

Annual Report of the Haffkine Institute

1984 – 1985



HAFKINE INSTITUTE
PAREL, BOMBAY-400 012
INDIA

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Honourable Minister for Public Health and Family Welfare,
Government of Maharashtra,
Mantralaya, Bombay-400 032 upto 11-3-1985

Vice-President :

Shri B.A. SAWANT,

Honourable Minister of State for Medical Education & Drugs
Mantralaya, Bombay-400 032 upto 11-3-1985

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Director, Haffkine Institute, Parel, Bombay-400 012.

Members :

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Education and Drugs Department, Mantralaya,
Bombay-32.

Smt. SHANTA SHASTRY upto 10-1-1985

Shri K.B. SRINIVASAN from 11-1-1985.

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

Smt. CHANDRA IYENGAR upto 10-1-1985.

Shri N.W. KARNIK from 11-1-1985.

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

Dr. S.K. SENGUPTA

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

Shri S.D. HIRUD

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

Smt. SHANTA SHASTRY upto 2-8-1984

Dr. B. R. KATE from 3-8-1984

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

Prof. V. RAMALINGASWAMI

Vice-Chancellor, University of Bombay, Bombay-400 032.

Dr. M. S. GORE

Scientist Emeritus, 450, Ganeshkhind Road, Pune-7.

Dr. C. G. PANDIT

Dr. R. K. MENDA

Director of Health Services, Government of Maharashtra,
St. George's Hospital Compound, Bombay-1.

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Members :

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**STAFF OF THE HAFFKINE INSTITUTE
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V.R. DESHPANDE, MBBS., M.D.

Chief Administrative Officer :-

M.G. MEHENDALE, B.A.

Accounts Officer :

R.S. Joshi

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Junior Scientific Officer :

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

BIOCHEMISTRY .

Senior Scientific Officer Class I :

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INSTRUMENTATION CELL

Junior Scientific Officer :

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IMMUNOLOGY

Senior Scientific Officer Class II :

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CHEMOTHERAPY

Senior Scientific Officer Class I :

B.G. KHADSE, M.Sc., Ph.D.

Senior Scientific Officer Class II :

P.P. DALVI, M.Sc. Ph.D.

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N.T. JAOKAR, M.Sc.

CLINICAL PATHOLOGY

Assistant Director :

R.S. KAMAT, M.B.B.S., M.D.

Medical Officer :

Y. FERNANDES, MBBS., DPB, M.D.
CHHAYA H. PANDIT, MBBS., M.D.

IMMUNOHAEMATOLOGY**Senior Scientific Officer Class I :**

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TRAINING**Senior Scientific Officer Class I :**

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VIROLOGY**Assistant Director :**

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Medical Officer :

RANJANA A. DESHMUKH, MBBS, MD

Junior Scientific Officer :

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

ZOONOSIS**Assistant Director :**

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Senior Scientific Officer Class II :

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B.D. SATHE, M.Sc., Ph.D.
V.R. KAMATH, M.Sc., Ph.D.
N.K. SUTAR, M.Sc.

STATISTICAL SECTION**Statistician :**

A.S. AREKAR, M.Sc.

PHYTOCHEMISTRY**Senior Scientific Officer Class I :**

S.R. CHAVAN, M.Sc., Ph.D.

Junior Scientific Officer :

S.T. NIKAM, M.Sc., Ph.D.

HUMAN PHARMACOLOGY & BIO-AVAILABILITY**Senior Scientific Officer Class I :**

N.G. CHANDERKAR, MBBS, Ph.D., M.D.

RADIATION BIOLOGY**Senior Scientific Officer Class I :**

N.G. CHANDERKAR, MBBS, Ph.D., M.D.

RABIES UNIT**Senior Scientific Officer Class I :**

H.J. MANSHARAMANI, MBBS, Ph.D.

TESTING UNIT**Senior Scientific Officer Class I :**

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

Junior Scientific Officers :

H.K. DALAL, B.Sc.

S.S. JAIN, M.Pharm.

S.B. DHOLAM, M.Sc.

TOXICOLOGY**Assistant Director :**

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

Senior Scientific Officer Class II :

M.G. PHATAK, M.Sc.

CLINICAL RESEARCH DIVISION**Senior Scientific Officer Class II :**

A.P. KULKARNI, M.Sc.

CHAPTER

INTRODUCTION

The year 1984-85 continued to remain critical on account of financial stringencies. The problem of funds to run the Institute, as well as the labour problem, are yet to be solved. Efforts are still being continued by the Government of Maharashtra to solve the problems of the Haffkine Institute. Except for the salaries of the staff, practically no other funds were made available to run the Institute. The core funds, at least for maintenance of the Institute buildings, salaries of the staff and initial expenses for Research, Training and Testing activities have been requested again by the Institute authorities from the Government. In spite of the severe financial crisis, research activities continued because of grants-in-aid from research promoting organisations. The following are the highlights of the various activities during the year of report.

Research :

The Bacteriology department pursued their experiments on genetic recombination between enteric species in order to obtain new strains of academic and/or applied potential.

The department of Biochemistry after successfully completing purification of the blood sugar lowering (hypoglycaemic) factor from **Bordetella pertussis** cell extracts, continued studies on the mechanism of the hypoglycaemic effect.

The department of Chemotherapy, in their endeavour for screening different organic compounds with antitubercular, antimalarial, antifungal and antibacterial activities, have synthesised several organic compounds with promising **in vitro** activities. Of these, five are ready for **in vivo** screening. Antimalarial screening with triazine derivatives is in progress. 2-substituted aryl-4-(biphenyl) thiazole derivatives were synthesised and tested for antitubercular activity.

The departments of Clinical Pathology and Immunology continued their investigations on immune regulation in

Salmonellosis. The data obtained so far indicate that the outcome of immunisation by different routes is not the same. In their study of delayed hypersensitivity (DTH) eliciting antigen/s of atypical mycobacteria and *Salmonella*, presently efforts are being centered around purification of the antigen/s. Immunological studies in acute hepatitis B, revealed that humoral and cellular responses to hepatitis B, surface antigen (HBsAg) were found not involved in inducing liver cell necrosis in the early phase of clinical disease.

The phytochemistry unit was involved in survey and collection of plants from different parts of Maharashtra State and their investigation for mosquito larvicidal insecticidal activity. Extracts of *Annona squamosa* and *Pongamia pinnata* seeds showed encouraging activities.

In their immunological studies on rabies, the Rabies Unit carried out experiments to compare the protection offered by daily dose-schedules with that offered by an interrupted dose-schedule of the sample type vaccine in mice. The results indicate that the interrupted dose gave better protection to mice.

The department of Toxicology undertook neurotoxicity study of Benomyl technical, carbamate, by the oral route in chickens. No demyelination or degeneration was observed with 100, 250, 500 and 1000 mg/kg doses of the pesticide. However, egg production and body weights were reduced at the 500 and 1000 mg/kg dose levels.

The Radiation biology unit carried out bioavailability studies with irradiated sodium ampicillin injection in rabbits. At 20 mg/kg body weight, blood levels were obtained though they were lower than those with other conventional preparations.

The department of Virology established the use of the Indian grey langur as an original non-human primate model for various studies with an anti-leprosy vaccine developed by The Cancer Research Institute Bombay which could be used for testing the antigenicity of different batches of vaccine or to determine the differential antigenicity of the

various antigens contained in the ICRC bacillus. Plasma factor, an antiviral antibiotic believed to be distinct from the interferons, was developed further in the department of Virology. It has shown antirabic activity in a heterologous host, the mouse, protecting mice injected simultaneously with rabies virus. Its antiviral spectrum was studied against RNA and DNA viruses. It was demonstrated that PF was potentially active against the RNA viruses. Successful attempts were made for economically viable preparation of large-scale plasma factor from adult fowl, without reducing its potency.

The Department of Zoonoses carried out a trial in the golden hamster, with UV-irradiated infective larvae vaccine of *Ancylostoma ceylanicum*. A single oral vaccination of hamsters with 100 infective larvae irradiated by means of a UV-tube (390 nm), at different time intervals, induced the development of resistance. A high level of protection was achieved at 15 min UV-exposure, yielding, 99.0% and 95.0% worm reduction against challenge doses with 100 and 1000 normal larvae respectively. The drug mebendazole (20 mg/kg) and UV-irradiated larvae combination against challenge infection with *A. ceylanicum*, in hamsters, was found to be highly effective. Serodiagnosis of human filariasis was successfully performed using *S. cervi* (bovine filarid) antigen for the detection of serum antibodies. A study of the comparative effect of anthelmintics mebendazole, cambendazole, thiabendazole, oxbendazole and levamisole on *A. cantonensis*, in mice, was undertaken using single and 5 dose treatment schedules at the 15th day post-infection stage, when maximal number of preadult worms were present in the CNS of mice. Of the drugs tested, mebendazole was found to be superior to all others. Further studies showed that a combination therapy of levamisole (25 mg/kg) and Flubendazole (15 mg/kg) produced 100% efficacy.

Testing :

Various types of samples, such as drugs, chemicals, cosmetics, water samples, water filters and samples submitted by the Prohibition and Excise department were tested at the Institute during the reporting year, in all, 3473 samples being tested.

Patient oriented services :

Samples from patients for investigations are accepted in addition to those from the department of Virology, by the departments of Clinical Pathology and Biochemistry. In all, 4260 and 396 samples were tested in the two latter departments respectively. Their services were utilised by various hospitals, charitable and private organisations in Bombay City and suburbs and other places (Appendix-III).

An Immunisation Clinic is run by the department of Virology of the Institute, where patients of various age groups are immunised against Cholera, Typhoid, Tetanus, diphtheria, whooping cough, rabies and poliomyelitis MMR and hepatitis B, at nominal cost. In all, 4307 individuals were immunised during the year under report (Appendix-X).

The Immunohaematology department carried out 1985 blood transfusions, 162 Rh-antibody titre and 17 grouping tests (Appendix-V).

The Radiation Biology Unit undertook thyroid uptake studies for 30 referred cases.

Training Programme :

The Institute offered training programmes for paramedical and medical personnel (Appendix-XII). A list of students registered during the year under report for M.Sc., M.Pharm, M.D. and Ph.D. degrees and those who successfully completed their thesis and obtained their degrees is given in Appendix XIII A and Appendix XIV. Appendix XV presents a list of recognised university teachers at the Institute.

The Institute enrolls five students in each of the subjects of microbiology and biochemistry for the M.Sc. degree courses by papers. Another important training programme of the Institute is that it prepares para-medical personnel, particularly in the field of medical laboratory technology. A one year course in Drug Analysis is also offered by the Institute. A one year diploma course (DMLT), of the University of Bombay, is offered. Twenty students were trained for the DMLT and 16 in Drug Analysis during 1984-85.

Four students received short term training during the same period.

ponsoring Research Bodies :

Some of the research projects are financed by the Department of Science and Technology, Government of India, the I.C.M.R., Lady Tata Memorial Trust, and the Central Council of Research in Ayurveda and Siddha system. The Institute is grateful to the above Departments and gratefully acknowledges their financial support.

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 the Teachers' Council met several times to provide guidelines for the smooth functioning of the Institute.

CHAPTER II

GENERAL DEPARTMENTAL ACTIVITIES**Bacteriology :**

Students were registered for the M.Sc. (Part I and Part II) in microbiology. Five students were registered for the M.Sc. (Part II) in microbiology, by papers, out of which four students secured first class, and one second. Five students were registered for M.Sc. Part I, three of which secured first class and one a higher second. Training programmes were also conducted for the D.M.L.T., Drug Analysis students and short-term trainees.

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

Biochemistry :

The department conducted training courses for M.Sc. biochemistry (Papers) Part I, Part II and D.M.L.T. students of Bombay University, and short term courses in biochemistry as before. Regular lectures in biochemistry for the M.Sc. students of Bombay University were held at the Haffkine Institute, during the first half of the year.

In its testing activities, biochemical analyses of clinical samples were carried out. The samples were referred by various City hospitals, private practitioners and medical officers of the Institute. The total number of samples tested is shown in Appendix II.

hemotherapy :

Three students are pursuing their Ph.D. degrees in medicinal chemistry and three working for their M. Pharm degree in organic pharmaceutical chemistry. Theory and practical classes were conducted for Drug Analysis students. One student was awarded the Ph.D. degree of the University of Bombay, in the field of medicinal chemistry under the guidance of Dr. B.G. Khadse.

In their microanalytical section, nearly four hundred newly synthesised compounds were analysed for carbon, hydrogen and nitrogen. This facility was also provided to the students from outside on a payment basis.

Clinical Pathology, Immunology :

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

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

Human Pharmacology, Bioavailability and Radiation Biology Units :

The Human pharmacology and bioavailability unit undertook blood absorption studies of different samples of pharmaceutical formulations in human volunteers. The list of pharmaceutical samples accepted for the studies is provided in Appendix IV. The radiation biology unit continued the thyroid uptake studies for benefit of referred cases. A total of thirty cases was referred.

Immunohaematology :

The unit of immunohaematology continued its activity to provide transfusion to children suffering from thalassaemia major. There are 52 patients registered under the scheme. The department also carried out Rh antibody titre and blood grouping tests. It undertook teaching and training of D.M.L.T. and M.Sc. students in blood banking. Medical officers and technicians from different district hospitals are also trained in blood banking.

Rabies Research Unit :

The rabies research unit continued to provide diagnostic service facilities for rabies on postmortem brain specimens.

and CSF. Advisory services to general public and medical profession were given on problems regarding dog bites and immunisation. The unit participated in the training programmes for M.Sc. (Microbiology) and M.D. (Microbiology) of Bombay University. Both lectures and practical classes were held. One M.Sc. (Microbiology) student is presently registered with the Head of the Unit.

Toxicology :

The toxicology department undertook toxicological evaluation of different types of chemicals, growth promoters and pesticides. These samples were referred by various industries. The different types of products tested are shown in Appendix IX. The department was also involved in training and teaching programmes of Drug Analysis and postgraduate courses.

Virology :

Amongst the routine activities of the department of Virology the most important is the Immunisation Clinic. In all, 4307 patients attended the Clinic during the period under report. The vaccines administered included antirabic, triple, oral polio, typhoid, cholera, tetanus and measles. The vaccines administered and the number of patients vaccinated are shown in Appendix X. The Clinical Virology section initiated the following diagnostic tests; detection of CMV in urine samples and detection of hepatitis B and hepatitis A markers in the clinical material received from local hospitals.

As usual, the department conducted the M.Sc. and M.D. (Microbiology) and M. Phil. lecture and practical annual course for the students from Haffkine Institute and the University of Bombay. In all, eighteen lectures and 7 practicals were conducted. Two Ph.D. (Microbiology) and 2 Ph.D. (Applied Biology) students are registered with the Head of the Department. One M.Sc. (Microbiology) student is also registered with the Departmental Head.

Zoonoses :

The Department of the Zoonoses was actively engaged in screening synthetic agents and plant extracts for their

antifilarial, antihelminthic, antimalarial and insecticidal properties. A number of commercial insecticide and rodenticide formulations were investigated for their efficacy. Colonies of insect vectors of public health significance and strains of helminth parasites were maintained for immunological, parasitological and chemotherapeutic research. Various species of poisonous and nonpoisonous snakes and scorpions continued to be maintained. Snake and scorpion venoms were regularly collected.

Appendix XI provides information regarding the total quantity of venom extracted from snakes and scorpions of different species.

Out of 606377 rats collected and brought to Haffkine Institute by the Municipal Corporation of Greater Bombay, 82773 rats were examined for plague by the smear technique. None of the smears was positive for plague.

The Department actively participated in various training programmes undertaken by the Institute. A total of 6028 visitors visited the Snake Farm during the year under report (Appendix XIA). Twenty three snakes were received for identification during the year.

Academic Activities, Honours and Awards :

The Government of Maharashtra recognised the Tuberculosis Research Laboratory of the Department of Chemotherapy as a "Tuberculosis Research Centre" for the Government of Maharashtra. It will provide testing facilities for detection of resistance to drugs, in TB patients. Dr. B.G. Khadse, Head of the department of Chemotherapy was invited to deliver a lecture in the field of anthelmintics by the Organon Research Centre, Calcutta, on 20th August, 1984. Dr. Khadse is a member of the Denaturant Committee constituted by the Government of Maharashtra. He was appointed on the Post-doctoral Committee in Cancer Chemotherapy to examine the research of Ph.D. students by the Director, Cancer Research Institute, Parel, Bombay. He has also become a recognised guide for the Ph.D. degree in organic chemistry of the S.N.D.T. University, Bombay. Dr. H.J. Mansharamani, Officer-in-charge, Rabies Research Unit, was invited to

participate in a seminar on rabies organised by the Tamil Nadu Agricultural University, at the Madras Veterinary College, Madras. He presented a paper on "Enhanced Efficacy of Rabies Vaccine by optimisation of Dose Schedules".

Dr. V.R. Rao, Head of the Department of Toxicology as nominated by the Pesticide Registration Committee, Govt. of India, as a member of the Panel for finalising the Guidelines or Toxicological Data Requirements of Household Pesticides. He was, also, nominated a member of the Chemical/Biological Expert Panel of the Steering Committee on Accreditation of Testing Laboratories, DST, Government of India. Dr. Rao was invited to deliver a talk on "Experimental Evaluation of Pesticides in Laboratory Animals" at the W.H.O. sponsored training Workshop on 'Pesticide Management' organised by the National Institute of Occupational Health, Ahmedabad, in September, 1984. He was a Ph.D. examiner at the Indian Institute of Science, Bangalore, in August, 1984.

Dr. M.V.N. Shirodkar, Head of the Department of virology, attended, as a member of the National Committee, the Indian National Science Academy — International Union of Microbiological Society meeting, held between the 18th and 20th of April, 1984. From May 2nd-5th, 1984, he was a member of the Expert Committee at the WHO/NICD Workshop for the preparation of a **Manual on Viral Hepatitis**. He also attended these meetings in the capacity of the Programme Officer WHO/NICD Regional Centre for Viral Hepatitis Surveillance (Maharashtra and Goa). On June 21st, 1984, the Head of the Department participated in a nationally televised Doordarshan programme on Viral Hepatitis in the serial '**Vigyan Alok**'. He was a Guest Speaker at the **Symposium on Recent Advances in Viral Hepatitis**, under the auspices of the Association of Physicians of India, held on September, 23, 1984. As a delegate from Haffkine Institute, he attended the **Symposium on Trends in Cancer Chemotherapy** sponsored by M/s. Hoechst India (Ltd.) on November, 17th, 1984. He was a special invitee at the V.N. Shirodkar Memorial Oration by Prof. Turnbull, from U.K., held at the Jaslok Hospital, Bombay, on December 9th, 1984. He gave an introductory invited lecture **An Overview of**

Viral Hepatitis at Haffkine Institute, which was organised by the Association of Microbiologists of India (Bombay unit) and sponsored by M/s. MERIND on February 12th, 1985.

On September 6th, 1984, he gave the Welcome Address at the Felicitation Function for Dr. P.J. Deoras, on the occasion of his 75th birthday, on behalf of the Association for Scientists of the Haffkine Institute.

On October 5th, 1984, he attended the meeting of the General Body of the Haffkine Institute, at the Mantralaya, on behalf of the Director, H.I.T.R.T.

CHAPTER III

RESEARCH PROGRESS

BACTERIOLOGY

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

Attempts were made again to transform sphaeroplasts of *E. coli* C 600 and *V. cholerae* with highly purified pBr 322 plasmid in presence of PEG 6000. However, no transfectants could be obtained, by this procedure, due to difficulties in revival of sphaeroplasts.

A *Shigella* plasmid, coding for tetracycline and kanamycin resistance, could not be transferred to *E. coli* AB 1157 a to *V. cholerae* by conjugation, due to the large size of the *Shigella* plasmid.

This confirmed that "cloning" is the only possible mode of bringing about desired recombinations between these species. It was decided, therefore, to concentrate on standardization of reagents and vector preparation for cloning experiments.

Antibacterial activity of plant extracts :

Cold extraction of three plant extracts with petroleum ether, chloroform and methanol revealed that methanol extracts possess maximal activity.

Extracts were further purified by column chromatography, but did not show significant antibacterial activity. The nature of the active compound was confirmed to be steroid in nature by the Lieberman Burchard Reaction.

BIOCHEMISTRY

Biochemical studies on *Bordetella pertussis* cells :

It was reported (1983-84 Annual Report) that a protein component, both electrophoretically and immunologically pure, showing significant hypoglycemic effect in experimental

animals, was isolated from *B. pertussis* cell extracts. This is the first time purification of this protein factor from cell extracts of *B. pertussis*, standard vaccine strain 255525, has been achieved. The determined molecular weight was $70,000 \pm 2000$ daltons. It was, also, found to cause lowering of blood sugar in alloxan diabetic mice (alloxan dose 250 mg/kg body weight). This fraction was found to exhibit significantly increased glucose tolerance in mice. The maximal blood glucose levels were attained within 1/2 h after glucose load and normal levels were at the end of 3 h.

After completing phase I of the problem, as outlined above, studies on the mechanism of the hypoglycemic effect is under way.

2. Biochemical studies on the toxic effects of organophosphorous insecticides on experimental animals :

It is known that organophosphorus compounds, which are widely used as insecticides, can cause delayed neurotoxicity in experimental animals and man, characterised by degenerative changes within the central nervous system (CNS). In this context, pathological free radical mechanisms, leading to lipid peroxidation and degeneration of phospholipids, with loss of membrane integrity, are currently considered as important factors responsible. With this in view, the effect of malathion-T, an organophosphorus insecticide, was studied and different parameters such as lipid levels, lipid peroxidation and lipase enzyme levels analysed, in both brain and liver tissue, in experimental animals. Malathion-T (0.5 ml/kg body weight) biweekly was administered i.p. to rats and both short term (15 day) and long term (30 day) studies were carried out. The animals became lethargic early during the studies (8-10 days), which intensified with passage of time. In the brain, all lipid levels — total lipids, triglycerides and cholesterol, decreased significantly, while, in the liver only the triglyceride level showed significant decrease. Lipid peroxidation and lipase enzyme levels also increased, in both brain and liver. These studies thus indicated that long-term malathion-T toxicity leads to a general de-

rangement of lipid metabolism and, also, enhancement of endogenous lipid peroxidation, in the brain tissue in rats.

CHEMOTHERAPY

Newly synthesised organic compounds were tested for biological properties against antimalarial, anthelmintic and antitubercular activities.

(a) **6-Alkoxy-2-substituted arylamino benzothiazoles :**

From this series, two compounds, namely 6-ethoxy 2-(3', 4' dimethoxy phenyl)-4-(3'-phenyl-5' methyl isoxazolyl) (p-ethoxy phenylamino) benzothiazole, were tested for their *in vitro* activity and are awaiting *in vivo* testing.

(b) **2-(substituted phenyl)-4-(3'-phenyl-5'-methyl isoxazolyl) thiazoles :**

In this series, also, a promising compound, namely 2-(3', 4' dimethoxy phenyl)-4-(3'-phenyl-5' methyl isoxazolyl) thiazole, is ready for *in vivo* screening.

(c) **1-(4-(heteroaryl)-N,N' 2,3-bismethoxy carbonyl) guanidines :**

In this series, too, two compounds warrant further *in vivo* studies.

Succinimide derivatives — antitubercular agents :

The work on this series is being continued since it has provided active compounds in the antitubercular, anticancer, antifungal and anthelmintic fields. In order to prepare the newer compounds the following intermediates were prepared.

- (1) p — chloro-benzyloxyamine
- (2) p — bromo-benzyloxyamine
- (3) 4' — amino-4'-nitro diphenylamine

Synthesis and antimalarial studies of symmetrical triazine derivatives :

Some of the intermediates required for the preparation of the above compounds were prepared from basic chemicals. s-triazine-4,6-diamino, 1,2-dihydro-2-methyl, 2-phenyl 1-(m-

diethyl amino ethyl) O-cresol) dihydrochloride and four allied compounds, from this series, were prepared and forwarded for antimalarial screening.

Synthesis and antitubercular studies of 2-substituted aryl-4-(biphenyl) thiazole derivatives :

Ten compounds, from this series, were prepared and characterised by structure determination studies. They were all tested against the H₃₇Rv. strain of *M. tuberculosis*. One compound, namely 2-(2-methoxy-4-propoxy)-4-biphenyl thiazole, showed activity similar to the standard drug INH. The oral toxicity studies of this compound revealed that it is not toxic at 1.5 g/kg, while the standard, dry, INH is toxic at 0.8 g/kg.

Schemes :

Work on the scheme entitled "Microbial Testing and Chemical Evaluation of Resin" sponsored by M/s. Ion Exchange India Ltd., was completed. A scheme for the process development of clofazimine, as an antileprotic drug, was submitted to the Haffkine Bio-Pharmaceutical Corporation Ltd., for financial assistance. A scheme entitled "Synthesis and *in vitro* Study of Antimalarial Agents" was approved by the Lady Tata Memorial Trust, for financial assistance and the work has commenced.

Activity of Tuberculosis Research Laboratory :

Primary screening of antitubercular activity by the submerged culture technique —

About one hundred compounds were tested for their antitubercular activity, by this method. Some of these showed activity ranging between 12.5 ug/ml to 0.04 ug/ml. This *in vitro* tuberculosis testing facility was, also, provided to research students from outside the Institute on a payment basis.

CLINICAL PATHOLOGY, IMMUNOLOGY

1. Study of immune regulation in Salmonellosis :

Earlier (Annual Report 1983-84), it had been shown that there was a differential decline in delayed hypersensitivity (DTH) and protective cell-mediated immunity, as a function

of time, following immunisation by the intraperitoneal (ip.) route. Employing, cyclophosphamide treatment of animals prior to immunisation, the decline of DTH, as a function of time following immunisation could be abrogated. Thus, coupled with the phenotypic markers of the splenic cells responsible for suppression reported earlier (1983-84 Report), these data conclusively indicate generation of suppressor cells having preferential action on the DTH response, following i.p. immunisation.

Employing the established technology of adoptive transfer of immune splenocytes into syngeneic hosts, followed by challenge (1982-83 and 1983-1984 Reports), the functional characterisation of immune spleen cells after intradermal (i.d.) immunisation with killed *S. enteritidis* vaccine, was carried out. On i.d. immunisation, only a DTH response was generated; no protective CMI was observed. Further, the spleen cells from i.d. immunised mice, on adoptive transfer, prevented generation of protective immunity, but not DTH, in syngeneic mice immunised with live *S. enteritidis* by the i.p. route.

Thus, so far, the data seem to indicate that the outcome of immunisation by different routes, is not the same. The differences in the effector responses observed appear to be due to different sets of regulatory cells generated. This may be due to different antigen presenting cells encountering the antigen. This facet is currently being investigated. This study is being supported by a Fellowship Grant from the ICMR.

2. Study of DTH eliciting antigen/s of a typical mycobacteria :

As a continuation of the project on study of mycobacterial antigens, DTH eliciting antigen of atypical mycobacteria is being purified employing the techniques standardised for purifying similar antigen from *M. tuberculosis* H₃₇Rv (1980-1984 Annual Report). The project was initiated in December, 1984.

3. Study of DTH eliciting antigen/s of *Salmonella typhi* :

In the light of the earlier reports that the antigens responsible for CMI are likely to be different from a somatic

and flagellar antigens (1983-84 Annual Report), a project on purification and characterisation of DTH eliciting antigen from *S. typhi* was recently initiated. This study is also being supported by a Fellowship Grant from the ICMR.

4. Immunological studies in acute hepatitis B :

Profiles of antigenic markers of hepatitis B viral (HBV) infection and humoral and cellular responses to them were investigated in acute hepatitis B. Adult male, non-pregnant female patients were investigated at various points of time following onset of clinical diseases till 7 weeks. As an index of liver cell necrosis, levels of serum alanine aminotransferase were, also, studied in each patient.

Humoral and cellular responses to hepatitis B surface antigen (HBsAg) were found to be not involved in inducing liver cell necrosis, in the early phase of clinical disease. Liver necrosis at this point in time was associated with anti-core anti-e-antibody and cellular responses. In the late phase of acute hepatitis, the liver cell necrosis was associated with T cell response to HBsAg.

PHYTOCHEMISTRY

This unit is involved in the survey, collection and investigation of Indian plants, from different parts of Maharashtra, for mosquito larvicidal/insecticidal activity, phytochemical investigation of active principle/phytoconstituents (both extract and pure) and toxicological studies on the active material.

Annona squamosa :

Annona squamosa seeds were investigated for mosquito larvicidal activity. The seeds were dried and powdered to uniform mesh. The powdered material was then extracted, with various solvents of increasing polarity. Of these petroleum ether extract was found to be a potent mosquito larvicidal agent. The same extract was put on column chromatography, using silica gel and elution with benzene, benzene : acetone (95 : 5), benzene : acetone (80 : 20), benzene : acetone (50 : 50) and acetone. All elutes were

rendered free of solvents on a water bath. Fractions I and II showed 100% mortality with 0.008% concentration and 0.016% concentration, respectively. The remainder of the fraction did not produce significant results.

Pongamia pinnata :

Pongamia pinnata seeds were investigated for cyclocidal activity. The results were extremely encouraging. At the 0.02% concentration, *Pongamia pinnata* oil showed 100% activity. *Pongamia pinnata* oil was placed on a column for chromatographic separation. The fractions collected were (I) benzene, (IA) benzene elute — dark yellow band, (II) benzene-acetone (95:5), (III) benzene-acetone band, (IV) benzene-acetone (80:20) and (V) acetone. Of these, fractions IA gave significant results. One hundred per cent mortality was achieved at the 0.001% concentration, while the 0.005% concentration gave 96% mortality.

RABIES RESEARCH UNIT

1. Developmental work on Sample-type rabies vaccine in tissue culture :

The work reported last year (1983-84 Annual Report) was further carried on. Cell monolayers of the VERO cell line of monkey origin was employed. Seed virus (Paris strain) was used as centrifuged as well as uncentrifuged material. In addition to DEAE — dextran, the cultures were also treated with trace amounts of trypsin solution (5 ug per ml of medium), to help increase virus adsorption. Virus in the fluid phase at various intervals was monitored by mouse inoculations, over a period of days. Very minimal quantities of virus were traceable.

2. Immunological studies on Rabies :

Further experiments were carried out to compare the protection offered by daily dose-schedules and interrupted dose schedules of vaccine in mice. The animals were challenged by the intramuscular route. The results, which are presented in Appendix VII indicate that the interrupted doses provide better protection to mice against a challenge dose of approximately 20 LD₅₀ (tested intramuscularly in

mice). The low titre of the intramuscularly adapted virus did not permit a stiffer challenge.

In another experiment, the vaccine dose per mouse was doubled to see if better protection occurred. However, there was no significant difference in protection between the two groups.

3. Miscellaneous studies :

Our work has already been reported on the serologic response of human subjects following different courses of AR vaccination. These tests were carried out employing the CIEP techniques and antigens were prepared by ultrafiltration. Efforts were made to test if suitable antigens could be prepared by the less cumbersome centrifugation method.

Stock virus preparations of the CVS strain were subjected to different cycles of centrifugation at 1000Xg, at 7500Xg and at 50,000Xg. Sediments and supernates were removed at various stages and tested by CIEP against standard rabies immune sera. Some antigens were also treated with protamine sulphate to sediment the virus. Precipitin lines were either not noted, or were faint, as compared to our earlier preparations.

RADIATION BIOLOGY UNIT

1. Bioavailability of irradiated sodium ampicillin injection :

A study to test blood levels of this antibiotic was carried out in six adult rabbits. The route of injection was intramuscular. The dose of ampicillin preparation was 20 mg/kg body weight. Periodic blood levels of the antibiotic revealed adequate peak levels. However, as compared to conventional preparations, the values were significantly lower. There was a low rate of absorption suggesting probable denaturation of the preparation.

2. Study on irradiated cobra venom :

Venom solutions of different concentrations were exposed to increasing doses of gamma radiations in the presence of ice, until the toxicity was totally lost. Aliquots of solutions kept aside before irradiation served as controls.

The toxicity of venom solutions, both before and after the irradiation, was determined by injecting 0.5 ml of solution by the intravenous route into albino mice (Kasauli strain) weighing between 18 to 20 g. Two mice were used per dilution and the minimal amount of venom required to kill both animals in 24 h was taken as the minimum lethal dose (MLD) for the mouse. For the particular batch of venom it worked out to 10 g (IV route).

Immunisation of guinea pigs with irradiated cobra venom is in progress.

TOXICOLOGY

1. Subacute oral toxicity studies in rats :

Zolone DT an organophosphate-chlorinated hydrocarbon combination insecticide was incorporated in rat feed at levels of 700, 1375 and 2750 ppm and fed to groups of rats, along with a control group for 90 days, to study the subacute toxicity (Annual Report 1983-84). A withdrawal group was also kept, for the dose 1375 ppm, for evaluating the effect of stopping the feeding of zolone DT after 90 days and keeping the group of rats under observation for further 30 days.

The results obtained so far have shown that there was no effect on feed intake, or body weight, or haematological parameters, in any of the groups of treated rats. At 700 and 1375 ppm levels, there were no gross signs or symptoms of toxicity. At the 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 at the 1375 ppm level, also showed reduction in plasma and whole blood cholinesterase. The decrease in plasma cholinesterase was not observed in the 1375 ppm reversal group female rats, but the whole blood cholinesterase still remained below the initial level. Histopathological examination of the various tissues is in progress.

Subacute dermal toxicity in rabbits :

Zolone DT, an organophosphate-chlorinated hydrocarbon

combination insecticide, was applied to the depilated backs of rabbits (3 males and 3 females per dose) in doses of 0.14 ml/kg (154 mg/kg), 0.28 ml/kg (308 mg/kg) and 0.56 ml/kg (616 mg/kg) for a period of 21 days, along with a control group, which received normal saline (Annual Report 1983-84). The period of application was followed by two weeks of observation.

Findings of the study included excitation, loss of body weight and diarrhoea in zolone DT-treated animals, after the end of the 3rd week (application period). Repeated application of zolone DT on the skin caused erythema in all the treated groups and, also, eschar formation in the group receiving the 616 mg/kg dose. There was no effect on food intake, in any of the groups of treated rabbits. There was no effect on the haematological parameters in any of the treated groups. The plasma and whole blood cholinesterase were inhibited in all the test groups, after three weeks of application. The decreased plasma cholinesterase level in all the test groups increased after the 5th week. However, the whole blood cholinesterase remained low, even after the observation period (5th week). Histopathological study of the various tissues of the postmortemed animals is in progress.

3. Neurotoxicity of benomyl technical in chickens :

Benomyl is a carbamate type of pesticide whose neurotoxicity was determined in chickens by the oral route, at doses of 100, 250, 500 and 1000 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 used 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 the 100 mg/kg dose. The body weight and food intake were reduced at the 500 and 1000 mg/kg dose levels.

VIROLOGY

Antirabic activity of plasma factor (PF) in mice :

An experiment was performed to test whether PF, an antiviral antibiotic distinct from IFN, possessed any activity against the RV-CVS strain of rabies virus. Serial ten-fold dilutions of RV-CVS were carried out, using sterile 2% ovine serum saline (BSS) as the diluent, to yield a final dilution of $10^{-3.0}$. An equivolumetric mixture of PF and RV-CVS $10^{-3.0}$ was simultaneously inoculated intracerebrally into 15 mice (12-14 g ; 0.03 ml per mouse). In another group of 5 mice, an equivolumetric mixture of inactivated PF (IPF) (60°C $1\frac{1}{2}$ h) and RV-CVS ($10^{-3.0}$) was simultaneously inoculated, intracerebrally. The observation period lasted for 21 days. Sixty per cent of the mice injected with PF + RV-CVS, were completely protected, as compared to only 13% which survived in the control group.

Antiviral spectrum of PF :

The efficacy of the antiviral action of PF against several DNA and RNA viruses had been previously studied in our Department. Two more viruses, CELO (Chick Embryo Lethal Orphan), a DNA virus and NDV (Newcastle disease), an RNA virus were presently studied. CELO, a fowl adenovirus, and NDV, a fowl paramyxovirus, were obtained from the Bombay Veterinary College (BVC). Both viruses were passaged intra-allantoically in 10-11 day embryonated White Leghorn eggs, to prepare virus stocks. Titration of virus stocks was performed to determine the LD_{50} titre of both viruses. For CELO, this was found to be between $10^{3.0}$ and $10^{4.0}$; whereas, for NDV, the LD_{50} was between $10^{3.0}$ and $10^{4.0}$; whereas, for NDV, the LD_{50} was between $10^{2.0}$ and $10^{3.0}$. The certain lethal doses (CLD) for CELO and NDV were between $10^{1.5}$ and $10^{2.5}$, and between $10^{1.0}$ and $10^{2.0}$, respectively.

An experiment was designed, wherein twenty 10.11 day embryonated hen's eggs were simultaneously inoculated intra-allantoically with a mixture containing 0.1 ml each of CELO ($10^{1.5}$ — $10^{2.5}$ CLD/0.1 ml) and PF. A virus control group of another twenty 10.11 day old embryonated eggs, was inoculated by the same route with a mixture containing

.1 ml each of CELO ($10^{1.5}$ — $10^{2.5}$ CLD/0.1 ml) and PBS (phosphate buffered saline). An identical experiment was performed, using NDV. For both viruses, the end point was indicated by mortality of the embryos 48 h post-inoculation.

In the CELO-PF experiment, it was observed that PF had no effect on the mortality produced by CELO in the eggs, as evidenced by 100% mortality in both experimental and control groups — an unequivocal demonstration that PF afforded no protection against the DNA virus, CELO. On the other hand in the NDV-PF experimental group, it was observed that PF afforded 75% protection against the RNA virus, NDV to eggs, whereas in the controls, no protection was observed, as indicated by 100% mortality of the embryos.

In the present study, therefore, PF has been demonstrated, as in the past, to be potently active against the RNA virus studied, but not the DNA. Thus, this extends further compelling support to work done by previous investigators, in this field, in the Department of Virology.

A new economical source of PF :

Twenty to 30 ml of PF was prepared in a 2 kg fowl. This was tested for sterility and for antiviral activity against Rous sarcoma virus in chicks. After ensuring its excellent potency, it was stored at -186°C in the vapours of liquid nitrogen. This newly developed model for large scale preparation of PF has not only greatly enhanced the rate of progress in experimental work on PF, but has proved to be very economical from the point of view of effort and finance. It is, also, a handy method of procuring this novel antiviral antibiotic in large quantities : It has thus proved to be a real breakthrough in the Department's basic research work.

The mode of action of PF and its possible site of production in the fowl, following inoculation of rabies virus, was investigated in a preliminary fashion. Following inoculation of the RV-CVS into the wing webs of adult fowl (1.5 to 2 kg), the areolar wing web tissue, was excised aseptically and stored in liquid nitrogen at 186°C ; similarly, other organs such as liver, spleen, Bursa of Fabricius and the thymus were also

excised and stored in liquid nitrogen for future studies. Such studies would include attempted isolation of live rabies virus from these tissues, as well as aimed at demonstration of the presence of such virus intracellularly, *in situ*, using immunofluorescence.

2. Activities of the WHO/NICD Regional Viral Hepatitis Surveillance Centre (Maharashtra and Goa):

Samples of sera were collected from the following groups of individuals :

1. Acute viral hepatitis cases (hospitalised), from Aurangabad Medical College Hospital.
2. Acute viral hepatitis cases from village Chapner, 60 km from Aurangabad City (OPD).
3. Acute viral hepatitis cases from Kasturba Infectious Diseases Hospital, Bombay (OPD).
4. Apparently healthy contacts of a family focus from village Umarale, District Thane, Maharashtra.
5. Apparently healthy prison inmates donating blood to the Haffkine Bio-Pharmaceutical Corporation Ltd. Blood Bank.

From the Aurangabad area, where an outbreak of viral hepatitis had occurred, a total of 119 serum samples, from cases of acute viral hepatitis were collected. All were negative for HBsAg by CIEP. By ELISA, however, 18.4% were HBsAg-positive.

A single family cluster of three HBsAg-positive cases, of which 2 members died within 3 days of onset of illness, occurred at village Umarale, in District Thane, Maharashtra, in August, 1984. This focus was investigated. Of 145 sera from the contacts and neighbourhood of this focus, only one was HBsAg-positive by Counter Immune Electro-phoresis. In the meanwhile, the prescribed anti-HBV prophylactic vaccination of the surviving family members and intimate contacts and general precautionary measures adopted, resulted in successful control of the focus, no subsequent outbreak of this nature having been reported, till the time of writing of this Report.

The prevalence of HBsAg in sera from "healthy" (prisoner) blood donors, from six prisons in Maharashtra, was studied by CIEP. Of the 312 sera tested, the overall incidence of HBsAg was 4.8%. The HBsAg carrier range was between 2.4 and 11%. The figures were higher for inmates from urban prisons, as compared to rural areas.

3. Studies with the ICRC anti-leprosy vaccine :

Leprosy is an insidious disease, with a long incubation period. Before a vaccine is tried for its preventive efficacy in man, it should be shown to be capable of inducing protective immunity. *M. leprae* is an intracellular parasite and resistance is generated chiefly through T cell-dependent, cell-mediated immunity (CMI).

A well-established parameter for CMI is the lepromin skin test. There is enough evidence, both in man and experimental animals, that the skin tests, particularly the 3-week Mitsuda reaction, correlate well with the capacity of the host to handle the leprosy bacilli.

Recently, leprosy has been induced in different species of monkeys. The monkey model is of special importance to man, since the distribution of lesions is similar to that observed in human leprosy. In fact, the animals which were lepromin-negative, developed lepromatous leprosy.

In the present study, in collaboration with the Cancer Research Institute, Bombay, Indian Langur monkey (*Presbytis entellus*) from our Department were utilized to test whether their skin reactivity (Mitsuda reaction) could be used for screening the Indian Cancer Research Centre (ICRC) bacillus antigens.

Eight monkeys were vaccinated with the ICRC anti leprosy vaccine. Four of the five lepromin-negative monkey showed lepromin conversion, 6 weeks after vaccination. Four lepromin-positive ones showed enhanced activity following vaccination.

Four monkeys, which had been maintained as controls showed no change in their lepromin status during the same

period, thus showing that the lepromin conversion was not a consequence of the administration of lepromin.

Thus, the Indian grey langur is a useful non-human primate model, which could be used for various studies with anti-leprosy vaccine. For instance, it might be utilized to test the antigenicity of different batches of vaccines produced; further, it might also be utilized to determine the differential antigenicity of the various antigens contained in the ICRC bacillus, or other mycobacteria.

ZOONOSES

1. Malaria :

studies on *Plasmodium vivax* :

Twelve fresh strains of *P. vivax* were established by continuous culture. Nine of these strains were obtained from ashis and three from the Kasturba Infectious Diseases Hospital, Bombay. Studies to assess suitability of various animal sera for the cultivation of *P. vivax in vitro* were continued. The sera used were horse, calf, foetal calf, pig, sheep and rabbit. Among the sera used, rabbit, foetal calf and calf produced levels of parasitaemia higher than that with human serum. The growth with other sera was lower, when compared to that with human serum.

Studies were initiated to assess the susceptibility of erythrocytes, from various blood group donors, to *P. vivax* infection *in vitro*. Apart from human erythrocytes, susceptibility of various animal erythrocytes to *P. vivax* is, also, being investigated.

studies on *Plasmodium falciparum* :

A strain of *Plasmodium falciparum* obtained from National Institute of Virology, Pune, is being maintained *in vitro*. Susceptibility of different erythrocytes to *P. falciparum* infection *in vitro* is being studied.

studies on *Plasmodium berghei* :

Continuous *in vitro* culture of *P. berghei* was successfully achieved by the Trager — Jensen technique with mouse erythrocytes and modified RPMI medium. The parasite strain

as continuously maintained for 45 weeks. The virulence and infectivity of the strain of *P. berghei* cultivated *in vitro* as studied after different periods of maintenance. Suitability of various animal erythrocytes and eukaryotic cells for cultivation of *P. berghei* *in vitro* was, also, investigated.

circulating antigens in *P. berghei* infected mice by ELISA and CIEP tests :

Samples of urine from mice infected with *P. berghei* were examined for the presence of circulating antigens, by ELISA and CIEP tests, at different stages of infection. This study showed presence of antigen in the urine from the 4th day post-infection. When parasitaemia reached 5% and above both tests were found sensitive.

2. Hookworm :

A vaccination trial in golden hamsters with UV-irradiated infective larvae of *Ancylostoma ceylanicum* was carried out. A single oral vaccination of hamsters with 100 infective larvae, irradiated by means of UV-tube (390 nm), at different time intervals, induced the development of resistance. As the time exposure of irradiation increased, there was a corresponding decrease in worm establishment. A high level of protection at 15 minutes UV-exposure was recorded yielding 99.0% to 95.0% worm reduction against the challenge doses of 100 and 1000 normal larvae, respectively. There was no marked difference in worm establishment in hamsters when vaccinated either orally or subcutaneously, subjected to oral challenge infection. In vaccinated hamsters, manifestations of resistance, at 15 minutes UV-exposure, were shown by marked reduction in worm establishment and highly reduced eggs per gramme of faeces (epg) in pellet along with significantly higher haemoglobin level in blood as compared to normal larval vaccinates and challenge controls.

Therapeutic effect, using the drug mebendazole (20 mg/kg), UV-irradiated larvae and their combination against the challenge infection of *A. ceylanicum*, in hamsters, was studied. The use of the combination of both was found highly effective. There was no *A. ceylanicum* worm recovery with sublethal

(100 larvae), and a negligible worm count (0.01%), even with a lethal challenge dose of 1000 larvae. In control animals, there was high worm load and mortality. Another approach was made with the gamma irradiation attenuated larvae of *Ancylostoma ceylanicum*. These were used for inducing immunity in golden hamsters. The hamsters were immunized with two doses of irradiated larvae (50 k rad), given 15 days apart, and were subsequently challenged with normal larvae in days 15, 30 and 60 after the second dose of vaccination. One hundred per cent protection was observed in hamsters which were challenged on day 15 post-vaccination. The degree of immunity decreased and subsequent challenges revealed a lower degree of protection, when normal larvae were administered after exposure to 50 k rad irradiated larvae, 97.71% protection was noted on subsequent challenges with normal larvae, on day 15 post-treatment.

3. Filariasis :

Serodiagnosis of human filariasis was successfully performed, using *S. cervi* (bovine filarid) antigen for the detection of serum antibodies. Various cases of filariasis were classified according to the nature of infection viz. asymptomatic (Mf +) cases (20), symptomatic (Mf +) cases (10), symptomatic (Mf -) cases (9). Besides these cases from endemic and non-endemic (mf -) area, (10) uninfected persons were also examined.

The immunodiagnostic tests carried out were CIEP, IFAT and ELISA. The IFAT and ELISA tests were positive, in titres ranging from 1 : 64 to 1 : 156 and 1 : 200 to 1 : 12 800, respectively, in all cases of microfilaraemia. Among acute cases, 10% showed positive titres by ELISA (1 : 1, 28, 800-1 : 12, 5600) and IFAT (1 : 128 — 1 : 1028). In the chronic cases, also, IFAT and ELISA presented positive titres in 100% cases (1 : 128 — 1024 and 1 : 12, 800 — 1 : 102400). The CIEP test, on the other hand, was positive only in one mf + case with acute clinical symptoms, and none of the control sera samples from the endemic or the non-endemic areas gave positive tests. Sera of samples obtained from cases of other forms of helminthiasis also did not yield

positive titres. These studies show that the IFAT and ELISA tests, using *S. cervi* antigen, present a fair degree of reliability.

Angiostrongyliasis :

The effect of the anthelmintics mebendazole, cambendazole, thiabendazole, oxbendazole and levamisole on *A. Cantonensis* in mice was studied, using single and 5-dose treatment schedules, on day 15 post-infection, when maximal numbers of preadult worms were present in the CNS of mice.

ebendazole was effective at 50 mg/kg, when administered daily for 5 days. Levamisole at a dosage of 200 mg/kg was effective in a single treatment. Cambendazole, thiabendazole and oxbendazole were effective at repeated dose schedules of the anthelmintics studied. Mebendazole was found superior to others. The studies were further continued to evaluate combinations of various anthelmintic agents against *A. Cantonensis* infection in mice.

The drugs were evaluated on day 15 post-infection. Combined therapy with levamisole (25 mg/kg) and flubendazole (15 mg/kg) produced 100% efficacy. The prophylactic activity of flubendazole was 4.24% at 25 mg/kg dosage, prior to infection. This activity was enhanced to 1.5% when treatment was continued after the infection. Levamisole administered prior to infection showed a 2.2% reduction of worm-load at the 25 mg/kg dosage level. The efficacy reached a level of 75.3% when the treatment was continued after infection.

Schistosomiasis :

A comparative study of morphological features of various larval stages of the human schistosome parasite found in imvi village and *Schistosoma haematobium*, *Schistosoma ansoni* and *Schistosoma Japonicum*, from other areas, was carried out. There was a close resemblance between *Schistosoma haematobium* from Egypt and schistosome species found in Gimvi. Further studies are being continued to characterise the species.

As a part of surveillance, a pilot survey was carried out to determine the present programme of infection in village imvi.

In all, urine samples from 83 subjects were examined for the presence of schistosome ova. Out of 83 only one child (18 years old) was found to excrete terminal spine schistosome ova in the urine. There were 8 cases of microscopic haematuria. These observations suggest that the focus of schistosomiasis still exists in the area.

6. Vector Control Research :

Field Trials of *Bacillus thuringiensis israelensis* :

A small scale field trial of *B. thuringiensis israelensis* was undertaken in Kalina area in collaboration with Hindustan Lever, Bombay and Municipal Corporation of Greater Bombay. This was undertaken to assess the efficacy of *Bacillus thuringiensis israelensis* against the larval stages of mosquitoes. In this trial it was observed that *B.t.i.* produced 80% reduction in larval density in clear water whereas in highly polluted water the reduction was only 30%. Further large scale trials are being contemplated to evaluate the efficacy of *B.t.i.* under local conditions.

Laboratory efficacy of *B. thuringiensis* was tested against 3rd stage larvae of *Culex-p-fatigans* under laboratory conditions and found 100% reduction in larval density. The efficacy of *B.t.i.* was assessed at different levels of pH, salinity, temperatures and with different larval stages of *Culex-p-fatigans*. The results of this study are still to be analysed.

7. Plague :

In all 606377 rats were collected from Greater Bombay and brought to Haffkine Institute. Out of rats collected 82773 were examined for bacteriological evidence of plague. Besides rats from Bombay, 20 rats from Pune were also examined. None of the rats was positive for plague.

In addition to rats, fleas collected from seven villages of Maharashtra State were also examined for plague. Pools of fleas consisting fleas per pool were inoculated into experimental animals viz. guinea pigs and mice. These animal inoculation studies did not demonstrate any evidence of plague infection.

CHAPTER IV

GENERAL ACTIVITIES

Library :

The library has a collection of books on biomedical sciences and related fields. It provides reference facilities for scientists and students from the field of Biochemistry, communicable disease, Immunology, Pharmacology, Zoonosis, Virology, etc. It provides facilities to its Scientists and students as well as to outsiders, from Institutes all over India. The library remained open to readers on all working days except Sundays and Public holidays.

There are 7854 books in the library. The number of books added during the year were 232 out of which 101 were received as gift from different Organisations.

Books received from British Council	..	..	37
Books received from W.H.O.	..	..	45
Books received from Springer, ICMR and other Organisations	..	..	19

The number of foreign journals subscribed was 98 and Indian 33. Total number of bound volumes were 17490 and about 550 journals were bound during the year. The library also offered Interlibrary loan facilities. The library borrowed about 43 journals from neighbouring libraries for its staff and loaned out 243 journals to other libraries in the city. About 4600 outsiders visited the library for reference. The working of the library during the year 1984-85 is given in appendix VI.

reprographic Services :

The library provides reprographic facilities to its staff and outsiders. The library received requests for xerox facility for a total No. of 4576 pages during the year. The outside members are charged Rs. 1.25 per page and Rs. 0.25 per every extra page. The service is free for staff members.

Artist Section :

The artist section looked after the needs of the Institute concerning the preparation of slides, charts, photographs required by staff, research scholars, for teaching, thesis, conferences and others. This is a small section attached to the Clinical Pathology Department.

APPENDIX I
BACTERIOLOGY

Tests	Number
1. Bacteriological analysis of Chemicals, Disinfectants and miscellaneous products	49
2. Water samples	10
3. Testing of water filters	18
4. Lyophilization of cultures	32
5. Culture supply	29
Total ..	138

APPENDIX II
BIOCHEMISTRY

Tests	Number
1. Blood	211
2. Urine	82
3. Special tests	103
Total ..	396

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	.. I.S.P. Hospital, Nasik.
B.Y.L. Nair Charitable Hospital	.. Public Health Centre, Buldhana.
K.E.M. Hospital	.. Civil Surgeon, Sangli.
M.G.M. Hospital	.. District Hospital for Women, Akola.
L.T.M.G. Hospital	.. Civil Hospital, Akola.
E.S.I.S. Hospitals	.. Primary Health Centre, Jalgaon.
K.B. Bhabha Hospital	.. General Hospital, Osmanabad.
Kasturba Hospital for Infectious Diseases	.. Civil Hospital, Dhulia.

Bombay Area	From Maharashtra
J.J. Group of Hospitals ..	Civil Hospital, Nasik.
Police Hospital ..	
Fertilizer Corporation of India ..	
Greater Bombay Municipal Dispensaries ..	
B.J. Wadia Hospitals for Children ..	

APPENDIX IIIA
CLINICAL PATHOLOGY

Tests	Number
1. A. Histopathology	1,032
2. B. Haematology	895
3. C. Cytology for malignant cells	14
4. D. Clinical Pathology	
Urine for routine examination	305
Stool for routine examination	250
Semen examination	4
Immunological Pregnancy Tests	372
Biological (Rat test) Pregnancy Tests	11
5. E. Bacteriology	
Culture for Tubercle Bacilli	49
Animal Inoculation for Tubercle Bacilli	6
Smear and culture for C. diphtheriae	15
Cultures of Theatre swabs for Anaerobes	257
Urine culture	300
Pus culture	135
Sputum culture	43
Stool culture	55
Blood culture	18
Antibiotic Sensitivity Tests	331
Stool culture (Typhoid)	1
Vaginal swab	1
Synovial fluid	2
Aspirated fluid culture	6
Ear swab culture	1
C.S.F. culture	9
Throat swab culture	1
Conjunctivitis swab	7
Prostatic fluid culture	1
6. F. Serology	
Widal tests	78
Weil Felix tests	8
Brucellosis tests	7
V.D.R.L. tests	27
Paul Bunnel Tests	19
Total ..	4,260

APPENDIX IV
HUMAN PHARMACOLOGY AND BIOAVAILABILITY

Manufacturer			Preparation
M/s. Alkem Laboratories	..	..	Ampicillin
M/s. Cachet Pharmaceuticals	..	..	SMZ + TMP
M/s. Relief Lab.	..	..	Tetracycline
M/s. Relief Lab.	..	..	Ampicillin
M/s. Artichem	..	..	Rifampicin
M/s. Artichem	..	..	Ampicillin
M/s. Triumph	..	..	Ampicillin
M/s. Triumph	..	..	Rifampicin
M/s. Sanghavi	..	..	Ampicillin
M/s. Sanghavi	..	..	Tetracycline
M/s. Sanghavi	..	..	Tetracycline
M/s. Lupin	..	..	Rifampicin
M/s. Lupin	..	..	Ampicillin
M/s. Blue Cross	..	..	Ampicillin
M/s. Standard Organics	..	..	Amoxycillin
M/s. Duphar Interfran	..	..	Ampicillin
M/s. Cipla	..	..	Amoxycillin
M/s. Lupin	..	..	Rifampicin

APPENDIX V
IMMUNOHAEMATOLOGY

Tests					Number
Rh. Antibody titre	..	..	..	..	162
ABO grouping	..	..	..	..	17
Cross Matching Transfusion	..	..	..	..	1,085
Total				..	1,264

APPENDIX VI

The following figures indicate the working of the library during the year 1984-1985.

Number of outside readers visiting the Library	Number of books and periodicals issued to our staff.	Number of periodicals issued under inter-library loan service.	Books and Periodicals obtained for our staff from other libraries.
4611	848	243	43

Additions to Library

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.
232	550	133	75	7

APPENDIX VII
RABIES RESEARCH UNIT

Comparison of daily and interrupted dose-schedules of AR vaccine
for protection in mice given subsequent challenge by IM route

accine (1%) chedule (days)	Day of Challenge	Results after Challenge					
		Expt. 1			Expt. 2		
		MR	%M	AST	MR	%M	AST
0-1-2-3-4-5 6-7-8-9	11*	16/20	80	9.95	10/17	58.5	7.76
0-3-7-10	12**	8/18	44.4	12.38	7/19	36.8	11.84

IM = Intramuscular

Vaccine = Total 1 ml of 1% per mouse, in both schedules.

MR = Mortality Ratio.

AST = Average Survival Time (Days) per mouse.

Challenge dose * = 21 - 38 LD₅₀ ** = 22 - 34 LD₅₀

APPENDIX VIII
TESTING

Tests					Number
Prohibition and Excise Testing					
1. Indian made Foreign Liquors	..	..	..	..	1,270
. Beer	..	..	..	..	996
. Toddy	..	..	..	..	721
. Ganja	..	..	..	..	52
. Bhang	..	..	..	..	19
. Charas	..	..	..	..	7
			Total	..	3,065
Commercial Testing					
. Insulin (Bio-assay)	..	..	..	..	39
. Pyrogen test	..	..	..	..	79
. Germicidal tests	..	..	..	..	43
. Adrenaline	..	..	..	..	12
. Physical tests	..	..	..	..	45
. Assays	..	..	..	..	23
. Oxytocin activity	..	..	..	..	4
8. Potency of Immune serum	..	..	..	..	2
. Miscellaneous tests	..	..	..	..	4
			Total	..	251

APPENDIX IX
TOXICOLOGY

Classification of sample	Number Tested	Toxicity Parameter
1. Pesticides	1	Acute dermal toxicity.
	1	Primary skin irritation.
	1	Mucous membrane irritation
	1	M.L.D. in chicken.
	1	M.L.D. in pigeon.
2. Growth promoting hormone (plant)	3	Acute oral toxicity.
	1	Acute dermal toxicity.
	2	Primary skin irritation.
	1	Mucous membrane irritation
	1	Acute toxicity in fish.
	1	M.L.D. in chicken.
	2	M.L.D. in pigeon.
3. Chemicals	8	Acute oral toxicity.
	2	Primary skin irritation.
	1	Mucous membrane irritation

APPENDIX IXA
TOXICOLOGY

Sample	Species	LD ₅₀	Remarks/Fiducial Limits
. Acute oral toxicity			
1. Amrutsanjivani	Mice	> 20 ml/kg	...
2. Vipul	Mice	> 19,800 mg/kg	...
3. Gumex	Mice	> 16.55 ml/kg	15.83-17.29 ml/kg
4. Can lining			
'B'-Nabuval	Mice	> 20 ml/kg	...
5. Cap lining	Mice	> 20 ml/kg	...
'A'-PC 200 latex			
6. Chemifloc SA-2	Mice	> 2,500 mg/kg	...
7. Chemifloc	Mice	> 2,500 mg/kg	...
8. Dendrite HM 5009	Mice	> 5,000 mg/kg	...
9. Fumed Silica			
(Silicon Dioxide)	Mice	> 10,000 mg/kg	...
10. Vipul	Rats	> 19,800 mg/kg	...
1. Mearimaal TR			
(Pearl Essence)	Rats	> 15 ml/kg	...

Sample	Species	LD ₅₀	Remarks/ Fiducial Limits
B. Acute dermal toxicity			
1. Celmone (d-NAA) 4.5%	Rabbits	> 10,180 mg/kg	...
2. Vipul	Rabbits	> 9,900 mg/kg	...
C. Primary skin irritation			
1. Celmone (α -NAA) 4.5%	Rabbits	No irritation.	
2. Amrutsanjivani	Rabbits	No irritation.	
3. Vipul	Rabbits	No irritation.	
4. Cocodiethanolamide	Rabbits	Moderate skin irritation	
5. Moiya oil (MOEF)	Rabbits	Mild skin irritation.	
D. Mucous membrane irritation			
1. Celmone (α -NAA) 4.5%	Rabbits	No irritation.	
2. Vipul	Rabbits	No irritation.	
3. Gumex	Rabbits	No irritation.	
E. Minimum lethal dose (MLD)			
1. Vipul	Chicken	Min. lethal dose > 9,900 mg/kg	
2. Celmone (α -NAA) 4.5%	Chicken	Min. lethal dose > 10,180 mg/kg	
3. Malathion 50 EC	Pigeon	Min. lethal dose > 137.26 mg/kg	
4. Amritsanjivani	Pigeon	Min. lethal dose > 20 ml/kg	
5. Vipul	Pigeon	Min. lethal dose > 19,800 mg/kg	
F. Acute toxicity in fish			
1. Vipul	Fresh Water fish	96 hours LC ₅₀ — 2020 ppm	

APPENDIX X VIROLOGY

Vaccine administered	patients Number of
Antirabic	4,066
Oral polio	118
Triple	87
Measles	9
Cholera	6
Typhoid	6
Tetanus	15
Total	4,307

APPENDIX XI
ZOO NOSES

Animal	Animals procured (number)	Venom produced (gm)	Venom supplied (gm)
Snakes			
Cobra	141	109.08	165.07
Krait	47	5.00	4.95
R. Viper	35	7.28	37.00
Echis	3422	45.68	20.67
Total :	3645	167.04	227.69
Scorpions			
Red scorpions	7840	5.16	5.00
Black scorpions	18	—	2.01
Total :	7858	5.16	7.01

APPENDIX XI A
ZOO NOSES

No. of visitors that visited the snake farm :						
1. Students	..	..	..	..	..	2628
2. Staff of Scientific Institutions	..	..	..	..	..	150
3. Distinguished visitors	..	..	..	..	..	59
4. Foreigners	..	..	..	..	..	27
5. Thursday visitors	..	..	..	..	..	3013
6. Others	..	..	..	..	..	151
Total :						6028

APPENDIX XII
POST-GRADUATE COURSES OFFERED

M.Sc.	—	Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology.
M.Sc. (By Paper)	—	Microbiology, Biochemistry.
Ph.D.	—	Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology.
M. Pharm	—	Pharmacology and Pharmaceutical Organic Chemistry
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	..	..	..	..	9
Diploma in Medical Laboratory Technology (DMLT)	..	..	..	..	20
Course in Drug Analysis	..	..	..	..	6
Short term Training Course	..	..	..	..	5

Research Fellowships and Scholarships

Jr. Research Fellowships (Platinum Jubilee)	..	..	3
Jr. Research Scholarships (Chinchwad Land)	..	..	5

APPENDIX XIII A

LIST OF STUDENTS WHO REGISTERED DURING THE YEAR 1984-85
FOR M.Sc., Ph.D., M. Pharm and M.D.

M.Sc.	Ph.D.
(1) Kum. G.B. Akerkar	(1) Shri B.J. Mohite
	(2) Shri V.G. Shirke
M. Pharm	M.D.
(1) Kum. Z.N. Khety	(1) Shri P.S. Kothari
(2) Shri R.N. Ali	
(3) Shri A.B. Shirgaonkar	

APPENDIX XIV

DEGREE AWARDS DURING THE YEAR 1984-85

Name of Candidate	Subject	Topic
(1) Dr. D.P. Buduk	Ph.D. Applied Biology	Immunological studies in protein malnutrition with reference to Humoral and cell mediated Immune response in protein deficient wistar rats.
(2) Kum. R.S. Pavri	Ph.D. Biochemistry	Studies on the Mechanism of oxidative injury to the Red cells in some Haemotological disorders.
(3) Shri B.D. Sathe	Ph.D. Applied Biology	Studies on schistosomiasis with particular ref. to the focus in village Gimvi Dist. Ratnagiri, Maharashtra State.

Name of Candidate	Subject	Topic
(4) Kum. L.S. Shirsat	Ph.D. Biochemistry	Biochemical and Toxicology aspects of PVC Plasticizers.
(5) Shri M. Kishore	Ph.D. Biochemistry	Biochemical studies on the Hypoglycemic factor from <i>Bordetella pertussis</i> .
(6) Shri A.K. Patil	Ph.D. Applied Biology	Studies of halophilic vibrios in deep sea off Maharashtra State.
(7) Kum. S.R. Ghogale	M.Sc. Microbiology	Immunological studies on Multiple Myeloma.
(8) Kum. H.B. Tiwari	M.Sc. Microbiology	Cross reactions of enteric fever causing salmonella in cellular immunity.
(9) Kum. S.G. Kulkarni	M.Sc. Microbiology	Cross reactions of atypical mycobacteria.

APPENDIX XV POST-GRADUATE TEACHERS

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

Name	Qualification
1. Dr. V.R. Deshpande	M.D.
2. Dr. M.V.N. Shirodkar	M.B.B.S., A.B.Sc.D. (Johns Hopkins) P.B.K. (U.S.A.)
3. Dr. R.S. Kamat	M.D.
4. Dr. K.H. Dave	M.D., D.P.B.
5. Dr. D.M. Renapurkar	M.Sc., Ph.D., F.M.A.S., F.E.S.I.
6. Dr. V.R. Rao	M.V.Sc., Ph.D.
7. Dr. F.L. Saldanha	M.Sc., Ph.D., F.I.C. (Cal.)
8. Dr. A.J. Baxi	M.Sc., Ph.D.
9. Dr. F. Fernandes	M.Sc., Ph.D.
10. Dr. P. Ramakrishnan	M.Sc., Ph.D.
11. Dr. S.V. Gadre	M.Sc., Ph.D.
12. Dr. B.G. Khadse	M.Sc., Ph.D.
13. Dr. M.K. Bhopale	M.Sc., Ph.D.
14. Dr. H.J. Mansharamani	M.B.B.S., Ph.D.
15. Dr. R.P. Bhamaria	M.Sc., Ph.D.
16. Dr. S.R. Chavan	M.Sc., Ph.D.

APPENDIX XVI
PAPERS PUBLISHED 1984-85

V.M. Rangnekar, S.R. Lokhande, R.P. Bhamaria and B.G. Khadse :
Synthesis of substituted N-aryl/methyl/heteroaryl morpholino/
N'-methyl piperazinyl succinimides as anti-tuberculosis agents.

— Ind. J. Pharm. Sci. **46** (6): 196-198, 1984.

A.S. Bobade, C.N. Desai and B.G. Khadse. Synthesis and study
of 2,7-disubstituted benzothiazole (3,2-d)-s-triazines-1, 3-diones as
possible antituberculosis agents.

— Ind. J. Pharm. Sci. **47** (3): 132-134, 1985.

S. Hattikudur and R.S. Kamat. Characterisation of a target antigen
of cell mediated immunity in *M. tuberculosis* H₃₇Rv.

— J. Med. Microbiology, **18**: 17, 1984.

S. Hattikudur and R.S. Kamat. Polymorphism of a mycobacterial
target antigen of cell mediated immunity.

— J. Med. Microbiology, **19**: 69, 1985.

S.R. Rath and R.S. Kamat. Antigens of hepatocyte cell membrane
in normal and non-neoplastic diseases of liver.

— Ind. J. Med. Res. **90**: 469, 1984.

Kulkarni, A.P., Arekar, A.S., Chanderkar, N.G. and Shirodkar,
M.V.N. Periodically monitored SGPT and SGOT levels in rabbits
neonatally inoculated with HBV.

— Hep. Scient. Memo. (West Germany) **7**: 87-88, 1984.

Chirmule, N.B., Shirodkar, M.V.N., Deshmukh, R., Chanderkar,
N.G. and Deo, M.G. ICRC vaccine-induced lepromin conversion in
monkeys (*Presbytis entellus*).

— Lepr. Scient. Memo. (U.S.A.) L-11139/1, 5-6 Leonard wood
Memorial, Virginia, U.S.A.

Shirodkar, M.V.N. In Manual on Laboratory Procedures of
Common Epidemic-Prone Diseases: Viral Hepatitis. Eds. Basu S.N.,
Bhatia, R. and Ichhpujani, R.L.P. 35 Publ. National Institute of Com-
municable Diseases, New Delhi, 1984.

Shirodkar, M.V.N. Kaveel — Ek Pivale Sankat.

— Svaramala 9: 93-98, Ed. Rane, M. Publ. Maharashtra Goa Prakashan,
Bombay, India, 1984.

APPENDIX XVII
PAPERS SENT FOR PUBLICATION

A.S. Bobade, D.R. Ashtekar and B.G. Khadse. Synthesis and
study of 2-alkyl and substituted aryl-4-(3'-phenyl-5-methyl isoxazolyl)
or 4-(p-nitrophenyl) thiazoles as possible antituberculosis agents.

— Ind. J. Pharm. Sci. 1984.

A.S. Bobade, D.R. Ashtekar, and B.G. Khadse. Synthesis and study of 6, 7-dimethoxy-3-substituted-4-amino-2-thiotetra-hydroquinazolines as antituberculosis agents.

— Bull. Haff. Inst. 12 (1): 1984.

A.S. Bobade and B.G. Khadse. Synthesis and antitubercular properties of 2-(substituted-acetamido and p-acetamido benzene, aminobenzene sulphonamido)-6-substituted-benzothiazoles.

— Bull. Haff. Inst. 1985.

S. Kulkarni, S. Hattikudur and R.S. Kamat. Cell mediated immunity cross reactions of mycobacteria: polymorphism of target bacterial antigen.

— Clinical and Experimental Immunology 1985.

S. Kulkarni and R.S. Kamat. Cross reactions in cell mediated Immunity induced by a typical mycobacteria

— J. Med. Microbiology, 1985.

A. George, S. Rath and R.S. Kamat. Differential regulation of effector responses of cell mediated immunity in experimental Salmonellosis.

— Clinical and Experimental Immunology, 1985.

H. Tiwari and R.S. Kamat. Cross-reactions in cell mediated immunity to Salmonella causing enteric fever.

— J. Med. Microbiology, 1985.

V.R. Kamath and D.M. Renapurkar. Laboratory evaluation of *Bacillus thuringiensis* var *israelensis*. (H-14) against *Culex-p-fatigans* larvae.

— Jr. Communicable Diseases.

V.R. Kamath, Sushila Menon, M.K. Bhopale and D.M. Renapurkar, *Ancylostoma ceylanicum*: Resistance induced by gamma-irradiated infective larvae in golden hamsters.

folia parasitologica

Almeida, A.J., Kamath, V.R., Bhopale, M.K. and Renapurkar, D.M. Study of protection against *Leishmaniasis* infection in cotton rats (*Sigmodon hispidus*) by *Setaria cervi* antigen.

— Z. Parasitenkunde

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

— Helminthologia

