

Annual Report of the Haffkine Institute

1989-90



HAFFKINE INSTITUTE
PAREL, BOMBAY 400 012
INDIA

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CHAPTER I

INTRODUCTION

In the year 89-90 the Government of Maharashtra handed over the Haffkine Institute buildings to the Public Works Department for maintenance and repair of the existing buildings and the laboratories as a part of the revamping programme of the Institute. The Government of Maharashtra also started funding the Institute on a regular basis through an annual budget. Recruitment of scientific staff (vacant posts) are yet to be undertaken. The Centre for AIDS Research and Control has started to function in the Institute from April 1989.

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

Research:

The department of Bacteriology were involved in the studies on cloning of *Vibrio cholerae* antigen genes, and synergism between trimethoprim and furazolidone and pseudomonas ribosomal vaccine.

The department of Biochemistry continued studies on, the hypoglycaemic effect of *Bordetella pertussis* cell extract, preparation of the hypoglycaemic factor from *Bordetella pertussis*, and study of its mechanism of action and mechanism of protective effect of purified *Bordetella pertussis* against oxidative damage in mouse pancreas.

The Chemotherapy department continued research in the synthesis of Heterocyclic compounds of possible therapeutic value especially in the field of Tuberculosis, Malaria, Antibacterial, Antifungal and Anthelmintics. In the benzimidazole carbamate series 5 (N.N. diethyl carbamoyl) - benzimidazole-2-carbamate showed anti T.B. activity at 4 ug/mg. Two compounds of N-Methyl-3-substituted amino succinimide showed antifungal activity. Two compounds of the Benzimidazole - thio - quinoxaline derivatives showed antimalarial activity.

The Immunology department in their study on the regulation of immune response to mycobacteria, have earlier shown that ip immunisation with mycobacteria generates suppression. During the year under report the suppression phenomenon was investigated in great details. The data support a distinct dual mechanism of regulation by "Immune response" and "Immune suppressor" genes.

Purification and characterisation of DTH and lymphoproliferation eliciting antigens of *M.vaccae* was initiated in order to investigate regulation and cross-reactivity in CMI.

In the study on the role of idiotypic regulation in chronic HBV infection, the ability of the antibody to interact with anti - HBc and anti-HBs of human, rabbit and goat origin was investigated by competitive inhibition ELISA. Results suggest that the anti-HBc and anti-HBs share idiotypic determinants.

The department of Zoonoses continued their research work on evaluation of chloroquine sensitivity of *Plasmodium vivax* isolates by employing the micro test method developed by them. None of the samples revealed chloroquine resistance.

In their study on detection of *Plasmodium vivax* using total *P. vivax* DNA as a probe it was observed that although the total *P. vivax* DNA probe was genus specific and could detect low grade parasitaemia it was not found to be species specific.

Testing:

As an important activity of the Institute various tests were undertaken on products submitted by the prohibition and Excise department of the Government of Maharashtra and on samples such as drugs, biologicals, disinfectants, pesticides, cosmetics, plastics etc. In all 5708 samples of various types were tested during the year under report.

Patient oriented services:

The departments of Clinical Pathology and biochemistry investigated clinical samples from patients. In all 4560 were investigated in the two departments. Various hospitals, charitable and private organisations from Bombay, suburbs and other places utilised the services of the above two departments. (Appendix III-A).

Patients of various age groups are immunised against Cholera, Typhoid, Diphtheria, Whooping cough, Rabies and Polio myelitis in the immunisation clinic against nominal charge. In all 1226 patients attended the clinic and were immunised (Appendix VIII).

Training Programmes:

The Institute offers various courses and training programmes for medical and paramedical personnel (Appendix - X). Students

registered during 1989-90 for different degrees / courses are listed in Appendix XI and XII, and students who have successfully completed their thesis and have obtained their degree is given in Appendix XIII, Appendix XIV gives the list of recognised University teachers at the Institute.

Sponsoring Research Bodies:

The Department of Science and technology, Indian council of Medical Research, Maharashtra council on Science and Technology, University grants commission, M/s. D.J. Enterprises, Bangalore and M/s. Alkyl Amine Chemical Pvt. Ltd., Thane 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. Apart from this the executive committee (Administration) and the Teachers Council met several times to provide guidelines for the smooth running of the Insititute.

CHAPTER II

GENERAL DEPARTMENTAL ACTIVITIES

Bacteriology :

Five students were registered for M.Sc. (Part-II) in Microbiology by papers. Three students secured first class and two second class. Five Students were registered for M.Sc (Part-I). Four secured first class and one second class. Training programme were also conducted for D.M.L.T. and short-term training students.

Routine testing activities were also carried out. Bacteriological analysis of various products were undertaken. Water filters were tested and water samples analysed. Lypholization of culture were also undertaken (Appendix 1).

Biochemistry :

The department conducted courses in M.Sc. Biochemistry (by papers) Part I and II, D.M.L.T. and short-term courses in Biochemistry. The department also took lectures on special topics for M.Sc (Part I) at the Haffkine Institute.

Clinical investigations of samples referred by various city hospitals, private practioners and medical officers of the Institute were carried out in the department. The total number of samples tested are shown in Appendix II.

Chemotherapy:

Four students are working for their Ph.D. degree in organic chemistry. One student is registered for M.Sc. organic chemistry and one student is registered for M. Pharm degree in pharmaceutical chemistry. Two students received their M.Pharm degree in Pharmaceutical chemistry from Bombay University.

Four departmental projects on synthesis of anti-tubercular, antimalarial, antifungal and anthelmintic drugs are in progress. Two research projects are sponsored by outside agencies namely M/s. D.J. Enterprises and Alkyl-Amine Chemical Co. Pvt. Ltd.

In their routine activities nearly 170 newly synthesised compounds were analysed for their carbon, hydrogen and nitrogen, 112 analysed for carbon and hydrogen and 57 for nitrogen. Similar facility of analysis was also offered to outside research.

other Institutes and laboratories were tested for antifungal and antibacterial activities.

Clinical Pathology, Immunology :

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

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).

Toxicology :

The Toxicology department was actively engaged during the year under report in toxicological evaluation of different types of chemicals, drugs, cosmetics, plastic containers, surgical gloves etc. (Appendix VII). These samples were referred by various industries. The department was also involved in postgraduate teaching programme.

Virology :

Routine activities of the Virology department included the running of immunisation clinic as an important public services. In all 1226 patients attended the clinic during the period for vaccination. The vaccines administered and the number of patients vaccinated are given in Appendix VII. Three clinical samples were processed for herpes virus isolation studies. In the Rabies research unit, rabies virus was isolated from postmortem sample of one patient.

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 12 lectures and 8 practicals were conducted. Two Ph.D., two M.Sc. and one M.Phil (Microbiology) students are registered with the head of the department.

Zoonosis :

Colonies of various species of insects of public health importance along with strains of parasites were continuously maintained. At the venomous animals unit, snakes of the species *Naja naja*, *Vipera russeli*, *Bungarus caeruleus* and *Echis carinatus* were maintained for their venoms. Alongwith venomous snakes scorpions of the species *Buthus tamulus* and *Palamnaeus gravimanus* were also

maintained. Appendix IX provides details of quantity of venom procured and supplied to various institutions.

A large number of drugs, indigenous plant extracts alongwith Ayurvedic formulations were investigated for their anti-malarial, anthelmintic and insecticidal properties. Evaluation of bio-efficacy of commercial pesticide formulations was undertaken as a part of testing activity of the department.

Plague surveillance programme for the Greater Bombay area was continued as part of the departmental activity. In all 616830 rodents and shrews were received from different wards of Greater Bombay and 85752 were examined for plague. None of the rats examined revealed the evidence of presence of plague infection.

Academic Activities, Honours and Awards

Dr. R.S. Kamat, Assistant Director and Head of the Department of Clinical Pathology and Immunology was invited to deliver a lecture in the Indo-EC symposium on Leprosy jointly organised by the Dept. of Science and Technology Govt. of India and European community in Nov.89 at Lonavala. Dr. Kamat was invited to deliver a lecture in the Indo-British symposium on Hepatitis and Liver diseases organised by the University of Madras, at Madras in January 1990. He was also invited to deliver a lecture in the Indo-U.S. symposium on Immunology, organised by Dept. of Biotechnology Government of India and U.S. Government at All India Institute of Medical Sciences, New Delhi; in Jan, 1990.

Dr. V.R. Rao, Assistant Director and Head Dept. of Toxicology attended the Lecture / Discussion programme of the National society for Air pollution control on Air pollution hazards of the chemical Industries-short term and Episode control Measures at Bombay.

The Medical officer incharge of Immunization clinic Dr. R.A. Deshmukh attended the "International symposium on Non A-Non B Hepatitis and Blood Borne Infectious Diseases" at Nihon University, Tokyo, Japan and presented a paper. She also attended the Fourth Annual Review meeting of the programme officers of WHO/NICD viral hepatitis surveillance centres at Delhi.

Dr. D.M. Renapurakar, Asst. Director and Head Department of Zoonoses participated in the VIII International Congress of protozoology in Japan from 10 - 17 July 1989 and presented a paper on *In vitro* cultivation of *Plasmodium vivax* and *P. falciparum* at the congress.

CHAPTER III

RESEARCH PROGRESS

BACTERIOLOGY

1. Cloning of *V. cholerae* antigen genes

To clone *V. cholerae* antigen genes in order to understand immune mechanism in relation to various *V. cholerae* antigen and to develop a potentially oral cholera vaccine. A library of *V. cholerae* chromosomal DNA has been constructed using PBR 329 as a vector, the host being *E. coli* JM109 strain. The library is being screened for selection of the desired clone using "immune blotting" technique.

2 Studies on synergism between Trimethoprim and Furazolidone.

The two drugs Trimethoprim and Furazolidone showed pronounced synergism against *Pseudomonas aeruginosa*. The M.I.C. for Trimethoprim Furazolidone and a 2 : 1 combination of the two being 300, 300 and 65 ug/ml respectively.

Both the drugs individually and in combination were found to exert bacteriocidal effect.

Estimations of the levels of polysaccharides, DNA, RNA and protein in the drug-treated and control cultures indicated that while Trimethoprim brings down the RNA content (through well known action on folic acid synthesis which in turn leads to inhibition of pyrimidine synthesis), Furazolidone shows significant effect on protein synthesis.

3 Studies on *Pseudomonas* ribosomal vaccine

A ribosomal vaccine using all the eight immunotypes individually have been prepared. ED 50 value show that each individual vaccine is able to confer protection against the homologous but not against heterologous challenge.

The vaccine preparations were found to contain LPS in addition to RNA and protein. This is in accordance to the preparation reported by other workers.

BIOCHEMISTRY

1. Preparation of the hypoglycaemic factor *Bordetella pertussis* and study of its Mechanism of Action.

This study is being carried out with the following objectives :

- 1) Biochemical characterization of purified Hypoglycaemic factor from *B. pertussis* cells (strain No. 18323)
- 2) Study of its mechanism of action.

The study involves cultivation of *Bordetella pertussis* cells (strain No. 18323) followed by enzymetic lysis (DNase, RNase and lysozyme in Tris buffer system PH7.4. The extract is then dialyzed. (1) cultivation of *B. pertussis* cells (strain No. 18323) from lyophilised ampoules is being undertaken in the laboratory. Two batches of culture received earlier from Bacteriology department were processed for the extraction of hypoglycaemic factor.

Crude extracts were prepared and its hypoglycaemic effect confirmed in rats. Preliminary studies showed that at a dose level of 1mg/kg body weight, a lowering of about 40 % in Blood sugar levels were observed.

2. Biochemical studies on the hypoglycaemic effect on *Bordetella pertussis* cell extracts : Studies on enzymes of the metabolic pathway.

Bordetella pertussis extract has shown antidiabetic effect in experimental animals. Following these findings the studies are being pursued with following objectives :

- (i) Extraction of *B. pertussis* cells (strain No. 18323) using a combined enzyme extracts, and purification.
- (ii) To confirm the dosage-response effect in mice.
- (iii) To study the effect of the extract on the activities of key enzymes of the glucose metabolic pathway in the liver of mice. The enzymes include the kinases - Glucokinase (GK). Phosphofructokinase (PFK) and Pyruvate kinase (PK), which catalyse essentially irreversible reactions in the direction of glycolysis. Also Glucose-6-phosphate dehydrogenase (G-6 PH), lactate dehydrogenase (LDH) and glyceraldehyde phosphate dehydrogenase (GAPDH) will be studied.

(iv) Assessment of the hypoglycaemic action **B. pertussis** extract with respect to activities of the above enzymes which are of critical importance in the directional control of metabolic flow in the liver.

The purified extract from the strain No. 18323 showed maximum hypoglycaemic (Blood sugar level lowering) effect at the end of 12 hrs. after iv injection in mice, which was identical with all the doses studied. This was a significant finding since in contrast, the previously used **B. pertussis** vaccine strain (No. 25525) showed maximum hypoglycaemic effect only at the end of 72 hr in mice under identical conditions. All the assays were carried out in overnight fasted animals.

The mice were injected intravenously with the extract at a dose level of 1mg/kg b.wt. (protein concentration) and enzymes from liver homogenates assayed. The enzymes profiles were studied by spectrophotometric assay, at the end of 6 hr, 12 hr, 18 hr, 36 hr, and 48 hr respective. The three kinases viz., G.K., P.F.K. and P.K. which catalyse essentially irreversible reactions in the direction of glycolysis, and are of critical importance for the directional control of metabolic flow, showed significant increase in activities at the end of 18 hr. Similarly the other enzymes G-6 PD, GAPDH and LDH also showed significant increase in activities at the end of 18 hours.

This would indicate a significant aspect of the biochemical mechanism underlying the hypoglycaemic effect of **B. pertussis** extracts.

CHEMOTHERAPY

The department of Chemotherapy continued research in synthesis of Heterocyclic compounds of possible therapeutic value especially in the field of communicable diseases like Tuberculosis, Malaria, Antibacterial, Antifungal and Anthelmintic.

Antifungal / Antitubercular Agents

a) Considering the need of antifungal/antitubercular drugs new compounds on N-Methyl-3-substituted amino succinimide were prepared and tested against H37Rv strain of **M. tuberculosis**. One compound showed antitubercular activity at 0.1/ug/ml and the other at 1.56/ug/ml in vitro testing. According to Meyer's Hypothesis the compounds showing antitubercular activity are likely to show antifungal properties also. Hence, all the compounds synthesised in the above series have

been screened against *T. rubrum*, *T. mentagrophyte* and *C. albicans*. Two compounds showed antifungal activity at 10 mg/kg in vivo testing in rabbits. Nearly 60 compounds are synthesised for the above purpose.

Benzimidazole carbamate series

b) In the above series none of the compound showed antibacterial activity. However one compound viz. 5(N<N. diethyl carbamoxyl)-benzimidazole-2-carbamate showed anti T.B. activity at 4 ug/ml. About 15 compounds were synthesised and screened in this series.

Antimalarial Agents

Benzimidazole-thio-quinazoline derivatives

6-chloroquinolines were prepared as per literature methods and condensed with substituted benzimidazole thio compounds to obtain above mentioned compounds. These compounds were tested against *P. berghei* in mice. Two compounds showed activity. One at 40 mg/kg and the other at 20 mg/kg by Rane's method.

CLINICAL PATHOLOGY

IMMUNOLOGY

1. Study of regulation of immune response to mycobacteria

Earlier it was shown that ip immunisation with mycobacteria generates suppression. However in *vivo* outcome of immunisation by the ip route is dependent upon the efficiency of presentation of the antigen of the organism by the peritoneal cells. It was observed that, when antigen presentation was efficient, as with slow grower mycobacteria, concomittant stimulation of T suppressors, can not abrogate the immune response. In contrast, in the context of inefficient presentation, as with rapid growers, stimulation of T suppressors can abrogate the response in vivo.

During the year, the suppression phenomenon was investigated in great details. The suppressor cells were found to be expanded in mice immunised by ip route only; id immunisation failed to stimulate suppressors. Adoptive transfer of ip primed spleen cells responsible for suppression were T cells with Lyt 2 marker. Suppression was antigen specific. Nature of antigen recognition was also investigated. Ip immunisation of Balb/c, C3H/He, CBA, and DBA mice generated suppression on ip immunisation. Ability to generate suppression was

dependent upon expression of an MHC class II gene product, namely the IE glycoprotein. No suppression was observed in IE deficient, C57BL/6 mice. (Balb/c x C57BL/6) F1 mice generated suppression. The F1 suppressor cells when adoptively transferred to parental strains, suppressed immune response to *M. vaccae* in Balb/c (IE+ve) but not in C57BL/6 (IE-ve).

Addition of anti-IA antibody to the cultures of id primed spleen cells resulted into abrogation of antigen induced proliferative response. Addition of anti-IE antibody to the culture of ip primed spleen cells resulted into enhancement of antigen induced proliferative response.

Based on these results it was concluded that the suppressor cells in mice primed ip with *M. vaccae* were Lyt 2 + T cells and their antigen recognition was IE restricted. The in vitro proliferative response of id primed cells was due to L3 T4 + cells and their antigen recognition was IA restricted.

Thus the data support a distinct dual mechanism of regulation of immune response mediated by the so called "Immune response" and "Immune suppressor" genes.

This work was supported by a University Grants Commission Sr. Research Fellowship Grant.

2. Purification and characterisation of DTH and lymphoproliferation eliciting antigen of *M. vaccae*.

In order to investigate regulation and cross-reactivity in CMI more precisely, a programme to characterise one or more DTH eliciting antigens in *M. vaccae* sonicate, was initiated.

Based on our earlier publication on *M. tuberculosis*, the papain digested *M. vaccae* sonicate was subjected to preparative electrofocussing on agarose employing a broad range ampholine pH 3-10. On focussing, several precipitated protein bands were seen. The pI values of the bands were taken with a surface electrode, the bands were cut and eluted with PBS. The protein from region containing bands of pI 4.0 to 5.0 could elicit DTH in mice primed id with *M. vaccae*.

Preparative IEF of the papain digested sonicate was done on Sephadex using a narrow range ampholine pH 3.5-5.0. Band corresponding to pI 4.3 elicited DTH. The purified antigen was a pure peptide without any sugar or lipid side chains. It had a molecular wt. of 65

kd and there were no subunits. The antigen could elicit DTH at 1.0 ug/ml concentration. The antigen could also stimulate in-vitro proliferation of id primed spleen cells.

An antiserum was raised against the antigen. It was shown to contain genus and group specific epitopes. Judging from DTH cross-reactivity both the epitopes seem to be recognised by T. cells.

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

Earlier it was reported that anti-idiotypic anti-body to human ant-HBc was raised in rabbits.

Ability of the antibody to interact with anti-HBc and ant-HBs of human, rabbit and goat origin was investigated by competitive inhibition ELISA. The anti-id antibody not only reacted with anti-HBc, it also reacted with ant-HBs.

The data suggest that the anti-HBc and anti-HBs share idiotypic determinants.

DEPARTMENT OF PHYTOCHEMISTRY

Following plants were taken to evaluate its mosquito larvicidal (culex-p-fatigans) activity.

- 1) *Saraca indica* Linn. Fam : Leguminosae, Ashoka-seeds.
- 2) *Narium indicum*, Mill, Fam : Apocynaceae, Pila kanher-seeds.

***Saraca indica*, seeds**

Mosquito larvicidal activity of hexane and benzene extract of *Saraca indica* seed was confirmed. Hexane extracts showed 100 % mortality at 0.024 % concentration where as benzene extract showed 92 % mortality at the same concentration. Therefore both the extracts were taken for further studies for isolation and purification of active principle.

Column chromatography of hexane extract of *Saraca indica*, seeds.

Six fractions which were collected from silica gel column in previous year were further evaluated for mosquito larvicidal activity. These fractions did not show promising mosquito larvicidal activity as compared to crude hexane extract, except fraction - 5 (Benzene : methanol, 90 : 10). This fraction showed 80 % mortality of mosquito

larvae at 0.024 % concentration. Therefore, following solvent system were used as eluent to silica gel column to assess whether the activity can be improved by better fractionation of hexane extract.

Hexane	Fraction - 1
Benzene	Fraction - 2
Benzene; chloroform (80:20)	Fraction - 3
Benzene; Chloroform (50:50)	Fraction - 4
Benzene; Ethylacetate (80:20)	Fraction - 5
Benzene; Methanol (95:5)	Fraction - 6
Methanol	Fraction - 7

Above seven fractions were tested for mosquito larvicidal activity. Fraction - 1 and Fraction - 2 did not produce promising mosquito larvicidal activity, whereas Fraction - 6 (Benzene : Methanol 95 : 5) showed 100 % mortality at 0.008 % concentration which was significantly superior to the original hexane extract.

Narium indicum, seeds :

Three fractions of hexane extract of *Narium indicum*, seeds which were collected from Silica gel column previous year were tested for mosquito larvicidal activity. Out of three fractions, fraction-1 (hexane elute) showed 100 % mosquito larvicidal activity at 0.016 % concentration.

DEPARTMENT OF RADIATION BIOLOGY, HUMAN PHARACOLOGY AND BIOAVAILABILITY UNIT

An assessment of bioavailability of selected antibiotics in health and disease by application of microbiological assay.

Bioavailability variations have been identified as causative factors in certain failures in drug therapy. Such therapeutic failures were seldom contributed to the product and most often to some abnormality in the patient.

An actual correlate of the *in vitro* activity of the antimicrobial agent would be to assess the bactericidal activity of the patients serum with that of age matched healthy individuals receiving an equal dose of the antibiotic. The study is going on.

Platelet aggregatory function in health and disease.

The study was aimed at assessment of platelet aggregatory activity in different study groups. These were divided into those of metabolic disorders such as hyperlipidaemia, diabetes mellitus etc. and cases of bacterial and viral infections. The study was carried out in hospitalized cases suffering from various infection like pneumonia, enteric fevers, meningitis, viral hepatitis etc. Initial and follow-up samples were collected. Conclusive evidence of platelet abnormality associated with these diseases and its prognostic value is being studied by analysis of data.

TOXICOLOGY**In Vitro system for screening and evaluating pesticide toxicity.**

Red blood cells have been used for studying the haemolytic effect by different drugs and chemicals.

The present study is being undertaken to determine the effects of various pesticides (cholinesterase inhibitors) on red cell and determine the order of lethality (lysing) with various concentrations. This would reflect on the degree of toxicity and will be compared with the existing acute animal toxicity (LD50). This method, if proved sensitive and comparable to animal experimentation data with regard to LD50 will help in screening potentially toxic pesticides before elaborate animal experimentations are undertaken. The method could, if found suitable, also be applied for residue analysis in foods.

Preliminary work in screening the species variation and sensitivity was carried out with Malathion 50EC. Pesticides that were included in the present study were Ethion, Malathion, Dimethoate and Fenitrothion. Analysis of malathion residues in biological tissues blood (plasma) and liver were attempted. Plasma spiked with malathion in various concentration were analysed. Results indicate that analysis could be done on plasma containing malathion.

VIROLOGY**Antihepatotoxic activity of *Phyllanthus niruri* plant extracts and its active constituents in experimentally included hepatitis B virus infection in langur monkey model (*Presbytis entellus*)**

Standardization of all biochemical methods including LTT and DNA polymerase has been carried out. Normal liver biopsy of

monkeys has been studied. Twelve samples of HBsAg and HBcAg positive sera have been collected screened and stored.

Activities of the WHO/NICD Surveillance Centre for viral Hepatitis.

The Department being the WHO/NICD hepatitis Surveillance centre, continued its surveillance activity.

In all six outbreaks of viral hepatitis from rural and Urban area of Maharashtra were investigated by the Department.

The outbreaks were reported from Deolgaon Raja, Sindkhedraja (Dist. Buldana) Rahuri (Dist. Ahmednagar) Sahada (Dist. Dhulia) Virar and Usmanabad. Except the endemic focus in Usmanabad, all the outbreaks were due to ETNANB (enterially transmitted non A non B) hepatitis virus.

The department also undertook the study on prevalence of hepatitis B markers in Government Dental College Staff, Kasturba Hospital for infectious diseases, Staff, Prostitutes and Voluntary blood donors.

In Usmanabad Hepatitis B was found to be prevalent in hospitalized cases of jaundice. A long term study is awaited.

ZOONOSIS

Malaria

Evaluation of chloroquine sensitivity of *Plasmodium vivax* isolates

The study of chloroquine sensitivity of *P. vivax* isolates from Greater Bombay and its suburbs was continued by employing the micro test method developed in the Department. In all 26 samples were investigated. None of the samples revealed chloroquine resistance.

Evaluation of antimalarial activity of synthetic derivatives against *Plasmodium berghei*.

The study of antimalarial activity of synthetic derivatives against a virulent strain of *Plasmodium berghei*, in mice was continued. The Department received synthetic derivatives from Chemotherapy Department, Haffkine Institute, Bombay College of Pharmacy and The University Department of Chemical Technology, Bombay. About 50 compounds were evaluated during the year.

Detection of *Plasmodium vivax* in blood using total *P. vivax* DNA as a probe.

The sensitivity of the whole DNA probe for the detection of *P. vivax* parasites in blood samples was assessed. *P. vivax*, *P. falciparum* and *P. berghei* samples were serially diluted to yield parasitaemia ranging from 10^{-1} - 10^{-5} . 50 μ l of these diluted samples were processed and applied to nitrocellulose filters. The probe was found to be highly sensitive and could detect parasitaemia of the level of 10^{-4} . The whole DNA probe of *P. vivax* also hybridised with *P. falciparum* and *P. berghei* and could detect parasitaemia of 10^{-3} in the case of *P. berghei* and *P. falciparum*. The probe however did not hybridise with human leucocyte DNA. Thus it was observed that although the total *P. vivax* DNA probe was genus specific and could detect even low grade parasitaemia, it was not species specific. Hence efforts were continued to develop species specific DNA probe.

Filariasis

Collection of *Setaria cervi* parasites and extraction of parasite DNA.

Collection of *Setaria cervi* parasites was carried out from Deonar abattoir. These parasites were used in our study dealing with DNA probe technology. Standardization of various methods for obtaining maximum yield of parasite DNA of *S. cervi* was carried out. Phenol chloroform extraction method gave satisfactory results, both qualitatively and quantitatively. This was confirmed after measuring the optical density and running the samples on 0.77 agarose gel.

Maintenance of several strains of filarial nematodes viz, *Brugia malayi*, *B. pahangi*, *Acanthocheilonema vitiae*, *Litomosoides carinii* obtained from Hindustan Ciba Geigy Research Centre, Goregaon was carried out. These parasites (adult worms) were later utilized for the extraction of parasite DNA and their microfilaria for extraction of microfilarial DNA, after purification with percoll gradient density centrifugation.

Angiostrongyliasis

Seasonal prevalence of *Angiostrongylus cantonensis* in the intermediate host *Laevicaulis alte*.

Laevicaulis alte is a vector of *A. cantonensis* in the Greater Bombay area. Seasonal prevalence of *A. cantonensis* was investigated

to find out maximum prevalence of the parasite in the field population occupying an area of 400Sq. metres approximately.

Maximum prevalence of larvae was observed in rainy months, June - November. Investigations concerning *A. cantonensis* larval tolerance in slugs showed that *L. alte* tolerated infection with 1500 larvae / per 10 slugs and produced optimum infection in slugs.

CHAPTER IV

GENERAL ACTIVITIES

Library :

The construction of the new Library building is in progress. 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 other Institute in India.

Presently there are 8872 books in the library. The number of books added during the year were 117. The total number of journals subscribed were 143. The total number of volumes bound were 846 during the year.

Facilities :

- 1) The Library provides reprographic facilities to its staff and outside readers. The library received 892 requisitions for zerox.
- 2) **Inter library loan :** The library borrowed 223 journals from neighbouring libraries for its staff and loaned 228 journals to other libraries in the city.
- 3) **Visitors :** Around 4651 visitors utilised the library facilities for their reference work.

APPENDIX - I BACTERIOLOGY

Tests	Number
1. Water Samples	48
2. Disinfectants, Biocides	44
3. Cultures Supplied	105
4. Filters	9
5. Resin Samples	4
6. Sugar Cane (Beverages and Juice Samples)	10
7. Water Purifying (Ultra Violet) Units	5
8. Frooti (Beverages)	4
Total	229

APPENDIX - II BIOCHEMISTRY

Name of the body fluid	No of Tests
Blood	285
Urine	80
Special Tests	73
Total	438

APPENDIX - III CLINICAL PATHOLOGY

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

Bombay Area	From Maharashtra
Cama and Albless Hospital	Civil Hospital, Thane
St. George's Hospital	Civil Hospital, Alibag, Raigad
Jagjivanram Hospital	General Hospital, Sawantwadi
Central Railway Hospital	ISP Hospital, Nasik
B.Y.L. Nair Charitable Hospital	Public Health Centre, Buldhana

K.E.M. Hospital	Civil Hospital, Akola
M.G.M. Hospital	District Hospital for women, Akola
L.T.M.G. Hospital	Primary Health Centre, Jalgaon
E.S.I.S. Hospital	General Hospital, Osmanabad
K.B. Bhabha Hospitals	Civil Hospital, Dhulia
Kasturba Hospital for Infectious Diseases.	Civil Hospital, Nasik.
J.J. Group of Hospitals	
Police Hospitals	
Fertilizer Corporation of India	
B.J. Wadia Hospital for Children	
Greater Bombay Municipal Dispensaries	

APPENDIX - III-A CLINICAL PHOTHOLOGY

Tests		Number
A.	Histopathology	834
B.	Haematology	1040
C.	Cytology for malignant cells	13
D.	Clinical Pathology	
	Urine for routine examination	269
	Stool for routine examination	198
	Semen examination	5
	C.S.F. routine cell count	-
	Immunological Pregnancy Tests	47
	Biological (Rat Test) Pregnancy Tests	2
E.	Bacteriology	
	Direct smear for Tubercle Bacilli	2
	Culture for Tubercle Bacilli	29
	Animal inoculation for Tubercle Bacilli	12
	Smear & Culture for C. diphtheriae	1
	Culture of Theatre swabs for Anaerobes	94

**APPENDIX III-A (CONTD.)
CLINICAL PATHOLOGY**

Tests	Number
Urine culture	337
Pus culture	111
Sputum culture	25
Stool culture	113
Stool culture (cholera)	45
Blood culture	39
Antibiotic Sensitivity Tests	378
Aspirated Fluid culture	33
C.S.F. culture	32
Conjunctivitis swab	-
Throat swab culture	27
F. Serology	
Widal tests	146
Weil Felix Tests	122
Brucellosis tests	122
V.D.R.L. Tests	14
Paul Bunnel Tests	32
Total Tests	4122

APPENDIX - IV

HUMAN PHARMACOLOGY AND BIOAVAILABILITY

Name of the Company	Product
Concept	Rifampicin
Magnachem Pharm P. Ltd.	Sulphatrimethoprim Erythromycin
Concept Pharm	Rifampicin
Lyka Labs	Ampicillin caps Rifampicin caps Telracyclin caps
Concept Pharm	Amoxycilin caps Ampicillin caps Ampicillin

APPENDIX IV (CONTD.)
HUMAN PHARMACOLOGY AND BIOAVAILABILITY

M/s. Kopran Chemicals Geno Dr. Dogaunkar	Rifampicin Ampicillin + Probencid
Alembic Chemical Works	Alcephhin 250 Alcephhin 500 Bacipen 250 Bacipen 500 Bacipen Injection 250 Bacipen Injection 500
Lyka Labs	Amoxycillin syrup Cloxacillin Cloxacillin Ampicillin syrup Ampicillin Injection Ampicillin Injection Cloxacillin Injection
Synthico Organics	Comparative Bioavailability of cloxacillin + ampicillin, cloxacillin + amoxycillin
M/s. U.S. Vitamins	Doxycyclin Sulphatri Methoprim Erythromycin Amoxycillin
M/s. Alembic Chemical Works	Cephalexin
M/s. Alembic Chemical Works	Erythromycin Syrup

APPENDIX - IV

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
4651	228	233	117
	Number of volumes bound	Number of current periodicals subscribed	
	846	143	

APPENDIX - V
TESTING UNIT

Tests	Number
TC	125
IMFL	2160
BEER	481
TODDY	1852
GANJA	52
BHANG	13
CHARAS	1
FRENCH POLISH	134
DENATURANTS	495
O.D. SPIRIT	34
S.D. SPIRIT	32
COUNTRY LIQUORS	3
FOREIGN LIQUORS FOR EXPORT	11
Total	5393

APPENDIX - VII
TOXICOLOGY

Sr. Classification of No. sample	No. tested	Toxicity parameter
1. Drug	1	Acute intra-muscular toxicity (LD50) in rabbits.
	1	Acute intra-venous toxicity (LD50) in mice
	1	Abnormal toxicity
2. Surgical gloves	2	Primary skin irritation of rabbits

**APPENDIX VII (CONTD.)
TOXICOLOGY**

	1	Skin sensitization test in guinea pigs
3. Plastic containers	3	Biological tests in mice and rabbits
4. Cosmetics	1	Acute oral toxicity, LD50 in mice
	2	Acute oral toxicity, LD50 in rats
	1	Acute dermal toxicity, LD50 in rabbits.
	1	Acute oral toxicity in pigeons (MLD)
	9	Primary skin irritation in rabbits
	3	Mucous membrane irritation in rabbits.
5. Agricultural chemicals	3	Acute oral toxicity LD50 in mice
	1	Acute oral toxicity, LD50 in rats
	1	Acute dermal toxicity LD50 in rabbits.
	4	Mucous membrane irritation in rabbits.
6. Chemicals	4	Acute oral toxicity, LD50 in mice
	2	Acute oral toxicity, LD50 in rats
	5	Primary skin irritation in rabbits
	4	Mucous membrane irritation in rabbits.
7. Painted container	3	Tests for painted containers (Acute)

APPENDIX - VII-A TOXICOLOGY

Acute toxicity studies

A. Acute oral toxicity in mice

1. Jaan Harvest Protector	Oral	LD50 value > 15,150mg/kg
2. Waterless hand cleaner	Oral	LD50 value > 10,000 mg/kg
3. Anti-scorpion venom serum	i.v.	LD50 value > 50 ml/kg
4. Indfloc - 236	Oral	LD50 value > 15000 mg/kg
5. Indfloc - 237	Oral	LD50 value > 15000 mg/kg
6. Jeevan crop booster	Oral	LD50 value > 15150 mg/kg
7. Jeevan crop protective	Oral	
8. Neem marks / Neem guard	Oral	LD50 value 14250 mg/kg
9. Filcon - 208	Oral	LD50 value > 10,000 mg/kg

B. Acute toxicity in rats

1. Babool tooth paste	Oral	LD50 value > 10,000 mg/kg
2. Babool dry bark powder	Oral	LD50 value > 10,000 mg/kg
3. Indfloc - 238	Oral	LD50 value > 4200 mg/kg Lower limit > 3784 mg/kg Higher limit > 4662 mg/kg
4. Neem based antifeedent formulations	Oral	LD50 value > 10,000 mg/kg
5. Cat floc T	Oral	LD50 value > 10,300 mg/kg

C) Acute toxicity in rabbits

1. Babool dry bark powder	dermal	LD50 value > 10,000 mg/kg
2. Neem based antifeedent formulations	dermal	LD50 value > 15,000 mg/kg

D) Acute toxicity in pigeons

1. Neem mark/neem guard	Oral	LD50 value > 14250 mg/kg
-------------------------	------	--------------------------

E) Undue toxicity test/test as per pharmacopoea

1. Anti-scorpion venom	--	The sample complies with I.P. test for abnormal toxicity.
------------------------	----	---

F) Acute toxicity of Immuno-modulatory Agent for Leprosy in rabbits

i.m. ---

II. Test As Per Painted Container

Water was stored in these painted containers for 7 days, 14 days & 28 days and given ad lib to mice.

APPENDIX VII-A (CONTD.)
TOXICOLOGY

- | | | |
|-----------------------------|--|---------------------------|
| 1. Polytex water tanks | | |
| 2. Polycon LIDP water tanks | | No toxicity was observed. |
| 3. G.R.P. pipes | | |
- III. Test of Plastic container**
- | | | |
|--------------------------------|--|--|
| 1. Plastic compound PC:ME:3932 | | The sample complies with biological tests for plastic as per USP XVIII. |
| 2. Polypropylene (co-polymer) | | |
| Bottle 125 ml. | | The sample complies with the test for plastic container prescribed in I.P. 1985. |
| 3. 300 ml. | | |
- IV) Skin sensitization test in guinea pigs**
- | | | |
|-------------------|--------------|------------------|
| 1. Surgical glove | intra dermal | No sensitization |
|-------------------|--------------|------------------|
- V) Primary skin irritation study in rabbits**
- | | | |
|--------------------------------|---------|----------------------|
| 1. Babool bark dry powder | Topical | Mild skin irritation |
| 2. A.F.M. (Ready to use spray) | | No skin irritation |
| 3. Water less hand cleaner | | Mild skin irritation |
| 4. Indfloc - 236 | | Mild skin irritation |
| 5. Indfloc - 238 | | Mild skin irritation |
| 6. Godrej soap A | | Mild skin irritation |
| 7. Godrej soap B | | Mild skin irritation |
| 8. Godrej soap C | | Mild skin irritation |
| 9. Godrej soap D | | Mild skin irritation |
| 10. Godrej soap E | | Mild skin irritation |
| 11. Godrej soap F | | No skin irritation |
| 12. Neem mark/Neem guard | | Mild skin irritation |
| 13. Surgical gloves | Topical | No skin irritation |
| 14. Ardil latex glove | | |
| 15. Vamubion | | Mild skin irritation |
| 16. Soap G | | No skin irritation |

Mucous membrane irritation study in rabbits

- | | | |
|-----------------------|-------------------------------|-----------------|
| 1. Babool tooth paste | Vaginal
mucous
membrane | mild irritation |
|-----------------------|-------------------------------|-----------------|

APPENDIX VII-A (CONTD.)
TOXICOLOGY

2.	Babool Bark dry powde	- " -	mild irritation
3.	A.G.M. (Ready to use spray)	- " -	mild irritation
4.	Jaan Harvest Protector	- " -	No irritation
5.	Indfloc - 236	- " -	mild irritation
6.	Indfloc - 237	- " -	mild irritation
7.	Indfloc - 238	- " -	mild irritation
8.	Jeevan crop booster	- " -	mild irritation
9.	Jeevan crop protector	- " -	No irritation
10.	Neem mark/guard	- " -	mild irritation
11.	Neem based anti feedent formulations	- " -	mild irritation

APPENDIX - VII
VIROLOGY

Vaccines administered	Number of Patients
ARV	341
PCEC	427
Oral Polio	152
Triple	135
Tetanus toxoid	70
Dual	32
Typhoid	21
MMR	15
Measles	1
Cholera	32
Total	1226

APPENDIX - IX ZONOSIS

Snakes	No. procured	Venom produced (g)	Venom supplied (g)
Cobra	134	76.83	41.17
R. Viper	54	44.30	35.74
Krait	225	6.29	3.08
Echis	3030	22.52	20.00
Total	3443	149.94	99.99
Scorpions :			
Red Scorpions	3254	0.78	0.71
Black Scorpion	240	0.07	-
Total	3494	0.85	0.71

APPENDIX - IX-A ZONOSIS

Statistics of visitors to snake farm :

1.	Students	5242
2.	Staff of Scientific Institutions	168
3.	Distinguished visitors	17
4.	Foreign guests	6
5.	Thursday visitors	106
6.	Others	34
Total		5573

There were 21 enquiries regarding identification of snakes from Hospitals and individuals during the year. (1989-90).

APPENDIX - X
POST-GRADUATE COURSES OFFERED

M.Sc.	- 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 Organic Chemistry.
M. Phil.	- Microbiology
M.D.	- Pathology and Microbiology.
D.M.L.T.	- Diploma in Medical Laboratory Technology.

b2M
APPENDIX - XI
TRAINEES ADMITTED

M.Sc. by papers	10
Diploma in Medical Laboratory Technology (DMLT)	20
Research Fellowship and Scholarship	
Post Doctoral Fellowships	3
Sr. Research Fellowship	6
Jr. Research Scholarship	15

APPENDIX - XII
LIST OF STUDENTS WHO REGISTERED DURING THE YEAR 1989-90 FOR M.SC., PH.D., M. PHARM, M. PHIL AND M.D.

M.Sc.	Ph.D.
None	1. Ms. Meena Bhide
	2. Mr. S.D. Palav
	3. Ms. Suchitra Dani
	4. Mr. G.C. Parkar

APPENDIX XII (CONTD)

5. Ms. Shanthi V.P.
6. Ms. Shibani Basak
7. Ms. Mangala Sawant
8. Mr. S.D. Deshmukh
9. Ms. Sandhya Mainkar
10. Ms. Neha Betawar
11. Mr. N.M. Rahimi

M. Pharm

None

M.D.

Dr. V. N. Virani

M.Phil

1. Ms. Vijayanti Ranade

APPENDIX - XIII
DEGREE AWARDED DURING THE YEAR 1989-90

Name of Candidate	Subject	Topic
Ph.D.		
1. Shri B.J. Mohite	Microbiology	"Immune mechanisms in Hepatitis B"
2. Shri K.E. Shroff	Microbiology	"Study of role of antigen presenting cells in regulation of immune response to mycobacterial antigens"
3. Shri T.G. Tare	Appl. Biol.	"Studies on biology of four common poisonous snakes of India"
M.Sc.		
1. Shri S.N. Kashid	App. Biol.	"Comparative acute and subacute oral toxicity of combination product phosalone - DDT with malathion (technical) in rats"

-
2. Shri J.R. Samuel Biochemistry "Effect of *Annona squamosa* Linn seeds a mosquito larvicidal agent, on the biochemical parameters in albino rats"
 3. Smt. Rajashree Bhise Biochemistry "Studies on the mosquito larvicidal effects of *Ocimum sanctum* Linn seeds."
 4. Smt. Sadhana Mohile Microbiology "Studies on *corynebacterium mediolanum* and its phages".

M. Pharm

1. Shri S.H. Patil Pharm Chem. "Synthesis and study of heterocyclic compounds as possible antitubercular agents"
 2. Shri V.R. Lokhande Pharm Chem. "Synthesis and study of some benzimidazole derivatives as possible antitubercular agents".
-

APPENDIX - XIV

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. R.P. Bhamaria	M.Sc., Ph.D.

APPENDIX XIII (CONTD)

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 PRESENTED AT NATIONAL AND INTERNATIONAL CONFERENCES

1. Eighth International Congress of Protozoology held in Japan from 10 - 17 July, 1989.

In vitro cultivation of *plasmodium vivax* and *P. falciparum*
Dr. D.M. Renapurkar.

2. International Symposium on Non A - Non B hepatitis and Blood Borne Infectious Diseases held at Nihon University Tokyo, Japan from September 27 - 30, 1989.

Situation of Non-A Non-B in urban and rural Maharashtra, India Dr. R.A. Deshmukh and S.R. Sengupta.

APPENDIX - XVI

PAPERS PUBLISHED 1989 - 90

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

V.G. Shirke, A.S. Bobade, R.P. Bhamaria, B.G. Khadse and S.R. Sengupta.

Synthesis and Antiburcular activity of some New-2-substituted asyl Amino-5, 6-Disubstituted / 6-substituted Benzothiazoles. Indian Drugs 27 (6): 350-353, 1990.

APPENDIX XVI (CONTD)

K.E. Shroff, S.R. Sengupta and R.S. Kamat. Route related variation in immunogenicity of mycobacteria. *Int. J. Lepr.* 58 : 44, 1990.

K.E. Shroff, K.B. Sainis, S.R. Sengupta and R.S. Kamat. Variation in immunogenicity of mycobacteria, role of antigen presenting cells. *Int. J. Lepr.* 58 : 58, 1990.

K.E. Shroff, S.R. Sengupta and R.S. Kamat. Pathogenesis of Route related variation in T suppressor response on immunisation with mycobacteria. *Int. J. Lepr.* 58 : 50, 1990.

V. Haridas, S. Rath, V. Bal, A.P. Kulkarni, S.T. Plumber, S.R. Naik and R.S. Kamat. Immune response to hepatitis B viral antigens in chronic infection and relationship to liver necrosis. *Ind. J. Med. Res. (A)* 91, 247.

D.M. Renapurkar. **Suncus murinus** : Observation on ecology, distribution, status to plague in Bombay. *J. Hyg. Epid. Micro. Imm.* 33 : 45-49, 1989.

APPENDIX - XVII

Papers sent for Publication 1989-90

R.S. Kamat. Role of antigen presenting cells in the Immunogenicity of mycobacteria. *Trop. Med and parasitology.*

L.S. Limaye, M.K. Bhopale, S.R. Sengupta, D.M. Renapurkar and K.D. Sharma. Transmission of **Angiostrongylus cantonensis** infection to experimental albino rats. *Ind. J. Helminth.*

N.K. Sutar, and D.M. Renapurkar. Influence of some growth promoting substances on multiplication of **Plasmodium falciparum in vitro**. *Ind. J. Malariol.*

D.M. Renapurkar, A.V. Gosavi and A.S. Arekar. Infection patterns in multiple species of nematodes parasitic in **Periplaneta americana** L. **Helminthologia.**
