

**Annual
Report of the
Haffkine Institute**

1988-89



**HAFFKINE INSTITUTE
PAREL, BOMBAY 400 012
INDIA**

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RESEARCH AND TESTING**

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Chief Administrative Officer :

M.G. MEHENDALE, B.A.

Accounts Officer :

R.S. JOSHI - Upto 31-8-88

A.J. KHARE

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Junior Scientific Officer :

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BIOCHEMISTRY

Senior Scientific Officer Class I :

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CHEMOTHERAPY

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CHHAYA H. PANDIT, M.B.B.S., M.D.

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Senior Scientific Officer Class I :

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IMMUNOHAEMATOLOGY

Senior Scientific Officer Class I :

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INSTRUMENTATION CELL

Junior Scientific Officer :

J.V. SAWANT, M.Sc.

LIBRARY

Librarian

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Senior Scientific Officer Class I :

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Junior Scientific Officer :

P.R. SANGOLE, M.Sc., DPB.

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A.S. AREKAR, M.Sc.

TESTING UNIT

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H.K. SONI, M.Sc., Ph.D.

Junior Scientific Officers :

H.K. DALAL, B.Sc.

S.S. JAIN, M.Pharm.

S.B. DHOLAM, M.Sc.

TOXICOLOGY

Assistant Director :

V.R. RAO, BVSc & AH, MVSc., Ph.D.

Senior Scientific Officer Class II :

M.G. PHATAK, M.Sc., Ph.D.

TRAINING

Senior Scientific Officer Class I :

S.V. GADRE, M.Sc., Ph.D.

VIROLOGY

Medical Officer :

RANJANA A. DESHMUKH, M.B.B.S., M.D.

ZOONOSIS

Assistant Director :

D.M. RENAPURKAR, M.Sc., Ph.D. FMAS.

Senior Scientific Officer Class II :

M.K. BHOPALE, M.Sc., Ph.D. Upto 25-10-88

Junior Scientific Officers :

T.G. TARE, M.Sc.

B.D. SATHE, M.Sc., Ph.D.

V.R. KAMAT, M.Sc., Ph.D.

N.K. SUTAR, M.Sc.

CHAPTER I

INTRODUCTION

The year 1988-89 has been very promising, full of hopes and prospects for a very bright future for this internationally renowned Research Institute.

The Dev and the Iya committees instituted by the Government of Maharashtra went into the problems of the Institute. After thoroughly going into every aspect of the working problems, such as core funds, man power, research projects, testing activities, they have submitted their reports along with recommendations to the Government of Maharashtra. The Committees during their deliberations also met the various sections of the staff for their views about the problems they are facing in the Institute. The outcome of the recommendations are awaited.

With the appointment of a full time Director, the problems of the Institute were projected to the above expert committee. Projects that were shelved were revived and newer projects from funding agencies were received. Despite major limitations such as inadequate staff, shortage of funds and poor conditions of the laboratories some good work has been undertaken in the Institute. Projects such as isolation of bacterial mutants of industrial importance, in vitro cultivation of *P. vivax* the important malarial parasite, in depth immuological studies in tuberculosis and hepatitis B, epidemiological studies in viral hepatitis and toxicological studies on pesticides and their formulations are some of the research areas.

The following are the highlights of the various activities during the year under report.

Research:

The department of Bacteriology undertook studies on a) Genetic recombination studies between enteric species with a view to obtain new strains with high academic and applied potential b) Genetic studies on industrially important bacterial cultures and their phages c) Studies on Ribosomal vaccine of *Ps. aeruginosa* d) Synergistic activity of Trimethoprim and Furazolidone.

The Biochemistry department continued their study on the mechanism of action of the hypoglycaemic factor from *Bordetella pertusis*.

The programme for the development chemotherapeutic agents in the field of communicable diseases is under progress in the department of chemotherapy. A number of new synthesized compounds were selected for testing for antifungal anti tubercular, Anthelmintic, Antimalarial activities.

The clinical pathology and immunology departments in their research on DTH eliciting antigen/s of *S. typhi* studied the physicochemical and biological characteristics of the antigen. The purified antigen was identified to be a pure protein with a molecular weight of 65 kd and without any subunits. Serological analysis revealed group and genus specific determinants on the antigen.

In their study on regulation of immune response to mycobacteria it was found that, when antigen presentation is efficient, concomittent stimulation of T suppressors can not abrogate the immune response.

The study of role of idiotypic regulation, in chronic HBV infection was continued. Cross reactivity of anti-idotypic antibodies to antibodies against hepatitis B surface and core antigens was investigated. Anti-idiotypic antibody to human anti-hepatitis B core antigen (anti-HBc) was raised in rabbits.

The phytochemistry unit investigated seeds of *Serace indica* and *Narium indicum* for mosquito larvicidal / insecticidal activity. Hexane and benezene extracts of seeds of *sarace indica* showing 100% mosquito larvicidal activity at the concentration of 0.024 % with 24 hours. The hexane extractrs of seeds of *Narium indicum* showed 100% larvicidal activity at 0.032 % upto 24 hours.

The department of Zoonosis undertook the development of DNA probes for the diagnosis of *Plasmodium vivax* (malaria) and filariasis. The construction of the probes was carried out using *P. vivax* and *S. cervi* DNA respectively. The DNA isolated were purified and used for cloning. Studies are underway to select a colony. Several compounds synthesised by the department of Chemotherapy were screened for antimalarial activity.

Testing:

During the period under report the Institute conducted various tests on products submitted by the prohibition and excise department of the Government of Maharashtra and samples such as drugs, biologicals foods additives, disinfectants, cosemetics, chemicals etc. In all 5663 samples of various types were tested.

Patient oriented services:

The Biochemistry and Clinical pathology departments conducts investigations on clinical samples of patients. In all 4252 samples were investigated in the two departments. The services of the above departments were utilised by different hospitals, charitable and private organisations from Bombay, suburbs and other places (Appendix - II III IIIA)

In the immunisation clinic, patients of different age groups were immunised against Cholera, Typhoid, Rabies, Poliomyelitis, Tetanus, Hepatitis. In all 1305 patients attended and immunised (Appendix VIII)

Training Programmes:

Various courses and training programmes for medical and paramedical personnels are offered by the Institute (Appendix - X). A list of students registered during 1988-89 for different degrees / courses and students who have successfully completed their thesis and obtained their degrees are given in Appendix XI, XII and Appendix XIII. The list of recognised university teachers of the Institute are given in Appendix XIV.

Sponsoring Research Bodies:

The Department of Science and technology, Indian council of Medical Research, Government of India, Atomic energy commission, Maharashtra council on Science and Technology Education and employment department, Government of Maharashtra, M/S Organon Research Centre, Calcutta and M/S. D.S. Enterprises financed some of the research projects. The Institute is thankful and gratefully acknowledge their financial support.

Administrative bodies:

The Governing Council presided over by the Hon'ble Minister for public Health, Government of Maharashtra met once during the year under report. The executive committee (Administration) and the Teachers Council met several times to provide guidelines for the smooth running of the Institute.

CHAPTER II

GENERAL DEPARTMENTAL ACTIVITIES

Bacteriology :

Five students were registered for M.Sc. (by papers) in Microbiology. Out of the five students four appeared at the final examination and secured first class. Mrs. Sadhana Phanse (Nee Ms Sadhana Mohile) submitted her thesis on "Studies on *C. mediolanum* and its phage" for the M.Sc. degree to the University of Bombay.

The department was also involved in testing activities. Bacteriological analysis of various products were undertaken. Water samples were analysed and water filters were tested. Lyophilization of cultures was also undertaken (Appendix 1)

Biochemistry :

Training courses in M.Sc. Biochemistry (papers) part-I and II and D.M.L.T. of the Bombay University were conducted. Lectures in special topics in advanced biochemistry for M.Sc. biochemistry (papers) part-II were held in the Institute during the year. A Junior Research Fellow joined the department to pursue research studies leading to M.Sc. degree in biochemistry.

The department carried out clinical investigations of samples referred by hospitals, practitioners and the medical officer of the Institute (Appendix II)

Chemotherapy:

The department of Chemotherapy is assisting researchers from various universities and research organisation for *in vitro* testing in the field of Tuberculosis, Antifungal and Antibacterial activities on payment basis. Around one hundred new compounds have been tested and reported. Analysis for C, H, N. were also undertaken for other research organisation and reports sent to them. Two students have been registered for Ph. D. degree in organic chemistry and one student registered for M.Sc. degree in organic chemistry

Clinical Pathology, Immunology :

The department of clinical pathology received specimen from various hospitals and agencies (Appendix III A) for investigations. The services offered covered the areas of cytology of the various body fluids, routine, haematological examination, diagnostic serology, bacteriology, mycology and histopathological examination of biopsies

The D.M.L.T. and Short Term Training courses were conducted. The staff participated in the integrated Bombay University teaching programme for M.Sc. (Microbiology), and M.D. (Pathology).

Human Pharmacology, Bioavailability and Radiation Biology Unit.

During the year under report the Human Pharmacology and bioavailability unit undertook routine testing of samples for blood absorption studies in human volunteers. The list of Pharmaceutical samples accepted for the studies are given in Appendix IV.

Testing Unit :

The Testing Unit undertook various samples for analysis. the list and number of samples tested during the year under report is given in Appendix VI.

Toxicology :

The toxicology department was engaged in toxicological evaluation of different types of chemicals, cosmetics, drugs, surgical gloves, water storage tanks (Appendix VII). The samples were referred by different industries. The department was also involved in teaching and training of post graduate students for M.Pharm and M.Sc. Applied Biology.

Virology :

The department of Virology runs the immunisation Clinic and renders important public services. In all 1305 attended the Clinic during the period under report for vaccination. (Appendix VIII). The Clinical Virology section of the department carried out cytomegalo virus detection in urine of 7 patients and rubella test in one patient.

In the Rabies research unit, isolation of rabies virus in mice from postmortem tissues was carried out for 3 patients. Antirabies antibody titration was also carried out in one patient receiving antirabic vaccine.

The department conducted the annual virology course for M.Sc. PH.D., M.Phil and M.D. (Microbiology) students of the University of Bombay. In all twelve lectures and eight practicals were conducted. Two Ph.D., two M.Sc. and a M.Phil (Microbiology) students are registered with the Head. of the Department.

Zoonosis :

Various species of poisonous, non poisonous snakes and

scorpions were maintained for obtaining their venom and carrying out research on these animals. Appendix IX provides details regarding the total quantity of venom extracted from snakes and scorpions of different species and supplied to various Institutes.

Synthetic agents and extracts of indigenous plants were screened for their antimalarial, anthelmintic, and insecticidal activities. In addition several commercial insecticide formulations were tested for their efficacy. Strains of insect vectors of public health importance and parasites were maintained under laboratory conditions for efficacy testing. There were regular lectures and demonstrations of venom extraction methods for visitors to the snake farm. (Appendix IX A)

As part of the plague surveillance programme 6552817 rodents and shrews collected by the Municipal Corporation of Greater Bombay were identified. Out of these 96 313 rats were examined for plague and were found to be plague free.

Academic Activities, Honours and Awards

Dr B.G. Khadse, the Head of the Department of Chemotherapy attended the conference of Indian Pharmaceutical Congress held at Calcutta from 26th to 28th December 1988. He also attended the training programme for Research and Development Managers at Bellvista Administrative Staff College, Hyderabad.

Dr. V. Ramakrishna Rao was invited to lecture at the Summer school on Environmental pollution sponsored by UGC/DST/CSIR at School of Environmental Biology, Dr. Harisingh Gour Vishwavidyalaya Sagar in 1988. He delivered a lecture on Biological Evaluation of plastic Medical products at UNDP/IAEA/RCA Regional Training course on Industrial Radiation sterilization Quality control and compatibility of Materials at BARC, 6 - 16 December 1988.

Dr. R. Deshmukh, Medical officer from the department of Virology presented a paper at the International conference on "Advances in the treatment of Infectious diseases" conducted by the Indian Society for Clinical Pharmacology and Therapeutics. She also presented a paper in a Seminar on antiviral vaccines conducted by Hoechst India Limited at the 18th Annual Conference of general practitioners Association Greater Bombay.

Dr. D.M. Renapurkar, Asst. Director and Head of the Dept. of Zoonosis was awarded the ICIPE/TWAS Research Associateship. He

visited The International Centre for Insect physiology and Ecology. Nairobi under the auspices of the above associate programme. To commemorate the completion of 50 years of research in entomology at Haffkine Institute, a symposium on medical entomology was organised on 26th March 1989.

CHAPTER III
RESEARCH PROGRESS
BACTERIOLOGY

1. Genetic recombination between enteric species with a view to obtain new strains with high academic and / or applied potential :

Studies on transformation of *S. typhi* 21a with PBR 322 DNA were completed and the conditions for optimal transformation were standardized.

2. Genetic studies on industrially important bacterial cultures and their phages.

Studies on phage of *C. mediolenum* R18 were carried out. The phage morphology and its dimensions were studied by electron microscopy. The phage nucleic acid was isolated and its nature, structure and molecular weight was determined.

3. Studies on ribosomal Vaccine of *Ps. aeruginosa*

With a view to study the cross protection afforded by Ribosomal Vaccine of *Ps. aeruginosa*, vaccines from two serotypes have been prepared and these are being tested initially for their efficacy against homologous challenge.

4. Synergistic activity of Trimethoprim and Furazolidone

In vitro antibacterial activity of Trimethoprim and Furazolidone, individually as well as in several combinations of the two, was studied employing different gram positive and gram-negative organisms. Suitable combination of the two was worked out.

BIOCHEMISTRY

Biochemical studies on the mechanism of action of the hypoglycaemia causing factor from *Bordetella pertussis* :

Since the hypoglycaemic state involves increased glucose utilisation, it was initially decided to study the activities of the key enzymes of the metabolic pathway in mouse liver. The enzymes include Hexokinase, Phosphofructokinase, Pyruvate kinase, Glucose-6-phosphate dehydrogenase and Lactate dehydrogenase. These studies will be carried with reference to a standard reference enzyme Glyceraldehyde phosphate dehydrogenase.

These studies would indicate the rates of response of the regulatory enzyme in relation to the antidiabetic effect of *B. pertussis*, and in addition the direction of metabolic flow.

A new strain of *B. pertussis* (No 118323) obtained from the H.B.P.C.L., Bombay, showed a remarkable hypoglycaemic effect. The maximum blood sugar lowering occurred at the end of 12 hours as against the time of 72 hours given by the previously used strain N.25525, when both were administered by the i.v. route. The hypoglycaemic effect lasted for 24 hours. The strain no. 18323 was further used in the preparation of the hypoglycaemic factor.

Spectrophotometric assay procedures for the glycolytic enzymes mentioned earlier, have been standardised in normal mouse liver. The effect of *B. pertussis* preparations being studied in normal as well as alloxan diabetic mice treated with the preparation.

CHEMOTHERAPY

The new drug development programmes in the field of communicable disease is under progress in the department. Large number of new compounds are synthesised and few of them have been selected for their detail studies. These include Antifungal, Antitubercular, Anthelmintic, Antimalarial. This year more emphasis are given on the detail testings of these compounds.

1) Antifungal screening

The selected compounds were taken up for *in vivo* screening programme in guinea pigs against *T. mentagrophyte*, *T. rubrum*, *C. albicans*. The compounds were blended with petroleum jelly under aseptic conditions and the cream obtained were utilised for testing purpose. During these experiments 10 guinea pigs were taken per drug. The results for 2½ month study revealed that petroleum jelly is not a good vehicle for this testing. Hence P.E.G.-400 was taken as the vehicle and the drug was blended with it and applied to the infected animals under trials. This experiment revealed that PEG 400 is a good vehicle and can be used safely.

2) Antifungal screening in rabbits

Using PEG 400 as vehicle for drug under investigation it was observed that antifungal activity could be very well tested. However in some of the control rabbit autocuring was observed. This was assumed to be due to delayed type of Hyperimmunity. Hence it was proposed in the new experiments, that first all the rabbit will be tested

for DTH test and those found negative to it, would be only selected for antifungal programme.

3) Antimalarial screening programme

Promising antimalarial compounds were prepared upto 15 gram scale and method of preparations and purifications were worked out. Large quantities of these compounds will be required for screening in monkey, and for their detail toxicological and pharmacological studies.

4) Antitubercular screening programme

The promising compounds were prepared upto 10-15 gram scale. Since no *in vivo* facility exist in the Institute efforts are going on for collaboration with interested pharmaceutical company for possibility of exploiting these compounds.

5) Anthelmintic synthetic programme

The synthesis of new compounds as per proposed Anthelmintic synthetic plan is under progress. Four compounds of arylthiazoloimidazole were prepared and sent to Organon Research Centre, Calcutta for screening.

CLINICAL PATHOLOGY AND IMMUNOLOGY

Study of DTH eliciting antigens of *S.typhi*

Earlier it was shown that the DTH eliciting antigen in the sonicate of *S. typhi* was a protein antigen of pI 7.1 - 7.2.

Physicochemical and biological characteristics of the antigen were studied. The purified antigen was identified to be a pure protein; tests for lipids and sugar side chains were negative. Its molecular weight, based on its mobility in 7.5% SDS polyacrylamide gel electrophoresis, was 65 Kd. The antigen was found to have no subunits. The purified antigen elicited a DTH response in mice immunised with killed *S. typhi* and a lympho-proliferative response by spleen cells of mice primed with *S. typhi*. Serological analysis revealed group and genus specific determinants on the antigen.

Study of regulation of immune response to mycobacteria

Earlier it was shown that difference in immunogenicity of mycobacteria by the intraperitoneal (ip) route was due to inefficient presentation of rapid grower antigens by the peritoneal macrophages.

ip immunisation was also shown to stimulate immune suppression. The suppression was shown to be mediated by T suppressor cells of Lyt 2 phenotype. It was observed to exhibit a spectrum of cross-reactivity as a function of dose of immunisation. When mice were primed with 10^5 cells / animal, the ip immunisation resulted into suppression that was restricted to the group, either slow or rapid grower, depending upon the immunogen. At higher doses, suppression became cross-reactive to all the organisms of the genus *Mycobacterium*. However, the suppression induced by ip immunisation with mycobacteria did not affect the ability of the mice to respond to *Salmonella*, indicating that the suppression was antigen specific. The suppression was the only response of mice immunised by ip route with rapid growers or *S. typhi*. In case of slow growers such as *M. tuberculosis*, suppression was only a component of the immune response, the mice were also capable of responding to the ip immunisation by DTH and lympho-proliferative responses. Based on these data it was concluded that, when antigen presentation is efficient, concomittent stimulation of T suppressors can not abrogate the immune response.

3) Study of role of idiotypic regulation in chronic HBV infection.

Earlier it was shown that chronic HBV carriers mount a poor cellular and humoral response to hepatitis B surface antigen (HBsAg) and this is associated with cellular and humoral responses to idiotypic determinents, on the antibody to HBsAg (anti-HBs).

Cross reactivity of anti-idiotypic antibodies to antibodies against hepatitis B surface and core antigens was investigated. Antibody to human anti-hepatitis B core antigen (anti-HBc) was raised in rabbits. It was thoroughly absorbed with insolubalised normal human serum proteins. Assay for anti-idiotypic antibodies was standardised.

PHYTOCHEMISTRY

The following plant seeds were collected locally

- 1) *Saraca indica*, Linn Fam : Leguminosae (ver. name-Ashok)
- 2) *Narium indicum* Mill Fam : Apocynaceae (ver name-Pilakanher)

Saraca indica

The powdered seeds were extracted with hexane, benzene, chloroform, ethyl acetate, acetone and methanol. Various extracts were made free of solvent using water bath and tested for mosquito larvicidal (*Culex-p-fatigans*) activity as per WHO specifications.

It was observed that hexane and benzene extracts showed 100% mosquito larvicidal activity at the concentration of 0.024% within 24 hours. Other extracts at the same concentrations did not show promising activity. The extracts were purified by using column chromatography technique. Silicagel column was used and the active extract was allowed to adsorb on the top of the column in narrow band. Following solvent systems were used for elution and fractionation to purify the active extract.

Hexane

Hexane : Benzene (80:20)

Hexane : Benzene (50:50)

Benzene

Benzene : Methanol (90:10)

Benzene : Methanol (80:20)

These fractions will be evaluated for mosquito larvicidal activity.

Narium indicum :

Powdered seeds were extracted with various solvents as mentioned above and solvent free extracts were tested for mosquito larvicidal activity. It was found that hexane extract at the concentration 0.032% gave 100% mortality of 3rd or 4th instar mosquito larvae upto 24 hours. Active extract was subjected to column chromatography using silicagel and eluted with hexane. hexane : methanol (90:10) and hexane : methanol (70:30). Various fractions will be evaluated for mosquito larvicidal activity.

TOXICOLOGY

Teratogenic studies of indigenous pesticide formulations

This study is being conducted with a view to investigate teratogenic effects of some widely used indigenously manufactured pesticide formulations. The teratogenic effects of a widely used indigenously manufactured formulation Dimethoate 30 EC was studied in rats. Acute oral toxicity was initially conducted to obtain indications of maternal toxic doses for selection of toxic doses for subsequent teratogenic experiments. The doses higher than 38.8 mg/kg were found to be maternally toxic. The doses selected for teratogenic studies were 38.8 mg/kg 25.87 mg/kg and 15.52 mg/kg. Dimethoate

30 EC at 38.8 mg/kg was teratogenic. Its effect was similar to that produced with Aspirin at 250 mg/kg (positive control). A high percentage of resorption was observed in both the groups. The percentage of resorption was lower in low dose groups. The incidence of skeletal anomalies was high in the Dimethoate 30 EC group receiving 38.8 mg/kg dose. Visceral anomalies showed underdeveloped organs specially in high dose groups (38.8 mg/kg). Further studies are proposed with malathion 50 EC, fenitrothion 50 EC and ethion 50 EC.

In vitro system for screening and evaluating pesticide toxicity.

Testing of newer pesticides for toxicity involves animal experimentations which are elaborate expensive and many times objected by antivivisectionists. Animal experimentation can not be entirely substituted, if one desires to extrapolate to near human situation. However, one can look for tests that do not involve live whole animals especially for screening potential toxic pesticides in order to eliminate extremely toxic pesticides before detailed animal studies are undertaken.

The present study was undertaken to determine the effects of various pesticides (Cholinesterase inhibitions) on red cells and determine the order of lethality with various concentrations. This would reflect on the degree of toxicity and findings will be compared with available data on acute animal toxicity. Presently the preliminary study is being carried out with malathion 50 EC. Later, studies will also be undertaken with ethion, malathion, dimethoate and fenitrothion.

VIROLOGY

Activities of the WHO/NICD Surveillance Centre for viral hepatitis

The department being a WHO/NICD viral hepatitis Surveillance centre, continued its Surveillance activity. Various outbreaks of Viral hepatitis were investigated by the department.

The outbreaks were reported from Kolhapur, Sangli, Sindhu Durg.

All the epidemics of viral hepatitis were found to be due to contaminated water causing infection by feco-oral route, and the causative agent appears to be ETNANB (enterically transmitted non A non B) virus.

ZOONOSIS

Malaria :

Evaluation of chloroquine sensitivity of *P. vivax* strains

Collection of *Plasmodium vivax* strains was carried out to evaluate their sensitivity to chloroquine. Forty isolates of *Plasmodium vivax* were obtained from Goa. The microtest method developed at the Institute was used for this purpose. None of the isolates investigated revealed resistance to chloroquine.

Constructions of DNA probes for the diagnosis of *Plasmodium vivax* malaria.

A study was undertaken to evaluate the possibility of development of whole DNA probe for the detection of *P. vivax* positive cases. *P. vivax* DNA was isolated and purified by percoll gradient centrifugation. That *P. vivax* DNA is free from host cell contamination was shown by hybridization experiment. *P. vivax* did not hybridize with leucocyte DNA. This pure *P. vivax* DNA was then further used for cloning in PBR 322 plasmid.

Screening of antimalarial agents :

In vivo screening of synthetic compounds for antimalarial activity was carried out using *P. berghei* mouse model. In all 40 compounds were evaluated for antimalarial activity. Besides the evaluation of compounds synthesised at the Chemotherapy department, a large number of compounds submitted by the other institutions were also investigated.

Filariasis :

Development of monoclonal antibodies was initiated by immunization of Balb/c mice with *Setaria cervi* adult worm and microfilarial antigens. Fusions were tried with various murine myeloma cell lines viz. NSI Sp 2/0 and 653 using PEG 1500 alongwith presensitized antigen spleen cells. The feeder layers used for the hybrid cells were Balb/c erythrocytes. These hybrid clones were grown upto the 12th day in 96-well plates and their supernatants were screened for the desired antibodies. The hybrid colonies of interest were transferred to 24-well plates for expansion using the limiting dilution technique and the positive clones of interest were the cryopreserved. Further identification of these clones would be carried out after the availability of the ELISA hybridoma kit.

Construction of DNA probes was carried out using *S. cervi* DNA isolated and purified as per the method of Jayaraman & Jayseelan (1987). Construction of a genomic library of the parasite DNA in plasmid PBR 322 was carried out after partial digestion of purified DNA by *Sau* 3A, cloning of the above fragments was done into the *Bam*HI site of PBR 322, before transformation of *E. coli* HB 101 strain. The transformed colonies were identified by their sensitivity to tetracycline. About 748 transformed colonies obtained were screened by colony hybridization using Nick translated *S. cervi* DNA. 7 colonies that hybridized strongly were selected. Further studies are being carried out to select a colony out of 7 clones which contain highly repetitive DNA sequences. This would then act as a probable DNA probe for diagnosis of bancroftian filariasis and also as a taxonomic tool.

In vitro maintenance of filarial nematodes :

In vitro maintenance of filarial parasites *Wuchereria bancrofti*, *Setaria cervi* mf stages was carried out in RPMI 1640 (W/o NaHCO₃) buffered with NaHCO₃ 2 gms/lit and Hepes 5.94 gms/lit. at PH 7.2 to 7.4 with the addition of 10% in activated human AB serum/Foetal Bovine serum (Gibco). The feeder layers tried were LIC-MK₂, Vero and Hela cell lines of these LIC-MK₂ showed excellent results for the maintenance of the microfilariae upto 3 weeks.

Angiostrongyliasis :

Cryopreservation of third stage larvae of *A. cantonensis*.

Cryopreservation of third stage larvae of *A. cantonensis* has been successfully carried out to get 52% cryoprotection. DMSO was used as a cryoprotectant. Efforts are being made to get better cryoprotection, by using different media.

Evaluation of plant extracts for anthelmintic properties

Plant extracts are being screened for their anthelmintic activity against *A. cantonensis*. Aqueous and alcoholic extracts of *A. calamus* are found to reveal potent anthelmintic activity against third stage larvae of *A. cantonensis* during *in vitro* evaluation of anthelmintic activity.

CHAPTER IV

GENERAL ACTIVITIES

Library :

The Library has a collection of books on biomedical sciences and related fields. It collects and provides information to scientists, and students in the field of Biochemistry, Communicable diseases, Immunology; Pharmacology, Zoonosis, Virology etc. of this Institute as well as to the institutions all over India.

The library remains open to readers on all working days except on Saturdays, Sundays and all Public Holidays.

There are 8755 books in the library. The number of books added during the year were 157. Total number of journals subscribed were 146. About 450 volumes were bound during the year. (Appendix V).

The Library was given financial support by the Government of Maharashtra for construction of an independent library building (ground + 3 storeys) under the VII th Five Year Plan. The construction work commenced in 1988 and was inaugurated by the Hon'ble Minister Late Shri Bhai Sawant. Sir J.J. College of Architecture was nominated as our consultant and have undertaken the responsibility of planning and construction.

Facilities :

- 1) Library provides reprographic facilities to its staff and outside readers. The library received xerox requisitions for approximately 784 pages during the year.
- 2) Inter Library Loan : The library borrowed 205 journals from neighbouring libraries for its staff and loaned 156 journals to other libraries in the city.
- 3) Visitors : About 4725 outsiders visited the library for reference.

APPENDIX - I
BACTERIOLOGY

Tests	Number
1. Biocides including disinfectants and antiseptics.	26
2. Filter Candles	28
3. Water samples	91
4. Supply and lyophilization of bacterial cultures	77
5. Polyethylene tanks	3
6. Ultra-violet water sterilizing Units	3
7. Antibiotics (comparative studies on their activity)	3
8. Identification of bacterial cultures	4
9. Zero-B filters	2
Total	237

APPENDIX - II
BIOCHEMISTRY

Name of the body fluid	No of Tests
Blood	306
Urine	56
Special Tests	217
Total	579

APPENDIX - III
CLINICAL PATHOLOGY

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

	Tests
A. Histopathology	337
B. Haematology	1333
C. Cytology for malignant cells	7
D. Clinical Pathology	
Urine for routine examination	260
Stool for routine examination	249
Semen examination	5
C.S.F. routine cell count	-
Immunological Pregnancy Tests	62
Biological (Rat Test) Pregnancy Tests	1
E. Bacteriology	
Direct smear for Tubercle Bacilli	-
Culture for Tubercle Bacilli	47
Animal inoculation for Tubercle Bacilli	11
Smear & Culture for C. diphtheriae	4
Culture of Theatre swabs for Anaerobes	701
Urine culture	134
Pus culture	24
Sputum culture	22
Stool culture	25
Stool culture (cholera)	-
Blood culture	1
Antibiotic Sensitivity Tests	151
Aspirated Fluid culture	4
C.S.F. culture	3
Conjunctivitis swab	-
Throat swab culture	12
F. Serology	
Widal tests	112
Weil Felix Tests	75
Brucellosis tests	71
V.D.R.L. Tests	11
Paul Bunnell Tests	11
Total Tests	3673

APPENDIX - III-A
CLINICAL PATHOLOGY

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

Bombay Area

Cama and Albless Hospital

St. George's Hospital

Jagjivanram Hospital

Central Railway Hospital

B.Y.L. Nair Charitable Hospital

K.E.M. Hospital

M.G.M. Hospital

L.T.M.G. Hospital

E.S.I.S. Hospital

K.B. Bhabha Hospitals

Kasturba Hospital for Infectious Diseases.

J.J. Group of Hospitals

Police Hospitals

Fertilizer Corporation of India

B.J. Wadia Hospital for Children

Greater Bombay Municipal
Dispensaries**From Maharashtra**

Civil Hospital, Thane

Civil Hospital, Alibag, Raigad

General Hospital, Sawantwadi

ISP Hospital, Nasik

Public Health Centre, Buldhana

Civil Hospital, Akola

District Hospital for women,
AkolaPrimary Health Centre,
Jalgaon

General Hospital, Osmanabad

Civil Hospital, Dhulia

Civil Hospital, Nasik.

APPENDIX - IV
HUMAN PHARMACOLOGY AND BIOAVAILABILITY

Sr.No.	Name of the Company	Product
1.	Dr. shaligram Grant Medical College	Erythromycin
2.	Lupin Laboratories Kalina, Santacruz	Cephalexin cap. 250
3.	Lupin Laboratories Kalina, Santacruz	Cephalexin cap. 500
4.	Lupin Laboratories Kalina, Santacruz	Rifampicin cap. 150 mg
5.	Lupin Laboratories Kalina, Santacruz	Rifampicin cap. 800 mg
6.	Lupin Laboratories Kalina, Santacruz	Rifampicin cap. 450
7.	MAPL Jagdish Pharm.	Gentamycin Injection
8.	Aristo Pharm.	Ampicillin Injection
9.	Priest Pharm.	Ampicillin cap. 250 Tetracyclin cap. 250 Doxycyclin cap. 100 Amoxycillin cap., 250 Rifampicin cap. 450 Rifampicin cap. 150
10.	Pushpam Pharm.	Amoxycillin caps. Sulphalximethoxazole Ampicillin caps. Rifampicillin caps. Erythromycin tabs. Chloxamphenicol caps.
11.	Kopran chemicals	Rifampicin caps. 300

APPENDIX - V

LIBRARY

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

Number of outside readers visiting the library	Number of periodicals issued under inter library loan service	Books and Periodicals obtained for our staff from other Libraries	Number of books added during the year
4725	156	205	157
Number of volumes bound	Number of current periodicals subscribed	Current periodicals received free of charge	Number of reports received from Scientific Institutions
450	146	70	26

APPENDIX - VI

TESTING

Tests	Number
Drug samples	150
Beer	433
I.M.F.L.	2269
Ganja	083
Bhang	021
Toddy	1732
Denaturants	452
O.D.S.	099
S.D.S.	058
French Polish	046
Country Liquor	016
Other Liquor	005
Total	5364

APPENDIX - VII

TOXICOLOGY

Sr. Classification of No. sample	No. tested	Toxicity parameter
1. Chemicals	6	Acute oral toxicity in mice
	1	Acute oral toxicity in rats
	1	Acute dermal toxicity in rabbits
	6	Primary skin irritation
	2	Mucous membrane irritation
	4	Toxicity in fish
2. Cosmetics	3	Acute oral toxicity in mice
	3	Primary skin irritation
	1	Mucous membrane irritation

APPENDIX VII (CONTD.)

3. Drugs / Surgical gloves	1	MLD and potency determination
	1	Antiscorpion Venom against scorpion venom
	1	Primary skin irritation
	1	Tested as per I.S.I. specification
4. Water storage tanks	11	Tested as per painted container test.

APPENDIX - VII-A
TOXICOLOGY

Sr. Sample No.	LD ₅₀	Fiducial Limits/ Remarks
Acute oral toxicity in mice		
1. Godrej suruchi	7245 mg/kg	6646.79-7897.05 mg/kg
2. Ureflow-30	>16,800 mg/kg	
3. Aquafloc-1001	740.4 mg/kg	6414.1-8630.8 mg/kg
4. Deet	1686.4 mg/kg	1545.2-18405 mg/kg
5. PVC plastisol (paste compound)	>10ml/kg	
6. Denim after shave	13.5 ml/kg	11.66 - 16.66 ml/kg
7. Denim - Cologne	8.4 ml/kg	6.56 - 10.57 ml/kg
8. Denim De-odorant	3.3 ml/kg	2.84 - 3.83 ml/kg
9. Acilol TA 1618 WER	>10,000 mg/kg	
Acute oral toxicity in rats		
1. Deet	2728 mg/kg	2292.4-3246.3 mg/kg
Acute dermal toxicity in rabbits		
1. Deet	9721.6 mg/kg	7.777.3-12, 1520 mg/kg
Primary skin irritation		
1. Deet		mild irritation
2. Cholecalciferol 7.5%		No irritation
3. Kumkum		No irritation
4. DM 4.5 adhesive		mild irritation
5. KPSV adhesive		mild irritation
6. Denim cologne		mild irritation
7. Denim deoderant		mild irritation
8. Denim After shave lotion		No irritation

APPENDIX VII-A (CONTD.)

9. Varnalson CAI 2010	mild irritation
10. Surgical gloves	No irritation
Mucous membrane irritation	
1. Deet	mild irritation
2. Cholecalciferol 7.5%	mild irritation
3. Kajal	No irritation
Toxicity in fish	
1. Godrej suruchi	MLC-609.0 PPM
2. Ureflo 30	MLC-302.4 PPM
3. Cholecalciferol 7.5%	MLC-315.7 PPM
4. Acilot TA 1618 WER	No mortality of symptoms of toxicity at 15 PPM as a single dose or daily for 15 days.

Sr.No.	Sample	Type of Test	Results
1.	Antiscorpion Venom (AScV)	Testing of AScV against Red scorpion venom	i.v.MLD-60 mcg/mouse potency-1.0 ml of reconstituted AScV neutralised 3 mg of Red scorpion venom.
2.	Silcion Rubber tubing	Toxicity testing as per Indian standards specifications in mice	No mortality or signs of toxicity.

Testing of water containers as per painted containers :

1.	Water containers coated with epoxy slurry	Test as per painted container.	No mortality or signs of toxicity in the animals receiving stored water in these containers.
2.	Cement container bonded with polysafe plaston	•	•
3.	Glass fibre reinforced plastic pipes	•	•
4.	Tubes coated with anti corrosive paint systems (8 samples)	•	•

APPENDIX - VIII
VIROLOGY

Vaccines administered	Number of Patients
Antirabic	791
Oral Polio	168
Triple	117
Cholera	24
Typhoid	16
Tetanus toxoid	65
Dual	10
Hepatitis B	80
MMR	34
Total	1305

APPENDIX - IX
ZOONOSIS

Snakes	No. procured	Venom produced (g)	Venom supplied (g)
Cobra	66	—	51.90
R. Viper	50	—	33.07
Krait	11	6.52	6.52
Echis	2241	23.66	22.99
Total	2368	30.18	114.48
Scorpions :			
Red Scorpions	6772	4.32	4.53
Black Scorpions	27	—	—
Total	6799	4.32	4.53

APPENDIX - IX-A
ZOONOSIS

Statistics of visitors to snake farm :

1.	Students	5243
2.	Staff of Scientific Institutions	1179
3.	Distinguished visitors	50
4.	Foreign guests	31
5.	Thursday visitors	187
6.	Others	76
Total		6766

There were 17 enquiries regarding identification of snakes from hospitals and individuals during the year. (1988-89)

APPENDIX - X
POST-GRADUATE COURSES OFFERED

M.Sc. (by research)	- Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology.
M.Sc. (By papers)	- Microbiology, Biochemistry.
Ph.D.	- Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology.
M. Pharm.	- Pharmacology and Pharmaceutical Chemistry.
M. Phil.	- Microbiology
M.D.	- Pathology, Pharmacology and Microbiology.
D.M.L.T.	- Diploma in Medical Laboratory Technology.

APPENDIX - XI
TRAINEES ADMITTED

M.Sc. by papers	10
Diploma in Medical Laboratory Technology (DMLT)	20
Short Term Training Course	15
Research Fellowship and Scholarship	
Post Doctoral Fellowships (Platinum Jubilee Chinchwad)	3
Sr. Research Fellowship (Platinum Jubilee)	15
Jr. Research Scholarship (Chinchwad Land)	15

APPENDIX - XII

LIST OF STUDENTS WHO REGISTERED DURING THE YEAR 1988-89 FOR M.SC., PH.D., M. PHARM, M. PHIL AND M.D.

M.Sc.	Ph.D.
Ms. S. Sunderam	Mr. A.K. Manian
Ms. A. Korhalkar	Mr. R.K. Mahajan
Ms. M. Joshi	Ms. S. Khetan
Ms. A. Jain	
Mr. R.N. Shetye	
M. Pharm	M.Phil
None	Mrs. R.R. Sende
	Mrs. A.V. Muzumdar
M.D.	
None	

APPENDIX - XIII
DEGREE AWARDED DURING THE YEAR 1988-89

Name of Candidate	Subject	Topic
Ph.D.		
Mr. N.K. Sutar	Appl. Biology	Studies on cultivation of <i>Plasmodium vivax</i> and <i>Plasmodium falciparum</i> in vitro
Mr. S.B. Bhirud	Org. Chemistry	Synthesis and study of organic compounds as possible antimalarial agents
M.Sc.		
Mr. R. J. Rayanade	Microbiology	The <i>in vivo</i> antiviral activity of chick plasma factor in a heterologous system.
Ms. S. Tatke	Microbiology	Studies on Transformation is <i>S. typhi</i> Ty 21a.
Ms. G. Akerkar	Microbiology	Studies on the kinetics of production and action and the antiviral spectrum of chick plasma factor.
Ms. N. Chakraborty	Microbiology	Effect of variation of dose schedules on Immunogenicity of rabies vaccine.
M. Pharm		
Mr. A.B. Shirgaonkar	Pharmacology	Teratogenic effects of organophosphate pesticide Dimethoate 30 EC in rats.

APPENDIX - XVI
LIST OF POST-GRADUATE TEACHERS

The following post-graduate teachers are recognized by the University of Bombay

Name	Qualifications
Dr. S. R. Sengupta	M.D.
Dr. R.S. Kamat	M.D.
Dr. K.H. Dave	M.D., D.P.B.
Dr. D.M. Renapurkar	M.Sc., Ph.D., FMAS
Dr. V.R. Rao	M.V.Sc., Ph.D.
Dr. P. Ramakrishnan	M.Sc., Ph.D.
Dr. S.V. Gadre	M.Sc., Ph.D.
Dr. B.G. Khadse	M.Sc., Ph.D.
Dr. M.K. Bhopale	M.Sc., Ph.D.
Dr. R.P. Bhamaria	M.Sc., Ph.D.
Dr. S.R. Chavan	M.Sc., Ph.D.
Dr. S.T. Nikam	M.Sc., Ph.D.
Dr. B.D. Sathe	M.Sc., Ph.D.
Dr. (Mrs.) A.C. Desai	M.Sc., Ph.D.
Dr. D.P. Buduk	M.V.Sc., Ph.D.
Dr. (Mrs.) V.R. Kamath	M.Sc., Ph.D.
Dr. N.G. Chanderkar	M.B.B.S., B.Sc. (Med) Path. & Bact., M.D., Ph.D.

APPENDIX - XV

PAPERS PUBLISHED 1988-89

Anita Kala, A.G. Mitra and P. Ramakrishnan (1988). Elevation of Glucose - 6 - phosphate dehydrogenase levels in alloxan diabetic mice by **Bordetella Pertusis** cell extract. Ind. J. Biochem. Biophys. 25 : 413 - 415.

V.G. Shirke, S.V. Nalavade, B.G. Khadse and S.R. Sengupta (1989) Synthesis and study of 3,5 - Dinitro-o-toluamide derivatives as Antituberculosis Agents. Indian Drugs 26(9) : 464-467.

Zarine Khetty, Vijayalaxmi R. Kamat, D.M. Renapurkar and B.G. Khadse (1988). Synthesis and evaluation of 4, 6 - Diamino - 1, 2 - Dihydro - 2, 2' Dialkyl Aryl-1-(substituted) S-Triazine as Antimalarial agents. Ind. J. Malariology 25 : 119 - 124.

S. Rath, V. Bal. B.J. Mohite, V. Haridas, S.R. Naik, S.A. Kamat, A.J. Zukerman and R.S. Kamat (1988) Anti-idiotypic humoral and cellular responses to antibody to hepatitis B surface antigen in hepatitis B viral infections. Clin. and Exp. Immunol 73 : 360.

K.P. Rajadhyaksha, V. Haridas, S.T. Plumber, R.S. Kamat and S.R. Naik. (1988). Efficacy of low dose intradermal hepatitis B vaccine. Ind. J. Gastroentero. 7 : 111.

A. George, K.E. Shroff, S. Rath, S.N. Ghosh, S.R. Sengupta and R.S. Kamat (1989). Route related variation in the immunogenicity of killed *S. enteritidis* vaccine : role of antigen presenting cells. Microbiol and Immunol. 33 : 479.

Saroj Bapna, D.M. Renapurkar and S.R. Chavan (1988) Cyclopicidal activity of three indigenous plant extracts. Insect. Sci. Applic. 9 (2) : 211 - 212.

M.L. Ramaiya and D.M. Renapurkar (1988). Use of animal erythrocytes for *in vitro* cultivation of **Plasmodium berghei**. Int. J. Parasit. 18 : 639 - 641.

D.M. Renapurkar (1988) Observations on commensal rats and their status to plague in Bombay. J. Hyg. Epi. Micro. Imm. 32 : 407 - 413.

D.M. Renapurkar, V.R. Pradhan, N.K. Sutar, R.A. Deshmukh, C. Pandit and S.N. Marathe (1989). Microtest for assaying chloroquine sensitivity of **Plasmodium vivax in vitro**. Chemotherapy 35 : 160 - 163.

V. Zaman, S. Bapna and D.M. Renapurkar (1989) Scanning electron microscopy of **Dracunculus medinensis**. Proc. Helm. Soc. Wash. 56 : 60 - 63.

D.M. Renapurkar (1988). **Mus musculus** : observations on ecology, distribution, status to plague in Bombay. Pestology 12(11) : 5-7.

APPENDIX - XVI

Papers sent for Publication

K.E. Shroff, S.R. Sengupta and R.S. Kamat, Route related variation in immunogenecity of mycobacteria. Int. J. Leprosy.

K.E. Shroff, K.B. Sainis, S.R. Sengupta and R.S. Kamat. Variation in Immunogenecity of mycobacteria : role of antigen presenting cells. Int. J. Leprosy.

K.E. Shroff, K.B. Sainis, S.R. Sengupta and R.S. Kamat. Role of antigen - presenting cells in variation in immunogenecity of mycobacteria. Clin. and Exp. Immunology.

V.R. Pradhan, N.K. Sutar and D.M. Renapurkar. Antiplasmodial activity of antibiotics against **Plasmodium vivax in vitro**. Biovigyanam.

M.L. Ramaiyya, V.R. Kamath and D.M. Renapurkar. Virulence and infectivity of **in vitro** cultivated **plasmodium berghei**. Int. J. parasitology.

N.K. Sutar and D.M. Renapurkar. Effect of liver extract on growth of **Plasmodium vivax in vitro**. Ind. J. Expt. Biol.

N.K. Sutar and D.M. Renapurkar. Influence of some growth promoting substances on multiplication of **Plasmodium falciparum in vitro**. Exp. Parasitology.

