

Annual Report of the Haffkine Institute

1985-86



HAFFKINE INSTITUTE
PAREL, BOMBAY 400 012
INDIA

Pages**Administration :**

Governing Council	..	..	..	..	..	1
Academic Council	..	..	..	..	..	3
Advisory Council for Research	..	..	..	..	..	3
Executive Committee	..	..	..	..	..	3
Staff of the Institute	..	..	..	..	..	4

introduction :

Activities — Highlights of Activities of Departments	..	7
Departments	..	12

Research Progress :

Bacteriology	..	16
Biochemistry	..	16
Chemotherapy	..	17
Clinical Pathology and Immunology	..	18
Phytochemistry	..	20
Rabies Research Unit	..	21
Toxicology	..	22
Virology	..	23
Zoonoses	..	26
General Activities	..	30

Appendices :

I	Bacteriology	..	..	..	..	..	31
II	Biochemistry	..	..	..	..	..	31
III	Clinical Pathology (III, IIIA)	..	..	..	..	..	31
IV	Human Pharmacology and Bioavailability	..	..	..	..	..	33
V	Immunohaematology	..	..	..	..	..	33
VI	Library	..	..	..	..	..	34
VII	Rabies Research Unit (VII, VIIA, VIIB)	..	..	..	..	..	35
VIII	Testing	..	..	..	..	..	36
IX	Toxicology (IX, IXA)	..	..	..	..	..	36
X	Virology	..	..	..	..	..	39
XI	Zoonoses (XI, XI-A)	..	..	..	..	..	39
XII	Post Graduate Courses	..	..	..	..	..	40
XIII	Trainees Admitted and Students Registered (XIII, XIII-A)	..	..	..	..	..	40
XIV	Degree Awards during the year	..	..	..	..	..	41
XV	Post-Graduate Teachers	..	..	..	..	..	41
XVI	Papers presented in Conferences	..	..	..	..	..	42
XVII	Papers published	..	..	..	..	..	42
XVIII	Papers sent for Publication	..	..	..	..	..	43

HAFFKINE INSTITUTE FOR TRAINING RESEARCH AND TESTING

GOVERNING COUNCIL

President :

Shri B. A. SAWANT,
Honourable Minister for Medical Education and Drugs,
Government of Maharashtra,
Mantralaya, Bombay-32.

Vice-President :

Smt. RAJANI SATAV,
Honourable Minister for State for Medical Education & Drugs,
Mantralaya, Bombay-32.

Member-Secretary :

Dr. V. R. DESHPANDE,
Director, Haffkine Institute, Parel, Bombay-12.

Members :

Secretary to Government of Maharashtra, Medical Education
and Drugs Department, Mantralaya, Bombay-32.

Shri K. B. SRINIVASAN upto 6-1-1986

Shri GOPALCHANDRA TRIPATHI from 7-1-1986

Deputy Secretary to Government of Maharashtra,
Finance Department, Mantralaya, Bombay-32.

Shri N. W. KARNIK

Deputy Director-General of Health Services, Government of
India, Nirman Bhavan, New Delhi-11.

Dr. MAHENDRA DUTTA

Commissioner, Food and Drugs Administration, Maharashtra
State, Griha Nirman Bhavan, Bandra (East), Bombay-51.

Shri S. D. BHIRUD

Director of Medical Education and Research, Government of
Maharashtra, St. Georges Hospital Compound, Bombay-1.

Dr. B. R. KATE

Director-General, Indian Council of Medical Research,
Ansari Nagar, New Delhi-16.

Prof. V.

Vice-Chancellor, University of Bombay, Bombay-32.

Dr. M. S. GORE

Scientist Emeritus, Purshottam Building, 1st floor,
Opera House, Bombay-4.

Dr. V. S. SALASKAR

Director of Health Services, Government of Maharashtra,
St. Georges Hospital Compound, Bombay-1.

Dr. (Smt.) M. R. CHANDRAKAPURE

ACADEMIC COUNCIL**Chairman :**

Dr. V.R. DESHPANDE

Members :

Dr. B.R. KATE
Dr. R.S. CHANDRAKAPURE
Dr. G.B. PARULKAR
Shri S.D. BHIRUD
Shri G.M. RAJARSHI
Dr. R.K. MENDA
Dr. K.D. SHARMA

Convenor :

Dr. S.V. GADRE

ADVISORY COUNCIL FOR RESEARCH**Chairman :**

Dr. V.R. DESHPANDE

Members :

Dr. (Smt.) M.R. CHANDRAKAPURE
Dr. B.R. KATE
Prof. N.N. SEN
Dr. R.N. BASU
Dr. M.G. DEO
Dr. R.K. MENDA
Dr. K.D. SHARMA
Dr. K.K.G. MENON

Convenor :

Dr. D.M. RENAPURKAR

EXECUTIVE COMMITTEE**Chairman :**

Dr. V.R. DESHPANDE

Members :

Dr. M.V.N. SHIRODKAR
Dr. R.S. KAMAT
Dr. V.R. RAO

Convenor :

Shri M.G. MEHENDALE

**STAFF OF THE HAFFKINE INSTITUTE
ADMINISTRATION**

Director :

V. R. DESHPANDE, MBBS, MD

Chief Administrative Officer :

M. G. MEHENDALE, BA

Accounts Officer :

R. S. JOSHI

BACTERIOLOGY

Senior Scientific Officer Class I :

S. V. GADRE, MSc., PhD

Scientific Officer :

R. P. BHAMARIA, MSc., PhD

BIOCHEMISTRY

Scientific Officer Class I :

P. RAMAKRISHNAN, MSc., PhD

CHEMOTHERAPY

Scientific Officer Class I :

B. G. KHADSE, MSc., PhD.

Scientific Officers :

M. T. JAOKAR, MSc.

V. S. TAMORE, MSc.

CLINICAL PATHOLOGY

Director :

R. S. KAMAT, MBBS, MD

Officer :

Y. FERNANDES, MBBS, DPB, MD. (Pathology)

CHHAYA H. PANDIT, MBBS, MD. (Microbiology)

CLINICAL RESEARCH DIVISION

Scientific Officer Class II :

A. P. KULKARNI, MSc.

HUMAN PHARMACOLOGY & BIO-AVAILABILITY

Senior Scientific Officer Class I :

N.G. CHANDERKAR,
MBBS, BSc.(Med) Path & Bact., MD, PhD.

IMMUNOHAEMATOLOGY

Senior Scientific Officer Class I :

D. CHENGAPPA, MBBS, MD.

INSTRUMENTATION CELL

Junior Scientific Officer :

J.V. SAWANT, MSc.

LIBRARY

Librarian :

Ms. M.V. JOSHI, BSc., BLib. Sc.

PHYTOCHEMISTRY

Senior Scientific Officer Class I :

S.R. CHAVAN, MSc., PhD.

Junior Scientific Officer :

S.T. NIKAM, MSc., PhD.

RABIES RESEARCH UNIT

Senior Scientific Officer Class I :

H.J. MANSHARAMANI, MBBS, PhD.

STATISTICAL SECTION

Statistician :

A.S. AREKAR, MSc.

TESTING UNIT

Senior Scientific Officer Class I :

H.K. SONI, MSc., PhD.

Junior Scientific Officers :

H.K. DALAL, BSc.

S.S. JAIN, M Pharm

S.B. DHOLAM, MSc.

TOXICOLOGY

Assistant Director :

V. R. RAO, BVSc & AH, MVSc., PhD.

Senior Scientific Officer Class II :

M. G. PHATAK, MSc.

TRAINING

Senior Scientific Officer Class I :

S. V. GADRE, MSc., PhD

VIROLOGY

Assistant Director :

M. V. N. SHIRODKAR, MBBS, AB, ScD, PBK, FMAS.

Junior Scientific Officer :

P. R. SANGOLE, MSc., Dip. Bact (Virology)

Medical Officer :

RANJANA A. DESHMUKH, MBBS. MD

ZOONOSIS

Assistant Director :

D. M. RENAPURKAR, MSc., PhD, FMAS

Senior Scientific Officer Class II :

M. K. BHOPALE, MSc., PhD

Junior Scientific Officers :

T. G. TARE, MSc.

B. D. SATHE, MSc., PhD.

V. R. KAMATH, MSc., PhD.

N. K. SUTAR, MSc.

CHAPTER I

INTRODUCTION

The problem of adequate core funds for the smooth functioning of Haffkine Institute has not been solved inspite of repeated projections to the Government of Maharashtra. Efforts are still being made at governmental level to convince the importance of the existence of such an internationally reputed Bio-medical Research Institute with proper core funding.

The Institute since its inception in 1899 has been making notable contribution in the field of communicable diseases including diagnostic services, surveillance and regulatory expertise. This was possible because of the full support it had received by way of adequate grant-in-aid from the Government.

It is sad that, the pathetic condition that is prevailing today at the Haffkine Institute is because of the non realisation that a Research Institute cannot be totally self dependent, and has to rely on proper grant-in-aid. The public services offered by the Institute cannot be compared with a commercial oriented private organisation, for, most of the services offered are at no basis (e.g. diagnostic services, surveillance and expertise which are an essential part of public health programmes). In fact at no time has the Institute backed out of its possibility of providing such public services whenever required by various Government bodies in the interest of public health.

Inspite of severe financial crisis, the research activities continued because of some grant-in-aid from research organisations such as DST, ICMR and private commercial houses. But such funding does not take care of the maintenance of Institute, initial expenses for setting up facilities, major projects pertaining to the theme of the Institute, health, and salaries of the staff. At present except for the of the existing staff practically no other funds were made available to run the Institute.

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

Research :

The Department of Bacteriology was involved in relating to genetic recombination between enteric species. studies were aimed mainly at obtaining new strains with commercial application and also for basic research.

The Department of Biochemistry continued their study the mechanism of the hypoglycaemic effect which includes

markers of metabolic pathway in **B-pertussis** sensitized (both normal and alloxan diabetic) mice. The study was aimed to see the effect of the factor in maintaining the levels of the protective enzymes SOD and catalase, and also prevention of lipid peroxidation in both normal and diabetic animals.

The Department of Chemotherapy synthesised different organic compounds for testing their biological activity against tuberculosis, malaria, fungal infection and pathogenic bacteria. Several of these compounds showed promising **in vitro** activities. A few of the compounds are being studied for their **in vivo** activity at Central Drug Research Institute, Lucknow. New Benzothiazole heterocyclic guanidino, sym-triazine moiety compounds were synthesized and tested for various **in vitro** activities.

The Department of Clinical Pathology and Immunology continued their investigations on (a) immune regulation in Salmonellosis, (b) DTH eliciting antigen/s of *S. typhi* and (c) Immunological and epidemiological studies in viral hepatitis.

Their current study revealed that interferon inducers such as Poly I : Poly C and prostaglandin blockers such as Indomethacin enhanced the immunogenicity of killed *S. enteritidis* vaccine in experimental mice. The vaccines were non-immunogenic by intraperitoneal route but prior treatment with the above inducers and blockers corrected the problem. The cause of route related variation in immunogenicity was studied and currently a comparative study regarding presentation efficiency of the different antigen presenting cells is in progress and the effects of above agents on antigen presenting cells are being titrated.

A comparative study related to antibody response, T cell response, SALT profile in hepatitis B has been carried out. Epidemiological study investigating frequency of serological markers of different viruses responsible for hepatitis in sporadic acute viral hepatitis and in normal population is being pursued.

The Rabies Research Unit continued their investigations into the kinetics of rabies virus in cell culture and effect of dose-schedule manipulation of rabies vaccine on its immunogenicity in experimental animals. Preliminary work was started to study immune cellular response in volunteers against rabies vaccine.

Radiation Biology Unit carried out comparative study on detection methodology for HBsAg employing Macro-Elisa (Abbot), Micro-Elisa (NIV) and Radio immunoassay (Abbot) techniques, Radio immuno assay proved to be superior to all the above mentioned methods with regard to specificity and sensitivity.

The Department of Toxicology completed the sub-acute toxicity study of Zolone DT, an organophosphate — chlorinated hydrocarbon, combination pesticide. Three dose levels namely 700, 1375 and 2750 PPM in diet were studied. There were no effects on feed intake or body weight or haematological parameters in any of the groups of treated rats. At 700 and 1375 PPM Zolone DT level there were no gross signs or symptoms of toxicity. At 2750 PPM level, five females died during the experimental period. Signs of toxicity such as tremors and diarrhoea were observed in the female rats. At 2750 PPM the plasma and whole blood cholinesterases were inhibited in both male and female rats. Female rats belonging to 1375 PPM level also showed reduction in plasma and whole blood cholinesterases. The decrease in plasma cholinesterase level was not observed in the reversal group of female rats but the whole blood cholinesterase still remained below the initial level. Histopathological observations revealed no change, which could be attributed to the administration of Zolone DT and were comparable to control group. Female rats seem to be more sensitive to Zolone DT than male rats.

The department also completed the subacute toxicity of Zolone DT in rabbit. The whole blood and plasma cholinesterase were inhibited in all the groups (Annual Report 1984-85). The histopathological examination revealed alopecia, scarring and cedema at the site of application on skin.

The department undertook neurotoxicity study of Zolone DT by oral route in chicken. No demyelination or degeneration was observed with the dose of the pesticide. However, egg production and body weights were reduced at 110 and 275 mg/kg dose level.

The department of Virology in their WHO/NICD viral hepatitis surveillance centre activities were involved in investigations of viral hepatitis out break in Karad, Sangli, Raigad and Sholapur districts. The outbreaks were due to faecal contamination of water supply. Medical and paramedical personnel from Mahatma Gandhi Memorial Hospital, Bombay were tested for HBsAg and anti-HBs (ELISA) due to hepatitis incidence in their staff. The susceptible staff received hepatitis B vaccine.

The kinetics of production and action of antiviral antibiotic, plasma factor (PF) was studied by testing its potential to inhibit tumour production by BS-RSV, *in vivo* and *in ovo* when administered at various time intervals prior to (prophylactically), concurrently with or following (therapeutically) the RSV inoculum. Results showed significant anti-RSV activity when administered between 48 h prior till 48 h following the RSV inoculum, showing maximum anti-RSV activity on concurrent administration of RSV PF.

In their study of venoms on tumor production by Rous-sarcoma virus (RSV) it was found that inactivated venom, where spreading factor has been destroyed, no tumor spread could be demonstrated. It was postulated that there exists in the venom some heat labile substance/s other than hyaluronidase which is/are responsible for tumor enhancing activity of SR-RSV.

The Department of Zoonoses continued their efforts to improve the currently used medium RPMI for *in vitro* cultivation of *P. vivax*, *P. falciparum* and *P. berghei* by incorporating additional nutrients.

Nearly 55 compounds synthesised at the Department of Chemotherapy were screened for antimalarial activity using *P. berghei*-mouse model. Synthetic agents and plant extracts were also screened for their anthelmintic and insecticidal potential. A number of commercial formulations were screened for their insecticidal properties. Immune mechanisms in *P. berghei*, *A. ceylanicum*, *Necator americanus* and *A. cantonensis* were studied.

Testing :

Various types of samples such as drugs, chemicals, cosmetics, water samples, water filters, samples submitted by prohibition and excise department were tested during the year. In all 4,623 samples were tested during the year.

Patient oriented services :

The Departments of Biochemistry and Clinical Pathology accepts samples from patients for investigations. In all 460 and 3,957 samples were tested in the two departments respectively. Various hospitals, charitable and private organisations in Bombay and suburbs utilise the services of the two clinical oriented departments (Appendix IIIA).

The Institute runs an Immunisation Clinic where patients of various age groups are immunised against Cholera, Typhoid, Tetanus, Diphtheria, Whooping cough, Rabies and Poliomyelitis for a nominal charge. In all 907 patients were immunised during the year under report (Appendix X).

The clinical vrology section started performing newer tests (Elisa) for detection of Rubella and cytomegalo virus, IgG and IgM type of antibodies. In all 7 patients were tested for Rubella IgG and 10 patients were tested for CMV IgG antibodies.

The Immunohaematology Department carried out blood transfusions, Rh-antibody titre and grouping tests (Appendix V).

Thyroid uptake studies were carried out by Radiation Biology Unit in 20 referred cases.

Training programmes :

Training programmes for paramedical and medical personnel were offered by the Institute. (Appendix XII). A list of students who were registered during the year under report for various degrees and those who successfully completed their thesis and obtained their degrees are given in Appendix XIII A and Appendix XIV. The list of recognised university teachers is given in Appendix XV.

The Institute enrolls five students in each of the subjects of Microbiology and Biochemistry for M.Sc. degrees by papers. The Institute also caters for training paramedical personnel, particularly in the field of medical laboratory technology. A one year course in Drug Analysis is offered by the Institute. A one year diploma course (DMLT) of the University of Bombay is also offered. Seventeen students were trained in DMLT and 13 students in Drug Analysis during 1985-86. Six students received short term training during the same period.

Sponsoring Research Bodies :

Some of the research projects are financed by the Indian Council of Medical Research, Department of Science and Technology, Lady Tata Memorial Trust, Central Council of Research in Ayurveda and Siddha system and Maharashtra Council on Science and Technology, Government of Maharashtra, Education and Employment Department.

Administrative bodies :

The Governing Council presided over by the Hon'ble Minister for Public Health, Government of Maharashtra met twice during the year under report. Apart from this the Executive Committee (Administration) and Teachers Council met several times to provide guidelines for the smooth functioning of the Institute.

CHAPTER II

GENERAL DEPARTMENTAL ACTIVITIES

Bacteriology :

Students as in the previous year were registered for M.Sc. (Part I and Part II) in Microbiology. Five students were registered for M.Sc. (Part II) in Microbiology by papers out of which four students secured first class and of the five students registered for M.Sc. (Part I), two secured first class and three, second class.

Testing activities included Bacteriological analysis of various products, testing of water filters, analysing water samples and lyophilisation of cultures (Appendix I).

Biochemistry :

Academic activities included training courses in M.Sc. Biochemistry (Papers) Part I and Part II, DMLT and Short term courses in Biochemistry. As part of the M.Sc. programme, lectures in Biochemistry were held at the Institute during the first term of the year.

The testing activities of the department included biochemical analysis of clinical samples referred by various city hospitals, private practitioners and medical officers of the Institute. The various tests carried out and the total number of samples are shown in Appendix II.

Chemotherapy :

Five students are working for their Ph.D. degree in Medicinal chemistry and five students are working for their M.Pharm. degree in Organic Pharmaceutical Chemistry. Theory and practical classes were conducted for drug analysis course.

Clinical Pathology :

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

The D.M.L.T. and Short Term 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 Units :

The Human Pharmacology and bioavailability Unit undertook blood absorption studies of different drug formulations in human

volunteers. The list of pharmaceutical samples accepted for the studies is given in Appendix IV. The Radiation Biology Unit continued the thyroid uptake studies of cases referred to the Institute. Total 20 cases were referred.

Immunohaematology :

The department of Immunohaematology provided transfusion to children suffering from Thalassemia Major. There are 38 patients registered. The department also undertook Rh antibody titre and blood grouping. It was also involved in teaching and training of D.M.L.T. and M.Sc. students in blood banking. Medical Officers and technicians from district hospitals are also trained in blood banking.

Rabies Research Unit :

Diagnostic services were provided by the Rabies Research Unit for detection of rabies in post-mortem brain specimens and CSF. Total of six samples were received for confirmation of the diagnosis of rabies. The nature of the samples and the results of microscopy/biological tests are given in Appendix VII. Advisory services to patients (dog-bite cases) and physicians were given on problems relating to dog bites and immunisation against rabies. The Unit also participated in training and teaching programmes for M.Sc. (Microbiology) and M.D. (Microbiology) students of the Bombay University. Lectures as well as practicals/demonstrations were given to them. Students from various medical colleges of the city visited the Unit as part of their academic activity to learn about rabies.

Toxicology :

The toxicology department carried out toxicological evaluation of different chemicals, pesticides, drugs, cosmetics and plastics. The types of products tested are shown in Appendix IX. The department also took part in training and teaching programmes of Drug Analysis and postgraduate courses.

Virology :

The most important routine activity of the department of Virology is the Immunisation of patients in its clinic. In all 907 patients attended the clinic during the period under report. The vaccines administered included anti-rabic, triple, oral polio, cholera, tetanus and measles. The different types of vaccines administered and the number of patients vaccinated are given in Appendix X — Detection of CMV in urine samples, detection of hepatitis B and hepatitis A markers in clinical material were also undertaken.

In its other important activity the department conducted the M.Sc., M.D. and P.Phil lectures and practical annual course for the students of Haffkiné Institute and students of Bombay University.

In all 18 lectures and 8 practicals were conducted. New students were also registered under the guidance of the head of the department. In all two students for M.Sc. (Micro) and five Ph.D. (Micro) are registered with the Head of the Department.

Zoonoses :

Various species of poisonous and non poisonous snakes were maintained for the collection of venom. The quantity of venom collected and supplied is given in Appendix XI. The different species of snakes and scorpions from whom the venom was obtained is also given in Appendix XI. The venomous animals unit of the department proved to be a key attraction to visitors' like professional herpetologists, zoologists, scientists, students and other visitors. In all 6,629 visitors visited the snake farm (Appendix XIA). Twenty nine enquiries were received from hospitals and individuals for identification of snakes.

The department continued to participate in plague surveillance activities. In all it received 5,14,311 rats for identification and screening. Out of these 79, 669 rats were examined for plague.

Mr. A. J. Almeida was awarded Ph.D. degree in Applied Biology for his thesis on "Immune mechanism in experimental filariasis".

Academic activities, Honours and Awards :

Dr. B. G. Khadse, Head of the Chemotherapy department was invited by Walter-Bushnell Co. and Organon Research Centre to deliver a lecture in the field of Anthelmintic. For the department a scheme entitled "Synthesis and in vivo study of antimalarial agent" was approved by the Lady Tata Memorial Trust who will be funding the scheme. Mr. S. M. Patil of the department was awarded the Lady Tata Memorial Fellowship. A collaborative research programme with Central Drug Research Institute has been accepted for in vivo testing of antitubercular drugs. Dr. Khadse along with three students attended the Indian Asian Congress of Parasitology, held at CDRI, Lucknow between 9 to 12th February, 1985.

Dr. H. J. Mansharamani, Head of the Rabies Research Unit delivered the Dr. S. L. Manjrekar Memorial Lecture arranged by the Association of Microbiologists of India at Pune University in October, 1985. He was also a special invitee at the symposium on rabies held during the 9th Annual Congress of the Indian Association of Medical Microbiologists at the Banaras Hindu University at Varanasi in November, 1985. He delivered a lecture on 'Effective Immunoprophylaxis against rabies'.

Dr. V. Ramakrishna Rao, Head of the Department of Toxicology, as a WHO adviser attended the International programme on chemical safety, Task Group on Environmental Health Criteria for Ammonia in Geneva from 8 to 13 July, 1985. He delivered a lecture on experimental evaluation of Pesticides in Laboratory animals for the course of the summer Institute on pesticide management in Agriculture held at Marathwada Agricultural University, Parbhani. He was nominated as Member Working group on Health Effects of Pesticides by the Indian Council of Medical Research.

The Head of the department of virology presented the work Langur Model for Hepatitis B virus at the meeting held under the auspices of Bharatiya Vidya Bhavan on 13th January 1986. Meeting was presided over by the Nobel laureate Dr. B. S. Blumberg.

The medical officer from the department attended the WHO/NICD annual meeting of programme officers at Delhi on September 25, 26, 1985 and presented the work done by the Regional Hepatitis Surveillance Centre for Maharashtra and Goa.

Dr. D. M. Renapurkar, Head of the Department of the Zoonoses, participated as a nominee of Government of India, in the WHO Inter-Country Seminar on Epidemiology of Plague from 2nd to 6th December, 1985 at Rangoon, Burma. The second training course in Zoonoses and their control was organised by the Zoonoses Department in October, 1985. The course was attended by senior health and veterinary officers of the Government of Maharashtra.

CHAPTER III

RESEARCH PROGRESS

BACTERIOLOGY

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

V. cholerae, both log phase cultures and spheroplasts, were subjected to transformation with pBr 322 DNA. *V. cholerae* and *E. coli* were also subjected to conjugation using *S. flexneri* as a donor.

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

Phage resistant mutants of *C. medfolanum* R18 strain were developed.

BIOCHEMISTRY

Biochemical studies on the mechanism of action of the Hypoglycemic factor from *B. pertussis* :

Studies on the mechanism of action of the hypoglycaemic factor purified from *B. pertussis* cells, as reported earlier, is being undertaken in normal and alloxan diabetic animals. The first point of interest is the possible protective effect of the factor against the toxic manifestations of alloxan diabetes.

It is now established that the diabetogenic action of alloxan is mediated through the generation and release of highly reactive free radicals of oxygen like superoxide anion, hydrogen peroxide and the hydroxyl radical. These bring about cellular membrane damage and ultimately total inhibition of pancreatic cell function. The toxic effects of alloxan are however counteracted by the intracellular free radical scavenging enzyme superoxide dismutase (SOD) and catalase which form the primary defence mechanism against oxygen toxicity and lipid peroxidation.

The observation that the *B. pertussis* factor is able to attenuate the hyperglycaemia of the alloxan diabetic state suggests a possible protective action of the factor against alloxan toxicity. Hence as part of our study on mechanism, it is first aimed to study the effects of the factor in maintaining the levels of the protective enzyme SOD and catalase, and prevention of lipid peroxidation in both normal and diabetic animals. Such a study has not been undertaken before.

The assay of the enzyme superoxide dismutase was standardised in pancreas and liver of both normal and **pertussis** factor treated animals. Further studies are in progress.

CHEMOTHERAPY

The department of Chemotherapy synthesised and tested the biological activity of organic compounds. These newly synthesised compounds were tested for antimalarial, antifungal, antibacterial and antitubercular properties.

(1) **5,6-Disubstituted-2-alkyl amino or aryl amino benzothiazoles :**

All benzothiazole compounds were prepared by new synthetic route and about 40-50 compounds prepared were tested for their antimalarial and antituberculosis activity.

6-Fluoro-2-(p-butoxy phenyl amino) benzothiazoles and 6-fluoro-2-(benzyl amino) benzothiazoles were tested thoroughly for their *in vitro* activity, some of them have shown good antituberculosis activity. Few selected compounds were sent to C.D.R.I. Lucknow for *in vivo* testing.

(2) **1-4-(Heterocyclic or 4-substituted aryl) N,N',2,3-bismethoxy carbonyl) guanidines :**

New heterocyclic guanidino compounds were prepared by condensing different heterocyclic amines with 2-methyl thiopseudo urea, 1,3-dicarboxylic acid dimethyl ester, in solvent at reflux temperature. The compounds were tested for *in vitro* tuberculosis activity and one of them showed good antituberculosis activity similar to standard drug INH.

(3) **5 or 6-Nitro-1-alkyl or hydroxyethyl-2-benzimidazole carbamate :**

Different benzimidazoles are characterised by elemental analysis were tested for antituberculosis activity. All the compounds are to be studied for antimalarial activity against *P. berghei* in mice.

Synthesis and Antimalarial activity of :

(i) **5-Substituted 2-(2,3-Bis-(methoxy carbonyl) guanidino) substituted benzimidazoles :**

(ii) **5-Benzoyl, 2-methyl substituted benzimidazole :**

Beginning from basic raw materials available, thirty derivatives of the above mentioned series were synthesized. These compounds are being screened for antimalarial activity against *P. berghei* in mice. Two compounds of the benzimidazole series have been found to be active. Further investigations are in progress.

Synthesis and Antimalarial evaluation of :

1,3,5-Triazine of the type : 4,6-diamino 1,2-dihydro-2,2-dialkyl or aryl (1-substituted)-phenyl-s-triazine :

Compounds with the symmetrical triazine moiety as the basic nucleus have been synthesized and tested for their *in vivo* anti-malarial action against rodent malarial strain *P. berghei* in mice. Four compounds have been found active at doses from 20-80 mgs/kg. One compound namely 4,6-diamino 1,2-dihydro-2,2-dimethyl-1,1-(p-bromo benzyl) oxy phenyl s-triazine was found to be active at 20 mgs/kg, both orally and subcutaneously, and was also studied for Preliminary Acute Toxicity.

CLINICAL PATHOLOGY AND IMMUNOLOGY

(1) Study of immune regulation in Salmonellosis :

Earlier it was shown that outcome of immunisation by different routes was not the same and that the differences in the effector responses observed were due to generation of different sets of regulatory cells.

Effects of interferon inducers such as poly I : poly C and prostaglandin blockers such as Indomethacin on the immunogenicity of killed *S. enteritidis* vaccine were studied in experimental mice. It was shown that both the agents enhanced the immunogenicity of killed vaccine. Particularly the vaccines were non-immunogenic by intraperitoneal route and prior treatment of animals with these agents corrected this defect.

Further the route related variation in immunogenicity was shown to be due to poor antigen presentation efficiency of peritoneal macrophages employing *in vitro* LTT assay. Employing the same assay it was also shown that poly I : poly C improved the antigen presenting efficiency of peritoneal macrophages. Currently researchers are comparing the presentation efficiency of the different antigen presenting cells and the effects of agents such as poly I : poly C on antigen presenting cells are being titrated. The project received a fellowship grant from I.C.M.R.

(2) Study of DTH eliciting antigen/s of *S. typhi* :

From the sonicate of *S. typhi*, by repeated gel filtration chromatography, a large m.wt. antigen (200,000 daltons), capable of eliciting DTH in guinea pigs and mice immunised with *Salmonella* was isolated. The antigen is still not pure; it shows several bands on isoelectrofocussing. The project is receiving a fellowship grant from I.C.M.R.

(3) Immunological and epidemiological studies in Viral Hepatitis :

(a) Immunological studies in acute hepatitis B :

Markers of hepatitis B viral (HBV) infection, immune

responses against them and levels of serum alanine aminotransferase (SALT) were studied in males, non-pregnant and pregnant females, in an attempt to correlate immune response with morbidity of the disease. The following were the significant findings.

- (i) Contrary to published reports the disease hepatitis B was definitely mild in pregnant patients, and recovery was earlier, though the markers of HBV infection did not disappear from their sera.
 - (ii) The antibody responses to HBV antigens were similar in pregnant and non-pregnant patients.
 - (iii) Non-pregnant patients mounted a late phase T cell response to hepatitis B surface antigen (HBsAg) which was absent in pregnant patients.
 - (iv) In early phase of acute hepatitis, the sALT profile was parallel to the profile of T cell response to hepatitis B 'e' antigen (HBeAg). In the late phase, the sALT levels correlated with T cell response to HBsAg.
 - (v) Absence of T cell response to HBsAg in pregnant patients is probably the result of non-specific immunosuppression known to occur in pregnancy, which may also be the cause of low morbidity of hepatitis B observed in our cases.
- (b) **Regulation of HBsAg specific immune-response in acute hepatitis B :**

Earlier it was observed that both humoral and cellular responses to HBsAg were delayed till 3rd-4th week after the onset of acute hepatitis B. Since the disease has a long incubation period, this observation was considered to be indicative of active suppression of immune response to HBsAg. Hence an investigation on the idiotypic network in relation to HBsAg was initiated.

Using affinity purified antibody to HBsAg (anti-HBs) patients of acute hepatitis B were investigated for evidence of expanded anti-idiotypic responses. Preliminary results show expanded T cell response to anti-HBs idiotypes, in patients in whom T cell responses to HBsAg had not developed.

(c) **Seroepidemiological studies in Viral Hepatitis :**

The major objectives of this study are to investigate frequency of serological markers of different viruses responsible for hepatitis, in sporadic acute viral hepatitis and in normal general population.

(i) **Sporadic viral hepatitis in adults:** 362 cases were investigated. Male to female ratio was 7:1. 61.6% were HBsAg positive, and 5.8% were anti-HAV IgM +ve; tentatively the remaining 32.6% were labelled as non A, non B (NANB) hepatitis pending further investigations for markers of Epstein Barr, Cytomegalo and Herpes virus infections and tests for IgM antibody against hepatitis B core antigen.

(ii) **Cases of fulminant hepatitis:** So far 31 cases of fulminant viral hepatitis have been studied. Nine were HBsAg +ve, two were anti-HAV IgM +ve. Two out of the nine HBsAg +ve cases only were anti-HBc IgM +ve. Thus the preliminary evidence seems to rule out hepatitis B as a major cause of fulminant hepatitis in Bombay.

This project has been receiving financial grant from Department of Science and Technology, Govt. of India and Overseas Development Administration, Govt. of U.K. The Project involves active collaboration between the laboratory of Prof. A. J. Zuckermann of London School of Hygiene and Tropical Medicine, Prof. N. C. Nayak of All India Institute of Medical Sciences, New Delhi and the Department of Clinical Pathology, Haffkine Institute.

PHYTOCHEMISTRY

Michelia Champaca Linn :

Red and yellow flowers of champha were collected from Haffkine Institute campus. The flowers were dried in shade. They were then cut into small pieces separately. Both the varieties were subjected to steam distillation for extracting the essential oils. The oils were then extracted from aqueous distillate, with petroleum ether 40-60°C. Petroleum ether extracts were dried using anhydrous sodium sulphate. The petroleum ether was recovered carefully using water bath, and the essential oils were collected separately. The essential oil from red flowers and yellow flowers was evaluated for mosquito larvicidal activity individually. Also their synergistic activity was assessed.

Biological studies :

Various concentrations of essential oil of red flowers were evaluated for mosquito larvicidal activity (culex-p-fatigen) as per W.H.O. specification.

It was observed that 0.002, 0.004, 0.006, 0.008 per cent of essential oil produced 64, 88, 100 and 100 per cent mortality, respectively.

In case of yellow flowers essential oil following observations were recorded with 0.002, 0.004, 0.006 and 0.008 per cent

concentrations. They resulted in 24, 24, 68 and 96 per cent larval mortality. While carrying out these studies solvent and water controls were kept which did not show any mortality of mosquito larvae.

Synergistic study of essential oils :

The following concentrations were pooled together in each beaker of essential oil of red and yellow flowers.

(1) 0.0008 + 0.002

(2) 0.0008 + 0.004

(3) 0.0008 + 0.006

(4) 0.0008 + 0.008

(1) and (2) showed no mortality, (3) and (4) showed 92, and 100 per cent mortality respectively.

Red flower essential oil alone with 0.0008 per cent concentration did not show any mortality of mosquito larvae (*Culex fatigans*).

RABIES RESEARCH UNIT

1. Adaptation of rabies virus to VERO cultures :

Not much progress could be made in adapting Paris strain of rabbit fixed virus to VERO cell culture system. However virus could be retrieved in the fluid phase upto the 6th post-innoculation day but it could not be passed into fresh cultures due to its low titre.

2. Immunologic studies on rabies vaccine in mice :

This work started earlier was continued. Daily dose-schedule of vaccine was compared with interrupted dose-schedule for protection in mice against challenge. Initially, the virus challenge was administered intra-muscularly (IM), but in later experiments, this was done by the intracerebral route. Results of a typical experiment representing each group are given in Appendices VII(A) and VII(B).

As can be seen from Appendix VII-A as against a mortality rate of 84.6% in unvaccinated mice, only 18.18% mice died after they had received 5 daily doses of vaccine. If the same amount of vaccine was distributed in 3 doses (given on alternate days), mortality was totally eliminated. Low infectivity titre of the IM-adapted challenge virus however, did not permit more stiffer challenge.

When mice were challenged intracerebrally (IC), as seen in Appendix VII-B the mortality of 100% (in control mice), was reduced marginally to 91.6% in the daily dose-schedule. Further

reduction in mortality (75%) was seen in the group of mice receiving the same total amount of vaccine in 3 divided doses given on alternate days. It therefore appears that interrupted doses of vaccine are clearly more protective against both IM and IC routes of challenge. The studies are being pursued further.

3. Immunologic studies in human volunteers :

If the rabies vaccine proves to be more protective when administered in an interrupted dose-schedule in experimental animals, the next logical step would be to extend this work to human subjects. Since challenge and protectivity cannot be carried out in man, the two other parameters of immunity namely cellular immunity (tested by leucocyte migration inhibition) and humoral immunity (tested by estimation of specific antibody) following vaccination are proposed to be undertaken. As a preliminary step, blood samples were collected from 17 normal human volunteers (age 23-48 years) for estimation of their baseline levels of CMI and antibody against rabies. None of them showed specific antibody against rabies. Their leucocyte migration inhibition ranged from 0-5.39% (mean 2.46%). Further trials of the response in human subjects following daily versus interrupted dose of ARV vaccine are being organised.

TOXICOLOGY

1. Sub-acute oral toxicity of Zolon DT in rats :

It was reported earlier (Annual Report 1984-85) that there was no effect on feed intake, body weight or haematological parameters in any of the treated groups. At 2750 ppm the plasma and whole blood cholinesterase were inhibited. Female rats belonging to 1,375 ppm also showed reduction in plasma and whole blood cholinesterase.

The histopathological examination of the various tissues were undertaken during the year under the report. Histopathological observations revealed no changes which could be attributed to the administration of Zolon DT and were comparable to control female rats.

2. Sub-acute dermal toxicity of Zolon DT in rabbits :

It was reported earlier (Annual Report 1984-85) that excitation, loss of body weight and diarrhoea were exhibited in all the treated groups. There was no effect on the haematological parameters in any of the groups of treated rabbits. Erythema and eschar formation were observed in animals due to repeated application of Zolon DT on the skin. The whole blood and plasma cholinesterase were inhibited in all the groups.

The histopathological examination of various tissues were undertaken during the year under report. Histopathological

observations revealed alopecia, scarring and oedema at the site of application in skin. Other tissues did not show any changes which could be attributed to the administration of Zolone DT and were comparable to control group.

3. Neurotoxicity of Zolone DT in chicken :

Zolone DT is an organophosphate chlorinated hydrocarbon combination pesticide. Its neurotoxicity was determined in chicken by oral route at doses of 44, 110 and 275 mg/kg body weight. The test substance was given orally for a period of 21 days or till ataxia developed. A separate group was kept as control which received distilled water in identical manner. Three birds were taken per dose.

Histopathological examination revealed no demyelination or degeneration in the tissues — brain, spinal cord and sciatic nerve. There was reduction in egg production above 44 mg/kg dose. The body weight and food intake were reduced at 110 and 275 mg/kg dose levels. The plasma and whole blood cholinesterase were inhibited at 275 mg/kg dose levels.

VIROLOGY

Activities of WHO/NICD viral hepatitis

Surveillance centre :

Several outbreaks of viral hepatitis were reported from different villages in the state of Maharashtra, of which four interesting ones were studied by our team of the WHO/NICD Regional Viral Hepatitis Surveillance Centre (Maharashtra and Goa). The investigations of the outbreaks at village Supne, District Karad, Village Bilashi, District Sangli, industrial town Khopoli, District Raigad and Mangalwedha, District Sholapur showed a predominance of NANB hepatitis. The sera samples from patients were extensively studied for the markers of hepatitis A and hepatitis B viruses. All these epidemics were caused by faecal contamination of the water supply, after a spell of unseasonal rain during the summer. As soon as the contaminated water supply was identified by the team and hyperchlorination of the water sources was undertaken, the incidence of hepatitis declined. Medical and paramedical personnel from the Mahatma Gandhi Memorial Hospital, Bombay, were tested for HBsAg and anti-HBs (ELISA), as a pre-requisite for vaccination against HBV infection. This became mandatory for the staff because of the death of a Surgical Registrar, who developed fulminant and fatal HBV infection.

A total of 172 samples were screened for HBsAg and anti-HBs by ELISA. Most of the susceptible staff members received hepatitis B vaccine. Their immunological response after completion of 3 doses was also studied.

Preliminary study to see the site of replication, (if any) of Rabies Virus in chicken :

The novel antiviral antibiotic, plasma factor (PF), was induced on administration of rabies virus (RV-CVS) into the subcutaneous areolar wing web tissue of adult White Leghorn chickens.

A preliminary study was performed to study the replication of RV-CVS at the site of inoculation, (Wing Web) or other tissues, such as the spleen, liver, Bursa of Fabricius, brain and blood of chickens. The various tissues including the local wing web, were harvested at 0, 3, 6, 12, 24, and 96 h post-RV-CVS inoculation.

Groups of mice were inoculated intracerebrally with each of the timed serum, or tissue suspensions, and observed for typical signs of paralysis and death by rabies. The virus was inoculated only from the local wing web tissue up to 6 hr.

Effect of repeated RV-CVS stimulus on induction of PF in chickens production of neutralizing antibody :

The effect of repeated RV-CVS inoculation on PF production in chicken was studied. It was found that seven repeated inoculations did not lead to the production of neutralizing antibody ; and the induction and production of PF was not adversely affected. The ability of chicken to induce neutralizing antibody on three consecutive one ml doses of PCEC vaccine is presently under investigation.

Kinetics of production and action of PF :

Plasma factor (PF) was tested for its potential to inhibit tumour production by BS-RSV, *in-vivo* and *in ovo* when administered prior to (prophylactically), concurrently with, or following (therapeutically) the RSV inoculum. PF was administered prophylactically at various time intervals (-120 h, -96 h, -72 h, -48 h and -24 h) prior to the BS-RSV inoculum. Similarly, PF was given therapeutically at different time intervals (+24 h, +48 h, +72 h, +96 h and +120 h) after the BS-RSV was inoculated. In every case, a single dose each, of PF and RSV, was used. Both *in vivo* and *in ovo*, the results indicated that PF showed statistically significant ($p < 0.01$ and $p < 0.001$) anti-RSV activity when administered from between 48 h prior till 48 h following the RSV inoculum, showing maximum anti-RSV activity on concurrent administration of RSV and PF.

Anti-rabic activity of plasma factor (PF) in mice :

Experiments were performed in continuation to previous experiments where PF and RV-CVS¹⁰⁻³ were simultaneously inoculated intracerebrally in mice. In this case however the RV-CVS, was further diluted to 10^{-4} and then inoculated intracerebrally

in mice. It was seen that the overall mortality between day 0 to day 21 was 15.2% in experimental as compared to 81.7% in control. Thus a highly significant difference ($p < 0.001$) between both the groups is established.

Next, two fold dilutions of PF ranging from 1:2-1:64 was done. Simultaneous inoculation of each dilution of PF and RV-CVS (10^{-4}) was given via the intracerebral route in 12-14 g adult mice (0.03 ml/mouse). In the control group simultaneous injection 10^{-4} dilution of RV-CVS with undiluted, heat inactivated PF (60°C for $\frac{1}{2}$ h) was given in mice as above. A gradient response was seen in the overall mortality between day 0 to day 21 for all dilutions.

In group where PF was diluted 1:2 the mortality was 30%, that in 1:4-48%, 1:8-52%, 1:16-78%, 1:32-92%, finally in 1:64-99%. Thus the per cent mortality increased from 30% to 99% as the PF was two fold diluted from 1:2 to 1:64.

Action of Venoms on tumour production by Rous-Sarcoma Virus (RSV):

In order to test the possible action of venoms on the Rous sarcoma, series of experiments was conducted in 3-4 day White Leghorn chickens, using the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV).

The preliminary experiment was performed to test for any possible biological activity of the four commonly available venoms tumour. Venoms of *Echis carinatus*, *Naja naja* and black scorpion did not produce any effect on the RSV produced sarcoma. Russell's viper venom alone, showed blockade of the tumours, in 40 per cent of the chicks. This tumour blockade by venom, when compared with that of the control SR-RSV group, was found to be statistically significant. Since, in the above experiment, venom and virus inoculations were performed at the same time (ipsilateral) it was necessary to find out, whether tumour blockade might also be obtained by inoculating the venom into the opposite wing (contralateral). In the experiment an ipsilateral control was included. It was observed that tumour blockade in the ipsilateral experiment was significant ($p < 0.001$), whereas, in the contralateral experiment, it was not significant ($p > 0.05$).

In order to investigate temporal parameter, a timed experiment was designed. Venom was inoculated at varied time intervals 1, 4, 8, 12 and 24 h post-SR-RSV inoculation. In the first group, in which venom was inoculated 1 h post SR-RSV, 47.4 per cent of the chicks did not produce tumours. However, in all the other groups, no blockade of the tumours was observed. Contrary to our expectations there was great enhancement of the tumours in

100 per cent chicks, when venom was inoculated 4, 8, 12 and 24 h post SR-RSV. It has been proposed that *Viperidae* venom contains "spreading factor" (hyaluronidase) in much larger amount than the *Elapidae* and *Crotalidae* venom. Keeping in mind this report, different concentrations (1:1, 1:20, 1:40, 1:80) of purified hyaluronidase (commercial) were tested in chicks for any spreading factor like activity against SR-RSV tumour. The commercial hyaluronidase failed to enhance the tumour.

Since, in the experiments with inactivated venom, where spreading factor has been destroyed, no tumour spread could be demonstrated, it is postulated that there exists some heat labile substance(s) other than hyaluronidase which is/are responsible for such unique tumour enhancing activity of SR-RSV.

ZOONOSES

Malaria :

Studies on *Plasmodium vivax* in vitro

Modification of RPMI 1640 complete medium :

The medium RPMI 1640 enriched with additional glucose and peptone produced continued multiplication of *P. vivax* in vitro. Although this medium proved useful for establishing long term cultures of *P. vivax* in vitro it did not prove useful for enhancing parasitaemia in culture. Therefore further modifications in the medium were attempted. These modifications consisted of, incorporation of coconut milk or chick embryo extract in RPMI 1640 complete medium (10%). It was observed that the incorporation of coconut milk in the RPMI 1640 medium improved the parasitaemia significantly. With initial parasitaemia of 0.2% parasitaemia reached 3.98 per cent when coconut milk was incorporated in the medium at the end of 96 h. With chick embryo extract, although there was improvement in parasite multiplication in vitro, this increase was not as significant as with the coconut milk.

Growth of *P. vivax* and *P. falciparum* in erythrocytes from patients suffering from genetic disorders :

Erythrocytes from patients suffering from Beta thalassemia, sickle cell anaemia and G₆PD deficiency were obtained and used for observing parasite multiplication in these erythrocytes. It was observed that none of the erythrocytes from cases of Beta-thalassemia, sickle cell anaemia and G₆PD deficiency supported *P. vivax* and *P. falciparum* growth. These findings confirm the observations of previous workers that individuals suffering from

β -thalassemia, sickle cell anaemia and G₆PD deficiency are resistant to malarial infection.

Screening of antimalarial agents :

The screening of synthetic compounds for antimalarial activity was carried out in *P. berghei*-mouse model. About 55 antimalarial compounds synthesized at the chemotherapy department were screened. Out of these compounds, 6 compounds were found to reveal antimalarial activity.

Hookworm infection :

Development of any potent broad spectrum anthelmintic requires appropriate helminth models in the laboratory animals. A new approach to screen stage specific anthelmintic was devised in *Ancylostoma ceylanicum* — hamster model system in order to screen the efficacy of various broad spectrum anthelmintics against various developing stages i.e. 3rd, 4th, 5th (pre adult) and adult stage. Hamsters infected with laboratory-adapted *A. ceylanicum* were dosed (single dose, per os treatment) with 4 reference anthelmintics i.e. mebendazole, albendazole, flubendazole and parbendazole. Their efficacy was measured in terms of percentage cure of infected animals as well as percentage worm reduction following treatment. Mebendazole and flubendazole were highly effective against the 4th, 5th and adult stages but not against the 3rd stage; whereas parbendazole was effective against all the stages. Albendazole showed promising results and was found effective against all the stages. The comparative results revealed that *A. ceylanicum* hamster model is sensitive and reliable for identifying and characterizing these broad spectrum anthelmintic agents, particularly against the developing stages.

Filariasis :

Use of ES antigens in the diagnosis of human filariasis :

Setaria cervi adult worm (2 females and one male) were maintained in RPMI 1640 incomplete medium and ES antigens were isolated from the media after weekly collections. These were then sonicated, centrifuged and the supernatant was then lyophilised and stored at 4°C in aliquots. Each aliquot having a protein content of 220 mg/ml estimated by Lowry's method (1951). This ES antigen was then used in the immunodiagnosis of human filariasis by CIEP, and FLISA.

None of the samples showed positive reactions by CIEP except the one, which was similarly detected by the somatic antigens earlier. ELISA tests showed the ES antigens to be highly reactive in the detection of filarial antibody from sera of non-microfilaraemic (clinical filariasis and endemic normal) (1:25000

to 1:10,2400) and microfilaremia (Mf +ve) cases (1:5400 to 1:25600) respectively.

Controls from non-endemic (10 cases), endemic (10 cases) and helminth infested sera samples showed negative reactions by the ELISA test with the ES antigens.

Detection of circulating antigens by CIEP and ELISA in human Filariasis :

To detect the circulating antigen from microfilarial positive and disease positive (Clinical) *Wuchereria bancrofti* infected patients, hyperimmune serum was raised in rabbits against *S. cervi* adult worms. All mf +ve patients (21 Nos.) showed a positive reaction and disease positive (10 Nos.) showed negative reaction by the CIEP test. While in ELISA test, the mf +ve showed higher titres from 1:25600 to 1:51200 whereas the clinical cases showed lower titres i.e. 1:200 to 1:12800, thus proving the fact there was no adult worm infestation in these patients. The mf +ve carriers showed high titres due to the presence of adult worm in the lymphatics of these patients. The presence of circulating antigens in mf positive cases thus revealed the presence of active worm infestation or worm load. The endemic normals (10 cases) and non-endemic normals (10 cases) did not show a positive reaction for circulating antigens. There was also no cross reaction between *S. cervi* and other helminthic infections viz. *Ancylostoma ceylanicum* and *Angiostrongylus cantonensis*. Thus it could be concluded that both CIEP and ELISA are useful in immunodiagnosis of filariasis.

Angiostrongyliasis :

Studies on angiostrongyliasis were mainly directed towards screening of anthelmintic agents. The drugs screened during the year were albendazole and perbendazole. These were screened in mice. Perbendazole was found to have good larvicidal activity against larval stages of the worm whereas albendazole was found effective against larval as well as preadult stages of *A. cantonensis*.

Schistosomiasis :

A resurvey of the focus of schistosomiasis situated in the village Gimvi was carried out to assess the schistosomiasis situation and work out a strategy for elimination of the focus. Of 352 persons examined by the urine filtration technique using Nucleopore filters, 2 were found infected. *Ferriisia tenuis* the presumed snail intermediate host, was found in all water bodies of major contact. Treatment of the infected persons is being undertaken and additional research on the snails intermediate host and potential mammalian reservoirs is being planned.

Snake research :

(A) Snakes :

1. Rearing of young ones of Russell's viper in captivity :

As stated in the earlier report the remaining two females of **R. viper** from group "A" were kept under observation. However, both the snakes died on 13.5.1985. This study shows that it could be possible to rear the young ones in laboratory. In the present investigation we could rear them upto the adult stage till they reached the age of 2 years 10 month and 26 days.

2. Standard method for venom collection :

Various methods of venom extraction like application manual pressure to the venom gland were used for extraction of venom from snakes maintained in laboratory and in an open farm. The venom was collected from Cobra, **R. viper** and snakes. The yield of venom with different methods was compared. It was observed that the vacuum method coupled with application of pressure on the venom glands is a better venom extraction method for Kraits. The method of pressing the venom glands however, more useful for extracting venom from Cobra and viper snakes.

(B) Scorpions :

Rearing of young black scorpion in captivity :

The scorpions were continued to be maintained in laboratory in the scorpion cage in the usual medium. T sheddings and three deaths were observed among the during the year. No courtship or mating in scorpions was The scorpions have attained the age of 3 years 7 months and days. They are still being maintained in the cage for

CHAPTER IV

GENERAL ACTIVITIES

Library :

The Haffkine Institute Library is one of the oldest library housing a collection of books on biomedical sciences and related fields. It is much sought after as a reference library, not only by its staff but also by scientists and students from all over India. (Appendix VI).

There are 7,980 books in the library. The number of books added during the year under report was 225 out of which 54 books were received as gift from different organisations. Total number of bound volumes in the library is 17,727 and about 227 journals were bound during the year. In all 133 foreign and Indian Journals are subscribed for the library (Appendix VI).

Reprographic facilities :

The library provides reprographic facilities to its staff and outside readers. The library received requisitions for 11,925 pages during the year under report.

Inter library loan :

The library borrowed 48 journals from various libraries for its staff and loaned 207 journals to other libraries in the city.

Visitors :

About 4,710 visitors visited the library for reference work.

Artist section :

A small section attached to the Clinical Pathology Department looks after the needs of the teaching and research staff, research scholars and students by preparing slides, charts, photographs for teaching, thesis, conferences and other activities.

APPENDIX I
BACTERIOLOGY

Tests	Number
(1) Bacteriological analysis of Chemicals, Disinfectants and misc. products	51
(2) Water samples	28
(3) Testing of water filters	14
(4) Lyophilization of cultures	198
(5) Blood samples	2
(6) U.V. water purifying unit	1
(7) Catoxymag samples	6
Total ..	300

APPENDIX II
BIOCHEMISTRY

Tests	Number
(1) Blood	290
(2) Urine	58
(3) Special tests	112
Total ..	460

APPENDIX III
CLINICAL PATHOLOGY

Tests	Number
A. Histopathology	677
B. Haematology	1685
C. Cytology for malignant cells	14
D. Clinical Pathology	
Urine for routine examination	199
Stool for routine examination	125
Semen examination	2
Immunological Pregnancy Tests	247
Biological (Rat test) Pregnancy Test	1
E. Bacteriology,	
Culture for Tubercle Bacilli	74
Animal inoculation for Tubercle Bacilli	17
Smear and Culture for C. diphtheriae	5
Cultures of Theatre swabs for Anaerobes	84
Urine culture	193
.	62

APPENDIX III (Continued)

Tests	Number
Sputum culture	46
Stool culture	19
Blood culture	13
Antibiotic Sensitivity Tests	220
Amniotic fluid culture	1
Plural fluid culture	1
Vaginal swab	1
Synovial fluid culture	2
Aspirated fluid culture	8
Ear swab culture	1
C.S.F. culture	1
Conjunctivitis swab	9
F. Serology	
Widal Tests	118
Weil Felix Tests	56
Brucellosis Tests	47
V.D.R.L. Tests	13
Paul Bunnell Tests	16
Total ..	3957

APPENDIX IIIA
CLINICAL PATHOLOGY

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

Bombay Area	From Maharashtra
Cama and Albless Hospital	Civil Hospital, Thana
St. George's Hospital	Civil Hospital, Alibag, Raigad
Jagjivanram Hospital	General Hospital, Sawantwadi
Central Railway Hospital	I.S.P. Hospital, Nasik
B.Y.L. Nair Charitable Hospital	Public Health Centre, Buldhana
K.E.M. Hospital	Civil Hospital, Akola
M.G.M. Hospital	District Hospital for Women, Akola
L.T.M.G. Hospital	Primary Health Centre, Jalgaon.
E.S.I.S. Hospitals	General Hospital, Osmanabad
K.B. Bhabha Hospitals	Civil Hospital, Dhulia
Kasturba Hospital for Infectious Diseases	Civil Hospital, Nasik.
J.J. Group of Hospitals,	
Police Hospital	
Fertilizer Corporation of India	
B.J. Wadia Hospital for Children,	
Greater Bombay Municipal Dispensaries	

APPENDIX IV
HUMAN PHARMACOLOGY AND BIOAVAILABILITY

Name of Company				Product
Pilgaonkar	..	..	..	Tetracycline with Dilapsin
Dhootapapeshwar	..	..	..	Amoxyllin Capsules
Jules	..	..	..	Doxycycline Capsules
Jules	..	..	..	Tetracycline
Pilgaonkar	..	..	..	M. Dopa Tablets
Cross	..	..	..	Ampicillin Tablets
Jules	..	..	..	Rifampicin Capsules
O.L. Pharmaceuticals	..	..	..	Ampicillin Capsules
				Cephalexin Capsules
				S.T.M.P. Tablets
				Rifampicin Capsules
Laboratory	..	..	..	Ampicillin Capsules
				Tetracycline Capsules

APPENDIX V
IMMUNOHAEMATOLOGY

Tests	Number
Transfusion	1000
Rh-antibodies titre and Blood group	100
Total	1100

APPENDIX VI

LIBRARY

The following figures indicate the working of the Library during the year 1985-86

Number of outside readers visiting the library 1	Number of books and periodicals issued to our staff 2	Number of periodicals issued under inter-library loan service 3	Books and periodicals obtained for our staff from other libraries 4
4710	1837	207	48

Q

Number of books added during the year	Number of volumes bound	Number of current periodicals subscribed	Current periodicals received free of charge	Number of reports received from Scientific Institutions
225	227	133	53	4

APPENDIX VII
RABIES RESEARCH UNIT

Results of tests carried out on samples received for rabies diagnosis

Sr. No.	Ref. No.	Specimens		Test Results		Remarks
		Species	Nature	Negribody	Biol. Test	
1.	6046	Human	Brain	N.D.	N.D.	
2.	6047	Human	Brain	(6)	(+)	Found(-) by N.T.
3.	6048	Human	CSF	N.D.	(-)	
4.	6049	Bull Calf	Brain	N.D.	N.D.	
5.	6052	Human	Brain	(-)	(+)	
6.	6055	Human	Saliva	N.D.	(-)	

N.D.: Not done as specimens received in putrified and unsuitable condition.

N.T.: Neutralisation test in mice for confirmation of diagnosis.

APPENDIX VIIA
RABIES RESEARCH UNIT

Comparison of daily and interrupted dose-schedules of ARV Vaccine for protection in mice given subsequent challenge by I.M. Route

Vaccine (1%) Schedule (Days)	Challenge			Results			Remarks
	Route	Day	Dose (LD ₅₀)	M.R.	%M	AST	
0-1-2-3-4-	IM	7	17.38	2/11	18-18	13.09	
0-2-4	IM	7	17.38	0/11	0	14	
Nil (Control)	IM	7	17.38	11/13	84.6	8.53	

Vaccine: Total of 1 ml. was given per mouse in each schedule.

I.M.: Intramuscular (0.2 ml.)

M.R.: Mortality Ratio. AST:— Average Survival Time in days per mouse.

APPENDIX VIIB
RABIES RESEARCH UNIT

Comparison of daily and interrupted dose-schedules of ARV Vaccine for protection in mice given subsequent challenge by I.C. route.

Vaccine (1%) Schedule (Days)	Challenge			Results			Remarks
	Route	Day	Dose (LD ₅₀)	M.R.	%M	AST	
0-1-2-3-4	I.C.	7	31.62	11/12	91.6	8.2	
0-2-4	I.C.	7	31.62	9/12	75	8.8	
0-4	I.C.	7	31.62	11/12	100	5.5	
Nil (Control)	I.C.	7	31.62	6/6	100	5.0	

IC: Intracerebral (0.03 ml.)

Vaccine: Total of 1 ml. was given per mouse in each schedule.

M.R.: Mortality Ratio. AST: Average Survival Time in days per mouse.

APPENDIX VIII TESTING

Tests					Number
& Excise samples :					
(1)	Indian Made Foreign Liquors	..	..	..	1948
(2)	Beer	..	..	..	912
(3)	Toddy	..	..	..	1108
(4)	Ganja	..	..	..	35
(5)	Bhang	..	..	..	6
(6)	Charas	..	..	..	3
Total					4012
Commercial Testing :					
(1)	Pyrogen	..	..	..	53
(2)	Adrenaline (Bio-assay)	..	..	..	4
(3)	Sterility	..	..	..	7
(4)	Other than physical tests	..	..	..	50
(5)	Physical tests	..	..	..	38
(6)	Assays (including antibiotics)	..	..	..	16
(7)	Rideal walker coefficient and staphylococcal coefficient	..	..	..	65
(8)	Insulin (Bio-assay)	..	..	..	3
(9)	Infra red/ultraviolet/polymorph	..	..	..	20
(10)	Other tests	..	..	..	3
Total					259

APPENDIX IX TOXICOLOGY

Classification of sample		No. Tested	Toxicity Parameter
1.	Pesticide, weedicide, insect repellent, plant growth promoter	3	Acute oral toxicity studies
		6	Acute dermal toxicity studies
		3	Primary skin irritation
		7	Mucous membrane irritation
		2	Acute toxicity in pigeons
		3	Acute toxicity in fish
		1	Sub-acute oral toxicity study in rats
		1	Sub-acute dermal toxicity study in rabbits
2.	Drugs	1	Neuro-toxicity in chicken
		1	Acute intra-muscular toxicity study
3.	Cosmetics	9	Primary skin irritation study
		1	Mucous membrane irritation.
Chemicals		2	Acute oral toxicity
		2	Acute toxicity in fish
		2	Mucous membrane irritation
Plastics		1	Biological tests on plastics as per U.S.P.

TOXICOLOGY

No.	Sample	Species	Route	LD 50 mg/kg.	Fiducial limits mg/kg	Remarks
Acute Toxicity Studies :						
1.	Active ingredient of Vipul	mice	oral	> 5,000		
2.	Active ingredient of Vipul	rats	oral	5,000		
3.	Hico Emulsion 110 R.	mice	oral	> 15,000		
4.	Goal 23.5% EC	mice	oral	3,360	3126 — 3612	
5.	Chempulp	mice	oral	> 10,000		
6.	Sodium Ampicillin	mice	i.m.	13,000	10740 — 15730	
7.	Methyl Parathion, 50 EC	Rabbits	dermal	610.7	321.37 — 1160.29	
8.	Chlordane 20% EC	Rabbits	dermal	1463.7	1030.83 — 2078.55	
9.	Endosulphan 35% EC	Rabbits	dermal	324	202 — 518	
10.	DDVP 76% EC	Rabbits	dermal	324.8	251.8 — 419.0	
1.	Active ingredient of Vipul	Rabbits	dermal	> 10,000		
2.	Goal, 0.35% granular	pigeons	dermal	> 10,000		
13.	Dimethoate 30% EC	pigeons	oral			M.L.D. : 114 mg/kg
15.	Celmone (NAA) 4.5%	fish	oral			M.L.D. : 15270 mg/kg
16.	Celmone (NAA) 4.5%	fish	—			L.C. 50 : 480 ppm
7.	Active ingredient of Vipul	fish	—			L.C. 50 : 2000 ppm
8.	Acilol TA 1618 WER	fish	—			L.C. 50 : 890 ppm
9.	Acilol TA 1618	fish	—			L.C. 50 : 2000 ppm
	Emulsifier of Vipul	fish	—			L.C. 50 : 8.3 ppm
Skin Irritation studies :						
1.	Ethion 50% EC	Rabbits				Mild irritation
2.	Active ingredient of Vipul	Rabbits				No irritation
3.	Karnataka Soap 8401	Rabbits				Mild irritation
4.	Karnataka Soap 8402	Rabbits				Mild irritation
5.	Karnataka Soap 85-A	Rabbits				Mild irritation
6.	Karnataka Soap 85-B	Rabbits				Mild irritation
7.	Karnataka Soap 85-C	Rabbits				Mild irritation
8.	Karnataka Soap 85-D	Rabbits				Mild irritation
9.	Karnataka Soap 85-E	Rabbits				Mild irritation
1.	Karnataka Soap 85-F	Rabbits				Mild irritation
	Blossom	Rabbits				Moderate irritation
	Goal 0.35%	Rabbits				No irritation

APPENDIX IXA (Contd.)

Sr. No.	Sample	Species	Route	LD 50 mg/kg.	Fiducial limits mg/kg	Remarks
Mucous membrane (vaginal) irritation :						
1.	Chlordane 20% EC	Rabbits				Mild irritation
2.	Dimethoate 30% EC	Rabbits				Mild irritation
3.	Ethion 50% EC	Rabbits				Mild irritation
4.	Methyl Parathion 50 EC	Rabbits				Mild irritation
5.	Lustrol IB 14/84	Rabbits				No irritation
6.	Goal 0.35% granular	Rabbits				No irritation
7.	Goal recipe	Rabbits				No irritation
8.	Pynamin Forte	Rabbits				No irritation
9.	Blossom	Rabbits				Mild irritation
10.	Lustrol G.P. Cleanser	Rabbits				No irritation
Sub-acute oral toxicity in rats :						
1.	Zolone DT	Rats				
Sub-acute dermal toxicity in rabbits :						
1.	Zolone DT by dermal route	Rabbits				
Neuro-toxicity in chicken :						
1.	Zolone DT by oral route	Chicken				
Toxicity of plastic container :						
1.	Plastic	Mice and rabbits				Complies as per U.S.P. biological tests for plastic container

APPENDIX X
VIROLOGY

administered					Number of patients
Antirabic	..	..	..	..	597
Cholera	..	..	..	..	11
Tetanus	..	..	..	..	15
Measles	..	..	..	..	4
Triple	..	..	..	..	111
Oral polio	..	..	..	..	161
Dual vaccine	..	..	..	..	8
				Total	907

APPENDIX XI
ZOO NOSES

Snakes	No. of Animals procured	Venom produced (g)	Venom supplied (g)
Cobra	151	75.07	103.40
Krait	20	5.41	7.10
R viper	62	5.19	35.50
Echis	2680	0.07	33.88
Total :-	2913	85.74	179.88
Scorpions :			
Red Scorpion	3208	0.84	8.66
Black Scorpion	390	0.22	0.55
Total :-	3598	1.06	9.21

APPENDIX XI A
ZOO NOSES

No. of visitors visiting the Snake Farm.					
(A) Students	..	..	..	..	3778
(B) Staff of Scientific Institute	..	..	..	..	179
(C) Distinguished visitors	..	..	..	..	64
(D) Foreigners	..	..	..	..	24
(E) Thursday visitors	..	..	..	..	2240
(F) Others	..	..	..	..	144
			Total	..	6429

There were 29 enquiries regarding identification of snakes from hospitals and individuals during the year.

APPENDIX XII
TRAINING
Post-graduate Course 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, Pharmacology and Microbiology.
D.M.L.T.	—	Diploma in Medical Laboratory Technology.
D.A.	—	Certificate Course in Drug analysis Short-term Training Course.

APPENDIX XIII
Trainees Admitted

M.Sc. by papers	..	..	..	..	8
Diploma in Medical Laboratory Technology (DMLT)	..	..	..	..	17
Course in Drug Analysis	..	..	..	..	13
Short Term Training Course	..	..	..	..	6

Research Fellowship and Scholarship

Jr. Research Fellowship (Platinum Jubilee)	..	..	..	1
Jr. Research Scholarship (Chinchwad Land)	..	..	..	1

APPENDIX XIII A

List of students who registered during the year 1985-86 for M.Sc., Ph.D.,
M.Pharm, M.Phil and M.D.

M.Sc.	Ph.D.
(1) Shri R.J. Rayanade	(1) Shri S.B. Bhirud (2) Shri T.G. Tare (3) Shri R.R. Hemkar (4) Mrs. Z. Zamani
M. Pharm	M. Phil
(1) Shri A.B. Shirgaonkar	Nil
M.D.	
Nil	

APPENDIX XIV
Degree Awards during the year 1985-86

Name of Candidate	Subject	Topic
(1) Smt. S.N. Hattikudur	Ph.D. Biochemistry	Physicochemical characterization and spectrum of cross-reactivity of a mycobacterial target antigen of cellular immunity.
(2) Kum. R.B. Hirwe	Ph.D. Biochemistry	Biochemical studies on experimental liver injury.
(3) Shri A.S. Bobade	Ph.D. Organic Chemistry	Synthesis of Heterocyclic compounds as possible antituberculosis agents.
(4) Shri V.R. Narvekar	M.Sc. Microbiology	Studies on bacterial and fungal contaminations in non-sterile biologicals injectables sterile areas and allied.
(5) Kum. S.P. Dandekar	M.Sc. Microbiology	Studies of two industrially important bacterial cultures and their phages.
(6) Dr. (Mrs.) Anita Gupta	M.D. M.D. Pathology	—
(7) Dr. (Mrs.) P.K. Samsi	M.D. Pathology	—

APPENDIX XV
Post-Graduate Teachers
The following Post-graduate teachers are recognized
by the University of Bombay.

Name	Qualifications
Dr. M.V.N. Shirodkar	M.B.B.S., A.B., Sc.D (Johns Hopkins), P.B.K. (U.S.A.)
Dr. R.S. Kamat	M.D.
Dr. K.H. Dave	M.D., D.P.B.
Dr. D.M. Renapurkar	M.Sc., Ph.D., F.M.A.S.
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.R. 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.) S.C. Desai	M.Sc., Ph.D.
Dr. D.P. Buduk	M.V.Sc., Ph.D.

APPENDIX XVI

Papers Presented at National and International Conferences

S.R. Chavan. *Pongamia pinnata* as a vector control agent. First Asian Congress of Pharmacology at New Delhi, 15-19th Jan., 1985.

Dr. H.J. Mansharamani. Late Dr. S.L. Manjrekar Memorial Lecture delivered at PUNE, organised by Indian Association of Microbiologists "Rabies Vaccines — Problems & Prospects".

Dr. H.J. Mansharamani. At the 9th Annual Congress of Indian Association of Medical Microbiologists at Varanasi. "Influence of dose-schedule manipulation of Rabies Vaccine on its immunogenicity".

APPENDIX XVII

Papers Published 1985-86

M. Kishore, A.J. Baxi & P. Ramakrishnan. Purification of a protein with hypoglycaemic effect from *Bordetella pertussis* cell extracts. *Indian J. Med. Res.* **83**, pp 139-147 (Feb. 1986).

A.S. Bobade, C.N. Desai and B.G. Khadse. Synthesis and study of 2,7-disubstituted benzothiazole (3,2d)-s-triazine-1,3-diones as a possible antituberculosis agent. *Ind. J. of Pharm. Sci.*, (3) (1985), 132-34.

V.M. Rangnekar, R.P. Bhamaria and B.G. Khadse. Synthesis of N-aryl/heteroaryl/substituted-methyl (p-substituted anilino) succinimides as Anti-tuberculosis agents. *Ind. J. of Chem.*, **25B** (March, 1986), 342-44.

Anil S. Bobade and B.G. Khadse. Synthesis and antitubercular properties of 2-(substituted acetamido and p-acetamido benzene, aminobenzene sulphamide)-6-substituted-benzothiazoles. *Bull. Haff. Instt.* (In Press), Dec., 30, (1985).

S. Kulkarni and R.S. Kamat. Cross-reactions of cell mediated immunity induced by atypical mycobacteria. *J. Med. Microbiology* **21**, 36, 1986.

S. Kulkarni, S. Hattikudur and R.S. Kamat. Cell mediated immunity cross-reactions of mycobacteria; polymorphism of target bacterial antigen. *Clin. Exp. Immunology* **63**, 111, 1986.

H. Tiwari and R.S. Kamat. Cross-reactions in cell mediated immunity to *Salmonella* causing enteric fever. *J. Med. Microbiol.* **21**, 233, 1986.

A. George, S. Rath and R.S. Kamat. Differential regulation of effector responses of cell mediated immunity in experimental Salmonellosis. *Clin. Exp., Immunol* **63**, 327, 1986.

M.K. Bhopale, L.S. Limaye, V.R. Pradhan, D.M. Renapurkar and K.D. Sharma. Studies on suspected clinical and experimental angiostrongyliasis: serological responses. *Journal of Hygiene, Epidemiology, Microbiology and Immunology*. **29**, 283-288 (1985).

Kamath, Vijayalaxmi R., Menon, Sushila; and Renapurkar, D.M. Effect of UV-irradiation, spleen cells and MLN cells on protective immunity against *A. cantonensis* infection in mice. *Z. Parasitenkd.* **72**, 221-226 (1986).

T.G. Tare. A study of snake venom yield by different methods of venom extraction. *Amphibia — Reptilia* **7**, 187-191 (1986).

D.M. Renapurkar and P.J. Deoras. Some observations on food habits of the Indian house rat, *Rattus rattus*. *Pestology*, **9**, 41-44, 1985.

D.M. Renapurkar and P.J. Deoras. Prevalence and distribution of commensal rats and rat fleas in Maharashtra State Part I: Rat and flea of Vidarbha region. *Pestology*; **10**, 17-21, 1986.

APPENDIX XVIII
Papers Sent for Publication

B.G. Khadse, S.R. Lokhande, R.P. Bhamaria and S.R. Prabhu. Synthesis Antitubercular studies of 4-(5-Nitro-2-furyl/2-Pyrazinyl/1-Adamantyl)-2-(substituted-Aryl/Alkyl amino) Thiazole. "Indian Journal of Chemistry" Section-B.

A. George, R. Nair, S. Rath, S. Ghosh and R.S. Kamat. Regulation of immune response in experimental *S. enteritidis* infection in mice. *J. Med. Microbiology*.

S.R. Chavan, Nirmala J. Kher and S.T. Nikam. Evaluation of mosquito larvicidal activity of petroleum ether extracts of indigenous plants *vitex niquundo*, *Bryonopsis laciniosa* and *Porana racemosa*. *Bull. Haff. Inst.*

A.J. Almeida, M.K. Bhopale, Vijayaiaxmi R. Kamath, Sushila Menon and D.M. Renapurkar. Immune responses in *Setaria cervi* implanted albino rats.

Vijayaiaxmi R. Kamath, Sushila Menon and D.M. Renapurkar. Effect of UV-irradiation, spleen cells and MLN cells on protective immunity against *A. cantonensis* infection in mice. *Parasitology Research*, **72**, 221-226 (1986).

B.D. Sathe and D.M. Renapurkar. Present status of schistosomiasis in village Gimvi of Maharashtra State, India. *Journal of Communicable Diseases*.

V.R. Kamath, R.P. Hemkar and D.M. Renapurkar. Laboratory evaluation of rodenticidal activity of cholecalciferol against Indian species of commensal rats. *Pestology*.

V.R. Pradhan, N.K. Sutar, M.V.N. Shirodkar and D.M. Renapurkar. growth of *P. vivax* in vitro. Effect of animal serum. *Indian Journal of Parasitology*.

Prevalence and distribution of commensal rats and rat fleas in Maharashtra State, Parts I, II, III, *Pestology*.

V.R. Pradhan, N.K. Sutar and D.M. Renapurkar. Factor affecting the Factor affecting the growth of *P. vivax* in vitro : effect of medium modifications *Experimental Parasitology*.

A.K. Patil and D.M. Renapurkar. Prevalence of halophilic vibrios in the deep seafish fauna of coastal areas. *Journal of General Microbiology*.

S. Bapna and D.M. Renapurkar. Laboratory evaluation of cyclopicidal activity of cypermethrin. *Current Science*.

S. Bapna and D.M. Renapurkar. Cyclops predation on first instar mosquito larvae. *Journal of Tropical Medicine and Hygiene*.

D.M. Renapurkar and P.J. Deoras. Some observation on food habits of *Rattus rattus* the house rat. *Pestology*.
