i>Institute Scientists' Lectures</i>		
Shri H. S. Ved	Biochemistry	Effect of Dietary Calcium and vitamin D on Lipid Metabolism.
Shri K. S. Pradhan	Pharmacology	Problem of Drug resistance in Maharashtra.
Kum. S. W. Awachat	Immunology	Studies on Mycobacterial antigens.
Shri A. P. Kulkarni	Virology	Study of Serum Transaminase levels in <i>Oryctolagus Cuniculus</i> inoculated with HIBOMA and Human Hepatitis B. Viruses.
Shri V. M. Rangnekar	Bacteriology	Studies on Conjugal Transferability and Genetic stability of Standard R-plasmids in recipient <i>S. typhi</i> .
Shri Mangal Vaidya	Blood Bank	Studies on Red Cell Catalase and Glutathione Peroxidase in the Haematological disorders.
Shri N. K. Sutar	Zoonosis	Studies on Snake Repellent.
Shri P. P. Chaudhari	Chemotherapy	Synthesis and study of substituted isothiocyanates and Pyridothiazoles as Anthelmintic Agents.
Kum. Rohini Nair	Clinical Pathology	Parameters of cellular immunity in salmonellosis.
Mr. S. Bhattacharya	D's Research Cell	Pharmacological & Biochemical studies on the Venom of <i>Echis carinatus</i> .
Mrs. S. N. Hattikudur	Immunology	Studies on Mycobacterial Immunity.
Mr. S. V. Gharat	Biochemistry	Purification of Hepatitis B surface antigen.
Mr. Milind Padki	Pharmacology	Studies with antibodies to organo-phosphorous compounds.

Speaker	Subject
<i>Guest Lectures</i>	
Dr. D. M. Storey Lecturer, Dept., of Biology University of Salford, SALFORD (U.K.)	The Effects of Nutrition on the Host-Parasite Relations in Cotton rat Filariasis.
Dr. M. J. Shield (Associate of Dr. Stanford) Middlesex Hospital & Medical School, U.K.	Environmental Mycobacteria in relation to infection with <i>M. leprae</i> .
Dr. R. N. Pithappan Reader in Immunology, School of Biological Sciences, Madurai Kamraj University, MADURAI	Towards Identifying T and B Cells in Lizard <i>Calotes</i> .
Dr. Phaiz Gokak, MBBS, Bombay.	New Aspects of Memory with Demonstrations.
Anthony T. Tu Prof., of Biochemistry Colorado University, U.S.A.	Chemistry and Action of Snake Venoms Followed by film on sea snakes.
Dr. Han's Lobel D.D. Centre, U.S.A.	Immunodiagnostic procedures for Malaria Surveillance.
Dr. M. W. Service Ph.D., D.Sc. (London) Head, Department of Entomology Liverpool School of Tropical Medicine, Consultant W.H.O., N.H.T. & U.S.A.I.D., C.D.C.	Problems of Malaria control.

APPENDIX XIX

Library

The following figures indicate the working of the library during the year:—

Number of outside readers visiting the library	Number of books and periodicals issued to our staff	Number of periodicals issued under inter library loan service	Books and periodicals obtained for our staff from other Libraries
1	2	3	4
7,549	4,768	268	48

Number of Books added during the year	Number of Volumes bound	Number of current periodicals subscribed	Current periodicals received free	Number of reports received from Scientific Institutions
192	562	193	110	44

APPENDIX XX

Paper presented in conferences

All India Medical Microbiologist Congress held from 23-11-79 to 25-11-79 at T. N. Medical College, Bombay.

- (1) "Significance of adhesion of *V. cholerae* to the intestinal mucus membrane in the pathogenesis of cholera", by Shree D. S. CHITNIS.
- (2) "The diagnostic utility of cytoplasmic antigens of yeast phase of *C. albicans*", by Shree J. P. MEHTA.
- (3) "The significance of continuous monitoring of hepatitis B surface antigen for the non-B viral hepatitis, Bombay epidemic of 1977-78", by Shree J. M. DESHPANDE.
- (4) "Studies on elevated SGOT levels as the biochemical marker of hepatitis in rabbits", by Shree A. P. KULKARNI.
- (5) "Studies on R-plasmids in Enterobacteriaceae", by Shree V. M. RANGNEKAR,
- (6) "Utility of radiolabelled antigen of *S. typhi* in seroepidemiology of typhoid fever", by DR. S. MUKERJI.

Annual Regional Chapter Meeting of the Indian Association of Pathologists and Microbiologists held on 8th March 1980, at Nagpur.

- (1) "An *in vitro* assay to measure protective cellular immunity", by Kumari ROHINI NAIR.
- (2) "Relationship between Colonisation Factor Antigen and serotypes of ETEC", by Kumari RADHIKA MIRCHANDANI.

APPENDIX XXI

Papers sent for publication

BHATTACHARYA, S., JADHAV, S. S., GAITONDE, B. B.: Variability in the toxicity of the Venoms of Indian land snakes.

Bulletin of Haffkine Institute.

CHAUDHARI, P. P., LOKHANDE, S. R., KHADSE, B. G., BHIDE, M. B. and GHOOI, SHAILA: Synthesis and anthelmintic activity of new pyrido (3-2-d) thiazoles.

Bulletin of Haffkine Institute.

DAVE, A. K., MUKERJI, S., GAITONDE, B. B. : Natural immunity against tetanus. Part I. A study in adivasi population in Virar.

Bulletin of Haffkine Institute.

GOSAVI, A. V., RENAPURKAR, D. M., KULKARNI, A. B. : Effects of Ascorbic acid on *in vitro* cultivation of *Hammerschmidtella diesingi*.

Bulletin of Haffkine Institute.

KHADSE, B. G., LOKHANDE, S. R., CHAUDHARI, P. P., BHIDE, M. B. and GHOOI, S. : Synthesis and anthelmintic study of new 2-substituted phenyl hydrazine and amino pyrido (3, 2-d) thiazoles.

Bulletin of Haffkine Institute.

KHADSE, B. G., LOKHANDE, S. R., PATEL, M. R., KHAINAR, V. L. : Synthesis and study of thiourea, N-(Substituted aryl or alkyl) -N 6-(2, 4-diamino) quinazolinyl as possible antituberculosis agents.

Bulletin of Haffkine Institute.

KHAIRNAR, V. L., LOKHANDE, S. R., PATEL, M. R. and KHADSE, B. G. : Synthesis and study of 5(6)-substituted-2-(substituted-benzyl) thio sulfonyl benzimidazoles as possible antituberculosis agents.

Bulletin of Haffkine Institute.

KHAIRNAR, V. L., LOKHANDE, S. R., PATEL, M. R. and KHADSE B. G. : Synthesis and study of 5(6)-substituted-2-(substituted-benzyl) thio sulfonyl benzimidazoles as possible antituberculosis agents.

Bulletin of Haffkine Institute.

KULKARNI, A. B., GAITONDE, B. B. : Thiamine levels in the Brain Regions of Normal and Thiamine deficient Rats.

Bulletin of Haffkine Institute.

MAHAJAN, R. J., GAITONDE, B. B., BABNE, V. B. and BODE, I. V. : Preliminary studies on the antifertility activity of *Lygodium flexosum*-I.

Bulletin of Haffkine Institute.

NERURKAR, C. B., CHAUDHARI, D. T., PATEL, M. R. and SABNIS, S. S. : Synthesis and study of thiocarbanilides derived from 2-(4-aminophenyl) thiazoles and 4-(4-aminophenyl)-thiazoles for *in vitro* antituberculosis activity-I.

APPENDIX XXI (Contd.)

Bulletin of Haffkine Institute.

NERURKAR C. B., CHAUDHARI, D. T., PATEL, M. R., SABNIS, S. S. : Synthesis and study of schiff bases derived from 2(4-aminophenyl) thiazoles for *in vitro* anti-tuberculosis activity-II.

Bulletin of Haffkine Institute.

PENDELWAR, S. L., CHAUDHARI, D. T., and PATEL, M. R. : Synthesis and study of 2-aryloxy thioisonicotinamides analogous to ethionamide and thiazoles. Study of some new N-Substituted pyrazinamides as antituberculosis agents-II.

Bulletin of Haffkine Institute.

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