

Annual Report of the Haffkine Institute

1983-84



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CHAPTER I

INTRODUCTION

As in the previous year, the year 1983-84 was plagued with lack of financial support in the form of sufficient grant-in-aid to run our Research Institute. There was also labour agitation (strike) in May, 1983. Because of the poor financial position the activity of the Institute was severely affected. The financial commitment with regard to salaries, purchase of equipment, chemicals, glassware and animals has increased tremendously due to escalating costs.

In spite of the adverse conditions the Institute is making Herculean efforts to continue its primary functions with zeal and enthusiasm true to its motto of alleviating human suffering and hereby serve the welfare of the people.

रोगापहारी विज्ञानम् । लोककल्याण साधनम् ॥

The Research, Training and Testing activities are continued in accordance with the tradition and prestige of Haffkine Institute. The following are the highlights of the activities of the Institute.

Research :

The Department of Bacteriology continued their experiments of genetic recombination between enteric species in order to obtain new strains with academic and/or applied potentials.

The department of Biochemistry successfully completed the purification of the blood sugar lowering (hypoglycemic) factor from *Bordetella pertussis* cell extracts, which was the main objective of the I.C.M.R. Scheme. A new study on the enzyme markers of the metabolic pathway in *B. pertussis* sensitized (both normal and alloxan diabetic) mice, has been initiated.

In their quest for new organic compounds having antitubercular, antimalarial, antifungal and anti bacterial activities the department of Chemotherapy synthesised several organic compounds which showed promising effects *in vitro*. Nearly fifty compounds were screened for anti tubercular activity. Out of which 5 compounds showed activity similar to INH. Of nearly ten compounds screened for antifungal activity only two compounds showed activity at 180 µg/ml dilution. Twenty five new Benzimidazole derivatives were tested against *P. berghei* strain of Malaria in mice. Out of these one compound showed antimalarial activity at 20 mg/kg dose in mice.

The Clinical Pathology and Immunology department investigated the cross reactions of atypical mycobacteria amongst

themselves and with classical mycobacteria employing cellular immunity as test parameter. Extensive cross reactions were observed. Investigations regarding regulation of cellular immunity against *Salmonella* infection has shown that intradermal immunisation predominantly generates delayed type of hypersensitivity (DH) and minimum protective cellular immunity. In contrast i.p. immunisation with live cells generates both. Significantly oral immunisation with sub lethal doses of live salmonella generates good cell mediated immunity.

The department of Pharmacology continued its work on indigenous plants viz. *Ipoemea digitata*, *Dalbergia latifolia* and *Dioscorea hispida* for their pharmacological activity. Synthetic compounds KRNVS-E and J were studied on isolated Guinea pig tracheas and atria for possible agonistic and antagonistic activity on B₁ and B₂ adrenergic receptors. Crude chloroform extract of *sempinus anacardium* and its purified fraction II were studied for their acute toxicity, while fraction II was checked for cytotoxic effects.

The Phytochemistry unit was engaged in the investigation of vector control (Mosquito larvicidal) agents from indigenous plants. Leaves of *Vitex negundo* Linn, *Nerium indicum* Mill and *Lantana camara* Linn and seeds of *Pongamia pinnata*, (Linn) Merr *Annona squamosa* Linn and *Azadirachta indica* A. Juss were studied.

Rabies research unit pursued the immunological studies on rabies by administering a constant total dose of vaccine in different dose schedules to groups of mice and subsequently challenging with lethal doses of virus on different days. Work on humoral immunity after vaccination in man was also carried out during the year. It was observed that in high risk patients nothing less than a full course of 10 ml per dose and followed by a booster dose should be given for higher sero-conversion rates.

The department of Virology established a new model in the Langur monkey for leprosy research. In collaboration with Cancer Research Institute, Bombay it was shown to be 90% seroconverted following lepromin inoculation. It was also shown in the same model that ICRC strain of *Mycobacterium* strongly cross reacted with the human strain of *M. leprae* (killed bacilli). In their study with plasma factor it was observed that the large fowl (hen and cock) were capable of producing plasma factor following inoculation with rabies virus. Finally the important research project on the HIBOMA-new born rabbit model was completed, specifically demonstrating the elevation of SGOT-SGPT levels in rabbits infected acutely and chronically with HIBOMA virus.

Zoonosis department developed a test for immunodiagnosis of angiostrongyliasis infection in humans. An irradiated vaccine

has been prepared using *Ancylostoma ceylanicum*. This vaccine is being evaluated for its efficacy.

Testing : The Institute's multifarious testing activities included various tests conducted on products submitted by the Prohibition and Excise department, drugs, biological material, food and food additives, disinfectants, pesticides, paints, plastics etc. In all 3537 samples were tested during the year.

Patient oriented services : Clinical pathology and biochemistry departments accept samples from patients for investigations. In all 4955 and 485 samples were tested in the two departments respectively. The services were used by various hospitals, charitable and private organisations in Bombay city and suburbs and other places. (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 cost. In all 859 individuals were immunised during the year under report (Appendix — IX).

Immunohaematology department which was started in the year 1976-77 served the needy and the poor, carried out 758 Blood transfusions, 92 Rh-antibodies titre, 791 grouping and cross matching tests (Appendix — V).

Training Programme : The Institute offered training programmes for medical and paramedical personnel (Appendix XII). A list of students registered during 1983-84 for Ph.D. and M.D. degrees and those who successfully completed their thesis and obtained their degrees are given in Appendix XIII and XIV. Appendix XV gives 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 MSc degree courses by papers. In addition to the degree courses, one of the important training programmes of the Institute is to prepare para-medical personnel, particularly in the field of medical laboratory technology. A one year diploma course (DMLT) of the University of Bombay is offered besides a one year course in drug analysis. Twenty two students were trained in DMLT and 13 students in Drug Analysis during 1983-84. Apart from this 4 students received short term training during the year.

Sponsoring Research Bodies : 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 finance some of the Research Projects. The

Institute is thankful 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.

A Review Committee meeting under the Chairmanship of Dr. Ramalingaswamy, Director-General, I.C.M.R. was held on 29th June, 1983 to study the problems of Haffkine Institute. Hon'ble Minister for Public Health, Dr. Lalita Rao took active part in the deliberations of the meeting. After discussions it was decided to hold a second meeting to find a solution and finalise the proposal deciding the future of the Institute.

CHAPTER II

GENERAL DEPARTMENTAL ACTIVITIES

acteriology

Five students were registered for M.Sc. (Part II) in Microbiology (by papers). Three students secured first class, and one second class. Five students were registered for M.Sc. (Part I). Four of them secured first class, and one a higher second class. Training programmes were also conducted for D.M.L.T., Drug analysis students and short-term trainees.

Routine testing activities were also carried out. Bacteriological analysis of various products was undertaken. Water samples were analysed and water filters were also tested. Lyophilization of cultures was also undertaken (Appendix I).

Biochemistry

The regular training activities of the department which comprise M.Sc. Biochemistry (by papers) Part I and Part II, D.M.L.T., and short term course in Biochemistry, were conducted satisfactorily. On recommendation, two post-graduate (M.Sc.) research students from outside Bombay were given facilities and technical help to complete analysis (both electrophoretic and chromatographic) of several samples of abnormal haemoglobins in the department.

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

Chemotherapy

Three students are working for their Ph.D. degree in Medicinal chemistry. One student is registered and working for her M.Pharm. degree in medicinal chemistry. Theory and practical classes were conducted for the students of Drug Analysis course. The graduate students are working on compounds synthesised for testing their biological activity. Two hundred and two compounds were analysed for their carbon and hydrogen and three hundred and fifty three compounds were analysed for nitrogen. Seventy five compounds were screened for antibacterial activity and fifty compounds were screened for antifungal activity. None of the compounds showed significant activity.

Clinical Pathology-Immunology

The department received specimens from the various hospitals and agencies (Appendix IIIA) for investigation. The services offered

(Appendix III) covered the general areas of cytology of the various body fluids, routine haematological examination, diagnostic serology, bacteriology, mycology and histopathological examination of biopsies.

The staff participated in the integrated Bombay University teaching programme for M.Sc. (Microbiology), M.D. (Pathology), and M.D. (Microbiology). The department also participated in the D.M.L.T. training course and offered short term training course in Clinical Pathology. Two candidates received M.D. degree in Pathology.

Human Pharmacology, Bioavailability and Radiation Biology units

During the year 1983-84 the Human pharmacology and bio-availability unit undertook routine testing of all incoming samples for blood absorption studies in human volunteers. The list of pharmaceuticals whose samples were accepted for the studies is given in Appendix IV. The Radiation biology unit carried out on a trial basis tests with sera of known hyper and hypothyroid cases and the facility can now be offered to referred cases.

Rabies Research Unit

In their routine activities the seed virus CVS strain was prepared and maintained in adult mice while the Paris strain of fixed virus was prepared in rabbits. Tissue-culture cell lines were maintained by serial propagation and storage in liquid nitrogen at appropriate time. The unit continued to provide diagnostic service on post-mortem specimens of brains of animal and human origin. List of samples received and results obtained are given in Appendix XI.

General public and members of the medical profession from the city of Bombay as well as from outstations availed the Advisory Service on Rabies to solve their problems regarding dog bites and immunisation.

The unit also participated in the training programmes for M.Sc. (Microbiology) and M.D. (Microbiology) students of the Bombay University by arranging lectures and practicals in the department.

Toxicology

The Toxicology department was actively engaged during the period 1983-84 in Toxicological evaluation of different types of pesticides, drugs, chemicals, cosmetics (Appendix VIII). These samples were referred by various Industries and Government bodies.

The department was involved in training and teaching programme of Drug Analysis and Postgraduate courses.

Virology

Amongst the routine activities of the Department of Virology is the Immunisation Clinic. In all, 859 patients attended the Clinic during the period 1983-84. The vaccines administered included antirabic, oral polio, triple, cholera, typhoid and measles. Measles vaccination commenced in the Clinic from January, 1984. The vaccines administered and the number of patients vaccinated are given in Appendix IX. As usual, the Department conducted the M.Sc., M.D. (Microbiology) and Ph.D. lecture and practical annual course for the students from Haffking and the University of Bombay. In all, nineteen lectures and ten practicals were held. Three Ph.D. (Microbiology) and two Ph.D. (Applied Biology) students are registered with the Departmental Head. One M.Sc. student obtained her degree during this period.

Academic Activities, Honours and Awards

One of the major activities of the department of Zoonosis during the year 1983-84 was the organisation of the Fourth National Seminar on Zoonoses and their control, on January 9-11, 1984. Under the chairmanship of the Director Dr. V. R. Deshpande this seminar was jointly organised by the Haffkine Institute and the Bombay Veterinary College. This was the first ever seminar organised in Bombay on Zoonoses. About 100 participants from various parts of the country participated in the seminar. Fifty five papers were presented. Dr. A. Zahra, WHO, World Health Representative to India, delivered the keynote address. Besides playing a major role in organising the seminar, various members of the Zoonosis department actively participated in the proceedings of the Zoonoses seminar and presented papers.

Dr. M. V. N. Shirodkar, Head of the department of Virology, delivered a lecture on **Viral Hepatitis** in the Institute under the auspices of the AMI on the 31st of May, 1983. He attended a meeting of the National Committee on Viral Hepatitis sponsored by the WHO and the DGHS in New Delhi on the 29th of April, 1983. He was Examiner for a Ph.D. Thesis of the University of Cochin, in March, 1983 and was Examiner at the M.D. (Microbiology) Examination of the University of Bombay held in April-May, 1983. The Head of the Virology department was an Expert on an MPSC Selection Committee in Microbiology and on the Selection Committee for the post of E 1 Scientist at MACS, Pune in 1983. He delivered a lecture on plasma factor at the Rabies Seminar at the Bombay Veterinary College, on 21st October, 1983. He presided over the inaugural function of 24th Annual AMI Conference at Hyderabad on 12th November, 1983. He was Vice-Chairman, Organizing Committee of the Fourth National Seminar on the Zoonoses and their Control, January 9th-11th, 1984. He also acted

as a Moderator on a nationally televised programme on Doordarshan entitled 'Zoonoses'. He was delegate at the **International Symposium on Microbial Metabolites** organised by Hoechst on 17th February, 1984. He served as an Expert on MPSC interviews for post of Microbiologist, 28-30th March, 1984.

During the year 1983-84 the Head of the Rabies Research Unit, Dr. H. J. Mansharamani continued as a recognised post graduate teacher by the University of Bombay for M.Sc. Microbiology (By Research and By papers). Two students from the department were awarded degrees in Microbiology. Three papers were presented from the department at the Fourth National Seminar on Zoonoses and their Control in January 1984 at Bombay.

Dr. S. V. Gadre, Head of the Bacteriology Department attended the XV International Congress of Genetics at New Delhi during December 1983 and a Symposium on Eukaryotic Genetics in Poona in February, 1984. He also delivered a special guest lecture at the Annual Conference of the Association of Microbiologists of India at Hyderabad, in November 1983.

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 :**

Attempts were made to clone vibrio genes into *E. coli* by using *pst* I, a restriction enzyme and T_4 DNA ligase to cut and ligate fragments of vibrio chromosome to pBr 322 and transform *E. coli*. These attempts were unsuccessful.

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

Phage resistant cultures of *Flavobacterium dehydrogenans*, *Corynebacterium mediolenum* and *Corynebacterium simplex* have earlier been isolated in this department in fulfilment of a sponsored project.

It was shown that in case of *E. dehydrogenans*, the resistance is due to loss or alteration of phage receptors on the mutant bacterial cell wall while in case of *C. simplex*, the absorption proceeds normally, but the subsequent infection is blocked due to lack of penetration of phage DNA or its destruction inside the mutant bacterial cell.

BIOCHEMISTRY

1. **Biochemical studies on *Bordetella pertussis* cell extracts :**

Purification of an electrophoretically and immunologically pure protein fraction from the enzyme extracts of *B. pertussis* was achieved. The final step involved preparative polyacrylamide gel electrophoresis (PAGE, 7.5% gel in Tris-glycine buffer, pH 8.4, Stinson et. al., 1977). Preliminary studies indicated its molecular weight to be in the range 60,000 — 80,000. It caused a significant lowering of fasting blood sugar level in mice (about 50% lowering at 5/ μ g dose level as against the same lowering with 200/ μ g of semipurified fr. V). It was next decided to study the blood sugar lowering effect of the pure preparation in alloxan diabetic mice. Accordingly an alloxan diabetic mouse model was developed in the laboratory for the first time. Graded doses of alloxan monohydrate were administered to mice by the IP route and the fasting blood sugar level monitored at regular intervals. It was found that a dose level of 250 mg/kg body wt. maintained a pronounced diabetic state even after 15 days.

Preliminary studies with Fr. V showed that there is a lowering in fasting blood sugar level in alloxan diabetic mice, thus indicating in addition, the probable independent insulin-like action of the *B. pertussis* factor.

2. Studies of the effects of undernutrition and hepatotoxic agents on lipid peroxidation :

Molecular oxygen though essential for survival, has long been shown to damage plants, animals and aerobic bacteria such as *Escherichia coli* when supplied at concentrations greater than those in normal air. The effects observed vary considerably with the type of organism used, its age, physiological state and diet such as the presence in the diet of varying amounts of vitamins A, E, C, and transition metals, antioxidants and polyunsaturated fatty acids. There are fragmentary data on the role of adequate nutrition in the regulation of oxygen toxicity.

We have studied the effects of undernutrition (Calorie restriction) at different ages on the Oxido-reductase system with special emphasis on the enzymes superoxide dismutase, catalase and glutathione peroxidase, and lipid peroxidation. The conclusions drawn from this study are that undernutrition imposed early in life affects the activity of the enzymes superoxide dismutase, catalase and glutathione peroxidase both in the liver and brain of rat. Recovery of the activity of these enzymes on rehabilitation is suggestive of these changes being transitory and reversible in the neonatally undernourished. The severity of the changes observed in the activity of these enzymes appears to be dependent upon the time of onset of nutritional deficiency. Undernutrition imposed late in life does not affect the antioxidant enzymes greatly.

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

Organophosphates are widely used as insecticides to increase the yield of agricultural products and also to control the vectors of malaria and the organism interfering with human food. One of the more serious effects of some organophosphorous compounds is their ability to induce delayed neuropathy in human and other animal species. Recent reports indicate that lipid levels in brain are altered. Delayed neurotoxicity has been reported, which is characterised by leg weakness and degeneration of axons and myelin within the CNS.

Pathological free radical mechanism leading to lipid peroxidation and degeneration of phospholipids, with loss of membrane integrity, are currently considered as important factors responsible. Hence it was thought worthwhile to study the effect of organophosphorous insecticide (Malathion-T) on lipid levels, lipid peroxidation and

lipase enzyme in brain and liver tissue in experimental animals. Such studies assume significance since brain is a target tissue of pesticide toxicity and has a high lipid content. The study has been initiated.

CLINICAL PATHOLOGY — IMMUNOLOGY

1. Study of mycobacterial antigens :

Polymorphism of a mycobacterial antigen participating in cell mediated immunity (see annual report 1982-83) was correlated with the immunogenicity of the species. Rapidly growing mycobacteria were shown to be poorly immunogenic and this was correlated with distinct differences in the structure of their target antigen of cellular immunity when compared with the same antigen from slowly growing mycobacteria which are good immunogenes.

2. Study of cross-reactions in cell mediated immunity of atypical mycobacteria :

Extensive cross-reactions in delayed hypersensitivity response were shown amongst atypical mycobacteria and between them and the classical mycobacteria. It was further shown that in mice immunised with atypical mycobacteria cross-reacting mycobacterial antigens could generate activated macrophages, an index of protective immunity. These data indicate that atypical mycobacteria, on infection, can induce cross protection against classical mycobacteria such as *M. tuberculosis*.

3. Study of cross-reactions in cell mediated immunity of non typhoidal salmonellae with *S. typhi*, *S. paratyphi A* and *S. paratyphi B* :

Employing a delayed type hypersensitivity response extensive cross-reactions between non-typhoidal *Salmonella* and *Salmonella* of enteric fever were shown. Further the cross-reacting antigens of typhoidal *Salmonella* were shown to generate activated macrophages in mice immunised with the non-typhoidal *Salmonella*. This indicates that non-typhoidal *Salmonella*, on infection can induce cross-protection against enteric fever.

4. Study of immune regulation in Salmonellosis :

Employing an adoptive transfer model for delayed hypersensitivity and protective immunity by means of transfer of immune spleen cells in syngeneic mice, the regulation of two effector responses was investigated. The time kinetics of decline of two responses differed grossly. For example 9 weeks post-immunisation cells responsible for delayed hypersensitivity were non-existent whereas the protection could be effectively transferred at this point of time. It was also shown that intradermal immunisation even with Freund's adjuvant predominantly generated delayed hyper-

sensitivity response. Employing anti Lyt antisera the reasons for decline of immunity were further investigated and shown that the decline was due to generation of suppressor cells. Employing the same system, behaviour of regulatory responses following vaccination will also be investigated.

CHEMOTHERAPY

1. Antitubercular agents :

(a) 6-Alkoxy-2-aryl amino benzothiazoles :

In this series two compounds namely 6-methoxy-2-(p-methoxy phenyl amino) — Benzothiazole and 6-Ethoxy-2-(p-ethoxy phenyl amino) — Substituted Benzothiazole showed similar activity to the standard drug INH. So these two compounds were synthesised upto 5 gram for further testing.

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

In this series nearly twenty new compounds were synthesised and tested for Antitubercular activity. One compound namely 2-(3'-4'-Dimethoxy phenyl)-4-(3'-phenyl-5'-methyl isoxazolyl) thiazole showed similar activity to INH.

(c) Thiazole derivatives :

4-(p'-Nitrophenyl)-2-(N-N'-dimethyl-amino phenyl) thiazole showed similar activity to INH in *vitro* screening against H37Rv strain of *M. tuberculosis* out of twenty new compounds prepared.

(d) 1-(4-(Heteroaryl)-N-N'-2,3-bismethoxy carbonyl) guanidines :

Out of eight compounds synthesised in this series one compound namely 1-(4-Morpholino phenyl) N-N'2,3-bismethoxy carbonyl) guanidine showed similar activity to INH and needs further investigation.

2. Antifungal agents — (Imidazothiazole derivatives) :

In this series nearly ten compounds were synthesised and tested for their antifungal activity against *T. rubrum*, *T. Mentagrophyte* and *M. gypsiu*m. Two compounds showed activity at 100 µg/ml. dilution.

3. Antimalarial agents — (S. Triazine derivatives) :

Twenty five new Benzimidazole derivatives were prepared in this series and tested against *P. berghei* strain of malaria in mice. Out of these one compound showed anti-malarial activity at 20 mg/kg. dose in mice.

4. Activity of Tuberculosis Research Laboratory :

This laboratory was engaged in performing the following studies :

Development of Radiometric procedure for primary screening to understand structure activity relationship :

Fifty two compounds from various series were subjected for radiometric analysis. Only nine compounds were found to be active. The radiometric primary screening for anti-tubercular activity was completed within 48-60 hours in contrast to 7-9 days by conventional method.

Using radiometry, it is possible to calculate the per cent inhibition values, which indicate how many times the generation time has been retarded. The per cent inhibition values thus give better understanding of structural chemical modification of a drug. Whereas the conventional tube dilution test indicates MIC only. Thus radiometric technique so developed will help the pharmaceutical industries to achieve an early information on quality control of the drugs as well as for drug screening purposes.

Primary screening of Antitubercular Activity by submerged culture technique :

About 20 newly synthesised compounds were screened for (in vitro) antitubercular activity. Eighteen compounds were found to possess antitubercular activity ranging from 0.02 mcg/ml to 12.5 mcg/ml. The results were reportable within 7-9 days time as compared to 14-21 days by surface pellicle method.

Rapid drug susceptibility testing of clinical isolates of *M. tuberculosis* using radiometry with special reference to pyrazinamide (PZA) :

Pyrazinamide (PZA) is an important antitubercular agent and its use is recommended by W.H.O. for short-term chemotherapy course. However this drug is active at pH 5.5. Such a pH is detrimental to *M. tuberculosis*. Thus radiometric procedure using ¹⁴C-U-acetate was developed. Fifty nine strains were sensitive to 50 mcg/ml of pyrazinamide, whereas four strains were resistant to 50 mcg/ml of PZA. This testing was completed within 4-8 days in contrast to 40-45 days by conventional method. The technique showed 100% agreement with the conventional methods. The radiometric technique appears to be highly promising.

A systematised study on pyrazinamide (PZA) :

Since PZA is recommended in combination with INH, rifampicin (RMP), a radiometric evaluation was undertaken to evaluate the combination studies. PZA showed Antagonism with INH and synergism with rifampicin. When PZA, INH and RMP were mixed together the killing occurred rapidly and the combination was a cidal one. Occurrence of drug resistance was negligible when three drugs in combination were studied.

PZA kills selectively *M. tuberculosis* while the other mycobacteria are not affected by PZA.

Other Activities :

Since last six months the Tuberculosis Research Laboratory is engaged in evaluating a cheaper and potent combination of Trimethoprim and sulphonamides. The study was aimed to replace Sulfamethoxazole from the routine Trimethoprim and Sulfamethoxazole combination. Trimethoprim and Sulfadiazine were found to be excellent combination against *P. vulgaris*, *E. coli*, *Klebsiella pneumoniae*. Thus sulfamethoxazole can be replaced by sulfadiazine.

PHARMACOLOGY

1. S.A.S. Scheme :

This scheme is working on *Semicarpus anacardium*. The crude chloroform extract of *Semicarpus anacardium* and its purified fraction labelled as fraction II were studied for their acute toxicity. The LD₅₀ of crude chloroform extract and fraction II was 409 mg/kg and 1024 mg/kg respectively. Since fraction II was reported to have anticancer activity, it was checked for its cytotoxic effect: It produced 100% haemolysis of R.B.C. at 100 µg/ml. In addition to this general Pharmacology of crude chloroform extract and fraction II was carried out. Fraction II demonstrated histamine like activity on various isolated tissue preparations as well as *in-vivo* preparations, such as mouse paw oedema and vascular permeability study.

2. C.C.R.A.S. Scheme :

This scheme is working on various indigenous plants. Rhizomes of *Hedychium spicatum* (Sontakka) plant was powdered and serially extracted with pet. ether, chloroform and alcohol in a Soxhlet apparatus. The petroleum ether extract was observed to have non-specific antagonistic activity on various isolated smooth muscle preparations in *in-vitro* study.

3. *Ipoemea digitata* :

Fraction II, isolated from the petroleum ether extract was shown to exhibit estrogenic activity, i.e. increase in the uterine weight, glycogen content and water uterine retention. This activity was confirmed in both ovariectomized rats and mice. The fraction was about 50 times less potent than standard estrogen. The chemical investigation of the active fraction is under progress. The general pharmacology of the fraction II was done on the following preparations — guinea pig ileum, guinea pig tracheal chain, Rat uterus, Rat phrenic nerve diaphragm, cat B.p. and respiration and guinea pig isolated heart. Fraction II did not exhibit any effect on the above preparation.

4. *Dalbergia latifolia* (Biti) :

The seeds were collected, dried, crushed and extracted with boiling water. This water extract was tested for its pharmacological activity *in vivo* as well as *in vitro* preparations. Water extract was observed to have a positive ionotropic effect on isolated guinea pig heart. This work is continued using a serial soxhlet extraction of *D. latifolia* seeds with petroleum ether, chloroform, ethanol and water respectively.

5. *Dioscorea hispida* :

The potent depolarising type of alkaloid obtained from crude alkaline chloroform extract from the tuber of this plant was found to have a tentative structure of dimer of dioscorine or epidioscorine. The substance is very unstable, heat labile and with poor yield, so collected in large amount for structural elucidation.

6. Cholinergic involvement in histamine induced bronchoconstriction :

To study this, Konzett Roseller's method was standardised for studying airway resistance in guinea pigs using grass polygraph. Effect of physostigmine, an anti-cholinesterase agent, on histamine induced bronchoconstriction was studied. Physostigmine at 100 µg/kg. potentiated the effect of histamine induced bronchoconstriction.

PHYTOCHEMISTRY

1. *Vitex negundo* Linn :

Leaves were collected, dried in shade, powdered and thereafter extracted with petroleum ether and methanol. Also essential oil was obtained by steam distillation of powdered leaves. Experimental studies showed that with 0.05% pet. ext, 0.1% methanol ext. and 0.016% essential oil produced 100% mosquito larvicidal activity.

2. *Nerium indicum* Mill :

Nerium indicum, leaves were dried and powdered thereafter extracted with petroleum ether, chloroform and methanol serially. These extracts were evaluated for mosquito larvicidal activity, when petroleum ether extract was found most potent. Alumina column chromatographic fraction II (Benzene Methanol, 8:2) showed 100% mortality at 0.01% concentration.

3. Mosquito larvicidal (*Culex-p-fatigan*) activity of some fixed oil from indigenous plants :

Dried and powdered seeds of *Annona squamosa*, *Azadirachta indica* and *Pongamia pinnata* were extracted in soxhlet with petroleum ether, chloroform and methanol. All these extracts were

made free of solvents and tested for larvicidal activity. It was observed that petroleum ether extract of *Annona squamosa* produced 100% mortality at 0.04% concentration, *Azadirachta indica* pet. ether extract exhibited 100% mortality with 0.06% concentration while in case of *Pongamia pinnata* pet. ether extract 100% mortality was observed at 0.01% concentration. All observations were made at the end of 24 hours and experiments were done as per W.H.O. specifications.

Petroleum ether extract of *Pongamia pinnata* seeds was subjected to silica gel column chromatography. The elution was carried out using Benzene, Benzene : Methanol (95 : 5), Benzene : Methanol (1 : 1) and Methanol solvent systems. It was observed that fraction III (Benzene : Methanol, 95 : 5, Yellow band) showed 100% activity at 0.04% concentration. Residual studies of petroleum ether extract with 0.4% concentration produced 100% mortality for 16 days and 80% mortality for 23 days. This is very promising and will be further purified and can be developed as good vector control (mosquito larvicidal) agent.

RABIES RESEARCH UNIT

1. Developmental Work on Rabies Vaccine :

Adaptation of rabies virus to VERO Cultures — Three experiments were carried out using 10% or 20% of the seed virus employing absorption times of 30 minutes and two hours. The stock virus was clarified by centrifuging at 7500 x g before seeding and cultures were incubated at 32°C. Tissue culture fluids harvested at various intervals upto a period of 6 days did not yield any virus by inoculation in mice.

Immunologic studies on Rabies :

This work was pursued further. A constant total dose of vaccine was administered in different dose schedules to groups of mice. The mice were then challenged with a lethal dose of virus on different days.

It was observed that mice were better protected when they received two doses of vaccine three days apart (0-3 schedule) than when they were given a vaccine dose every day for five days (0-1-2-3-4 schedule). (Appendix XI A).

The study of the parameters of immunity led us to believe that the earliest protection was due to cellular immunity (on the 1st day) when neither specific antibody nor interferon were detectable.

Humoral Immunity after Vaccination in Man :

Further work on Humoral Immunity was carried out in the year under review. A total of more than 500 blood samples were

collected from human subjects undergoing different doses of AR Vaccine. Antibody tests were carried out by CIEP technique which was standardised earlier.

The results indicate that even after a full course of vaccination (5 ml x 14 injections), the sero conversion rate was less than 60%.

single booster dose given 7-10 days after the last dose increased the rate to about 80%. Surprisingly with 10 ml x 14 injections, (Appendix XI B) the conversion rate was around 94%. Partial and short courses (3, 5 or 7 injections) gave poor responses. The study warranted the conclusion that in high risk patients nothing less than a full course, preferably 10 ml per dose and followed by a booster dose must be recommended.

RADIATION BIOLOGY UNIT

Studies with labelled tetanus toxin :

The studies with labelled tetanus toxin were continued further. The aim was to find out the exact binding sites of the toxin to the various tissues either in the brain or in the spinal cord.

It was observed that no strict correlation exists between the binding to spinal cord *in vivo* and the production of local or general tetanus. It cannot be excluded that originally intact toxin is totally or partially converted in the body to derivative which becomes fixed to its target organ without maintaining a visible tetanus. That is why radioactivity in the spinal cord can outlast the duration of symptoms.

TOXICOLOGY

Sub acute toxicity studies in rats :

Zolone DT was incorporated in rats feed at the levels of 100 ppm, 1375 ppm and 2750 ppm and were fed to groups of rats along with the control group for 90 days. Further work including postmortem studies, haematological, biochemical and histopathological investigations in order to determine the toxic effects of the pesticide are in progress.

Sub acute toxicity studies in rabbits :

Zolone DT was applied to the backs of the rabbits in doses of 0.14, 0.28 and 0.56 ml/kg for the period of 21 days, along with control group. Further work including postmortem findings, haematological, biochemical and histopathological investigations in order to determine the toxic effects of the pesticide are in progress.

Toxicity studies of commonly used pesticides :

Completed acute dermal toxicity of Phenthoate 50 EC and Ethion 50 EC in rabbits. Ethion 50 EC was found to be more toxic

than Phenthoate 50 EC. The primary skin irritation effects of the following pesticides were studied — Phenthoate 50 EC, Methyl parathion 50 EC and Dimethoate 30 EC. All the three pesticides produced mild skin irritation when compared to control. Stomp 30% was studied for its effects on the mucous membrane. It was observed that Stomp 30% EC produced mild irritation to the mucous membrane (Appendix VIIIA).

Acute toxicity to chicken was conducted with Zolone EC and olone DT. Zolone EC was more toxic (MLD 1034 mg/kg) than olone DT (MLD 2200 mg/kg). Endosulphan technical and Zinc hosphide were tested for their toxicity to fish. Endosulphan technical was more toxic (LC_{50} 0.0025 ppm) in comparison to inc phosphide (LC_{50} 0.930 ppm) (Appendix VIII A).

VIROLOGY

In collaboration with the Cancer Research Institute, langur monkeys were shown to be 90% seroconverted following lepromin inoculation, thus showing, for the first time, that a locally, economically available non-human primate model for leprosy research exists. Secondly, also in the langur, it was shown that the ICRC strain of *Mycobacterium* strongly cross-reacts with the human strain, *M. leprae* (killed bacilli). The Director, C.R.I. is already in the U.S.A. to announce this global breakthrough and publications in international level journals are expected shortly.

It was shown that large fowl (hens and cocks) are capable of producing plasma factor following inoculation with rabies virus. Hitherto, only 1-6 day chicks were being used, but the volume of plasma from each bird was very small (about 0.5 ml). By using large birds, a single White Leghorn can yield upto 20 ml of plasma factor, thus making the preparation of this new antiviral antibiotic easier and more economical.

Another important research activity was the completion of the work on the HIBOMA-newborn rabbit model, specifically demonstrating the significant elevation of the SGOT-SGPT levels in rabbits infected acutely and chronically with HIBOMA virus.

Finally, it was discovered that the vanom from Russel's viper markedly enhances the spread of Rous sarcoma virus in chickens and markedly reduces the latent period of the tumour. This project, as well as others above are in progress.

ZOONOSIS

1. Plague :

The examination of rats for evidence of plague was continued. Every day 300-400 rats were examined. Besides rats, flea samples

from various localities of Maharashtra were also examined for plague by animal inoculation tests. The study of assessment susceptibility of rats to experimental plague was continued to record the susceptibility pattern of various rodents. *R. rattus* and *R. norvegicus* showed continued resistance to experimental plague infection, whereas *Bandicota bengalensis* was found to be highly susceptible.

2. Malaria :

The cultures of *P. vivax* were continuously maintained. The strains were used for studying nutritional requirements of the parasite. The effects of nutrients like para amino benzoic acid, hypoxanthine, glucose, peptones on parasite multiplication *in vitro* was studied. It was observed that the incorporation of glucose and peptones enhanced the parasite multiplication *in vitro*. Studies were carried out to observe parasite growth by using animal sera in place of human serum. These studies showed that with RPMI medium incorporated with extra peptone parasites could be maintained with animal sera.

Studies were also carried out to assess sensitivity of strains of *P. vivax*, obtained from Kasturba Hospital, to chloroquine.

3. Hookworm infection :

Ancylostoma ceylanicum was maintained in hamster model. Adult worms were collected from the intestine of the infected hamsters and antigen was prepared in PBS pH 7.2 after homogenization and centrifugation. Excretory and secretory antigens of adult worms were collected in MEM medium (without serum) at 37°C for 4 hours. Another lot of worms was replaced in the same medium for the further collection of ES antigen. Normal hamsters were inoculated intraperitoneally with such antigen and they were challenged with normal 100 larval dose orally after an interval of 5 days. Animals were sacrificed on the 20th day for the worm recovery. It was observed that there was a remarkable decrease in worm load in both ES and somatic antigen vaccinated hamsters.

4. Angiostrongyliasis :

The Slugs (*L. albae*) were collected, larvae were isolated from them and given to albino rats for the serology i.e. to detect serum antibodies. Experimental infection with 100 *A. cantonensis* larvae in albino rats indicated positive counterimmuno-electrophoresis test in serum from the day 5 to 375 after infection. Different rat groups infected with larval doses of 100, 500, 2000 and 5000 showed positive CIEP reaction on the 21st day post infection when preadult worms were seen in CNS. There was no positive CIEP reaction with dose of 15 larvae. Cerebral fluid of rats infected with heavy dose of 5000 larvae showed positive CIEP reaction on the 21st day.

Effect of 60 Co irradiated *A. cantonensis* larvae against the angiostrongyliasis in mice was evaluated with different exposure doses of 10, 20, 40, 60, 80 and 100 K rads. A lethal dose of 200 normal larvae was offered orally to each animal after 10 days post immunization. A remarkable worm reduction occurred in animals of 80 and 100 K rads irradiated larvae.

Schistosomiasis :

A fresh survey of schistosomiasis was carried out in the village Gimvi. There were two new cases of two male children 11 and 13 years old). They were showing terminal spine ova in the urine. Serological investigations for diagnosis of schistosomiasis were carried out using CIEP, IHA and ELISA tests. Attempts were made to infect laboratory animals with forked tail cercariae which have been collected from the snails (*Ferrissia tenuis*). Experiments are in progress.

Filariasis :

The vector mosquitoes (*Culex fatigans*) for human filariasis were collected from different wards of Bombay to study the presence of microfilariae. About 1000 mosquitoes were dissected and out of this only Khardanda and Bandra areas were found positive for microfilariae. Epidemiological survey was carried out in the endemic areas known as Papadi-Bassein to collect the positive sera samples for serological studies using different antigens.

7. Snake Research :

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

Two groups of six young snakes each were reared in captivity by offering them mice (8 to 10 gms) once a week as food. During the year 47 and 46 mice were eaten by each snake from group A and B respectively and 6 shedding of skins were observed in each snake in each group. Two snakes, from each group, were found dead. This was due to the fact that the casting of skin did not occur in case of one snake and there was continuous starvation in the case of another snake. After the age of two years snakes will be confined for pairing them for reproduction.

B. Rearing of young ones of Black scorpion in captivity :

Twenty four young scorpions were continued to be reared in the cage in the medium consisting of red soil, pieces of barks and water. Ventrally cut mice were kept in the cage as food for scorpions. During the year nine scorpions were found dead. The remaining scorpions will be weighed after completion of two years.

CHAPTER IV

GENERAL ACTIVITIES

Library

The Library has been collecting books on biomedical sciences and allied fields. It caters to the needs of scientists and students working in the fields of Communicable diseases, Pharmacology, Biochemistry, Zoonoses, Virology, Immunology etc. of this Institute and of Institutes all over India. The library has 7731 books and 17290 bound journals.

The Library Committee Members met thrice during the year and discussed library matters for its smooth functioning.

The British Council Library presented 71 Books and 34 Books were received from the World Health Organisation. The Springer Verlag Co. gifted 13 Books to the Library.

The inter library loan facility is now extended to 15 libraries. The library is open from 9-30 A.M. to 5-15 P.M. on all working days except Sundays and Public Holidays. The working of the library during the year 1983-84 is given in Appendix VI.

Reprographic Services

The library has a panaprint xerox machine and provides xeroxing facilities to the Institute as well as to outside members. 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. Total No. of pages xeroxed during 1983-84 are 6930 (approx) including few additional copies.

Artist section

This is a small section attached to the Department of Pharmacology. This section looks after the needs of the Institute concerning the preparation of slides, charts, photographs required by staff, research scholars, for teaching, thesis, conferences and also for exhibitions and demonstrations arranged by the Institute from time to time.

Sports club

The Haffkine Institute sports club was established on 2-10-58. It has ten different sections and each section is looked after by a separate secretary. There are facilities for Cricket, Badminton, Table tennis, Volley Ball, Carrom, Chess and Bridge. Members participate in Tournaments.

APPENDIX I
BACTERIOLOGY

Tests						Number
(1)	Bacteriological analysis of Chemicals, Disinfectants and misc. products					52
(2)	Water samples					19
(3)	Testing of water filters					17
(4)	Lyophilization of cultures					76 tubes
Total						164

APPENDIX II
BIOCHEMISTRY

Tests							Number
(1)	Blood	..	..	..	..	..	246
(2)	Urine	..	..	..	..	..	83
(3)	Special tests	..	..	..	..	..	156
Total :							485

APPENDIX III
CLINICAL PATHOLOGY

Tests						Number
(1)	A. Histopathology					1,467
(2)	B. Haematology					1,107
(3)	C. Cytology for malignant cells					13
(4)	D. Clinical Pathology					
	Urine for routine examination					253
	Stool for routine examination					193
	Semen examination					5
	Immunological Pregnancy Tests					646
	Biological (Rat test) Pregnancy Tests					11
(5)	E. Bacteriology					
	Culture for Tubercle Bacilli					23
	Animal Inoculation for Tubercle Bacilli					18
	Smear & Culture for C. diphtheriae					23
	C.S.F. Culture					8
	Urine Culture					315
	Stool Culture					33
	Blood Culture					12
	Sputum Culture					47
	Antibiotic Sensitivity Test					330
	Pus culture					177
	Cultures of Theatre swabs for Anaerobes					203
	Plural fluid culture					9
	Vaginal swab					2
	Synovial fluid					1
	Aspirated fluid culture					8

	Ear swab culture	..	..	..	..	5
	Bone marrow culture	..	..	..	..	1
	C.S.F. for A.F.B.	..	..	..	..	1
	Urine for A.F.B.	..	..	..	..	3
	Sputum for A.F.B.	..	..	..	..	2
	Pus for A.F.B.	..	..	..	..	1
	Nasal swab	..	..	..	..	1
	Vi Antibody titre	..	..	..	..	1
(6)	F. Serology					
	Widal test	..	..	..	..	68
	Weil Felix	..	..	..	..	2
	Brucellosis Test	..	..	..	..	2
	V.D.R.L. Test	..	..	..	..	24
	Paul Bunnel Test	..	..	..	..	40
Total :						4,955

APPENDIX III A CLINICAL PATHOLOGY

Hospital 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 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 Hospitals	.. General Hospital, Osmanabad.
Kasturba Hospital for Infectious Diseases	.. Civil Hospital, Dhulia,
J. J. Group of Hospitals	.. Civil Hospital, Nasik.
Police Hospital	.. Hindustan Aeronautics Ltd., Nasik.
Fertilizer Corp. of India	
B. J. Wadia Hospitals for Children	
Greater Bombay Municipal Dispensaries	

APPENDIX IV HUMAN PHARMACOLOGY AND BIOAVAILABILITY

Name of Company	Product
M/s. Citadel Fine Pharma	.. Tetracycline
M/s. Cachet Pharma	.. Sulpha + TMP.
M/s. SDWD Pharma	.. Rifampicin
M/s. Pilgaonkar	.. MDopa
M/s. Alembic Chemicals	.. Rifampicin
M/s. Hindustan Antibiotics Ltd.	.. Ampicillin
M/s. Triumph Pharmaceutical	.. Erythromycin
M/s. Blue Cross Pharma	.. Rifampicin
M/s. Haffkine Biopharma	.. Rifampicin
M/s. Lupin Laboratory	.. Rifampicin
M/s. Alkam Laboratory	.. Rifampicin
M/s. Cachet Pharmaceutical	.. Sulpha + TMP

APPENDIX V
IMMUNOHAEMATOLOGY

Tests	Number
Transfusion	758
Cross matching	776
Rh-antibodies titre	92
Blood group	15
Total :	1,641

APPENDIX VII
TESTING

Tests	Number
Prohibition & Excise Testing	
(1) Indian made Foreign Liquors	983
(2) Beer	940
(3) Toddy	1,010
(4) Ganja	86
(5) Bhang	103
(6) Charas	2
Total :	3,124

Commercial Testing

(1) Polymorph	37
(2) Insulin (Bio-assay)	58
(3) Antitoxins	06
(4) Pyrogen	72
(5) Sterility	07
(6) Oxytocin activity	03
(7) Ultra violet scanning	11
(8) Adrenaline (Bio-assay)	11
(9) Rideal-Walker Co-efficient and Staphylococcal Co-efficient	20
(10) Complete analysis	14
Total :	229

APPENDIX VIII
TOXICOLOGY

Sr. No.	Classification of sample	Number Tested	Toxicity Parameter
1.	Chemicals	7	Acute oral toxicity.
		2	Primary skin irritation study.
	Pesticides	2	Acute dermal toxicity.
		3	Primary skin irritation.
		1	Mucous membrane irritation.
		2	M.L.D. in chicken.
		2	Acute toxicity in fish.
	Painted containers	1	Acute toxicity study in mice by feeding water (stored in these containers) for the period of 7, 14 and 28 days ad lib for 7 days.

APPENDIX VI

LIBRARY

The following figures indicate the working of the library during the year 1983-84

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.
2000	1346	56	90

Additions to Library

Number of books added during the year	Number of Vol. Bound	Number of Current Periodicals.	Number of Current Periodicals recd. free of charge.	Number of reports recd. from scientific institutions.
261	605	149	75	22

APPENDIX VIII A
TOXICOLOGY

Sr. No.	Sample	Species	LD ₅₀	Fiducial limits/ Remarks
Acute oral toxicity in mice :				
1.	Katarang-100	Mice	> 10 ml/kg	
2.	Linoxyl CS-40	Mice	> 10 gm/kg	
3.	Vytal MS 5	Mice	> 15 gm/kg	
	Dendrite HM : 5070	Mice	> 5 gm/kg	
	Vishwas flocks	Mice	> 10 gm/kg	
	Pidicryl 8716	Mice	> 10 ml/kg	
	Waste polypropylene wax	Mice	> 5 gm/kg	
Acute dermal toxicity in rabbits :				
	Phenthoate 50 EC	Rabbits	530.8 mg/kg	279.24-1008.5 mg/kg
	Ethion 50 EC	Rabbits	265.0 mg/kg	182.8-384.4 mg/kg
C. Primary skin irritation study in rabbits :				
1.	Phenthate 50 EC	Rabbits	Mild skin irritation	
2.	Methyl parathion, 50 EC	Rabbits	Mild skin irritation	
3.	Dimethoate 30 EC	Rabbits	Mild skin irritation	
	3 M Silicon Fluid-100 CST	Rabbits	No irritation	
5.	Pidicryl 8716	Rabbits	Mild skin irritation	
D. Mucous membrane irritation study in rabbits :				
1.	Stomp 30% EC	Rabbits	Mild irritation	
E. Minimum lethal dose (MLD) in chicken :				
1.	Zolone EC	Chicken	Minimum lethal dose-1034 mg/kg Maximum lethal dose (Killing 100%)-10340 mg/kg	
2.	Zolone DT	Chicken	Minimum lethal dose-2200 mg/kg Maximum lethal dose (100% mortality) 17,600 mg/kg	
F. Acute toxicity in fish :				
1.	Endosulphan technical	Fish	96 hrs LC ₅₀ — 0.0025 ppm	
2.	Zinc phosphide technical	Fish	96 hrs LC ₅₀ — 0.930 ppm	
G. Toxicity studies of painted containers :				
1.	Painted containers		No toxicity was observed in the animals when compared with the controls.	
	Tap water was stored in containers for the period of 7, 14 and 28 days and it was fed ad lib to mice for 7 days.			

APPENDIX IX
VIROLOGY

Vaccine administered							Number of patients
Antirabic	..	..	..	..	..	..	483
Cholera	..	..	..	..	..	..	13
Typhoid	..	..	..	..	..	..	29
Measles	..	..	..	..	..	..	10
Triple ..	..	..	..	..	..	..	158
Oral polio	..	..	..	..	..	..	166
Total :							859

APPENDIX X
ZOOONOSIS

Snakes	Animals procured	Venom produced	Venom supplied
Cobra	115 Nos.	68.80 gms	127.52 gms
Russell's viper	93 Nos.	16.75 gms	4.20 gms
Krait	42 Nos.	2.47 gms	15.69 gms
Echis	2 Nos.	—	30.03 gms
Total :	252 Nos.	88.02 gms	177.44 gms
Scorpions			
Red Scorpion	8728 Nos.	10.08 gms	1.38 gms
Black Scorpion	815 Nos.	0.31 gms	1.04 gms
Total :	9543 Nos.	10.39 gms	2.42 gms

APPENDIX X-A
ZOOONOSIS

No. of visitors that visited the Snake Farm						
A. Students	..	..	..	..	..	2,458 Nos.
B. Staff of Scientific Institutions	..	..	..	..	..	162 Nos.
C. Distinguished visitors	..	..	..	..	..	114 Nos.
D. Foreigners	..	..	..	..	..	59 Nos.
E. Thursday visitors	..	..	..	..	..	2,928 Nos.
F. Others	..	..	..	..	..	239 Nos.
Total :						5,960 Nos.

Sixteen snakes were received for identification from hospitals and individuals during the year.

APPENDIX XI
RABIES RESEARCH UNIT

results of laboratory tests on tissue specimens received for diagnosis of rabies

Type of sample	Number received	Number tested	Results	
			(+) for Negribody/ Histopathology	(+) By mouse inoculation
Human brain	7*	5	2	2
Cow brain (in formalin)	1	1	1	Not done
Dog brain (in formalin)	1	1	Nil	Not done

* One sample was contaminated and one sample highly toxic to mice.

APPENDIX XIA
RABIES RESEARCH UNIT

Study of early protection after 2 different Schedules of Rabies Vaccination

Vaccine schedule	Day* of Challenge**	No. of mice	Results of protection tests	
			Mortality Ratio (MR)	Average Survival Time (AST) Days
(0-3)	4	22	14/22 ($p < 0.05$)	13.27 ($p < 0.001$)
(0-1-2-3-4)	4	21	18/21	11.05 ($p < 0.01$)
(0-3)	6	21	4/21 ($p < 0.001$)	18.81 ($p < 0.001$)
(0-1-2-3-4)	6	22	12/22 ($p < 0.01$)	14.09 ($p < 0.001$)
(0-3)	8	19	1/19 ($p < 0.001$)	20.42 ($p < 0.001$)
(0-1-2-3-4)	8	21	9/21 ($p < 0.01$)	16.48 ($p < 0.001$)
Control	—	14	14/14	7.29

* Day from first dose of vaccine

** 25-30 LD₅₀/0.2 ml intramuscularly in adult mouse

APPENDIX XI B
RABIES RESEARCH UNIT

Antibody Response rates in 195 persons after full Course
(5 ml x 14 injections) of AR Vaccination

Sr. No.	Category V* + B**	No. of samples tested by CIEP	Number (+)	Percent (+)
1.	14 + 0	56	29	51.8
2.	14 + 7	52	30	57.7
3.	14 + 14	35	17	48.5
4.	14 + 30	34	17	50.0
5.	14 + 60	8	3	37.5
6.	14 + 180	10	1	10.0

* Number of Vaccine doses.

** Time interval (days) after last injection when blood sample was drawn for antibody assay.

APPENDIX XII
Post-graduate Courses Offered

MSc.	—	Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology.
MSc. (By papers)		Microbiology, Biochemistry.
PhD.		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)	..	..	..	..	..	22
Course in Drug Analysis	..	..	..	..	..	14
Short Term Training Course	..	..	..	..	..	4

Research Fellowship and Scholarship

Jr. Research Fellowship (Platinum Jubilee)	..	..	..	2
Jr. Research Scholarship (Chinchwad Land)	..	..	..	2

APPENDIX XIII A

**List of students who registered during the year 1983-84
for M.Sc., Ph.D., M. Pharm. and M.D.**

M.Sc.	Ph.D.
1. Kum. N. Chakraborty	1. Shri N. K. Sutar
2. Kum. S. M. Mohite	2. Kum. V. R. Pradhan
3. Kum. S. M. Tatke	3. Shri A. G. Mitra
	4. Shri A. P. Kahar
	5. Shri K. R. Pawar
	6. Smt. L. R. Khalid
	7. Kum. M. N. Nihalani
M. Pharm	M.D.
1. Kum. C. N. Desai	1. Smt. M. S. Ghan

APPENDIX XIV
Degree Awards during the year 1983-84

Name of Candidate	Subject	Topic
1. Mrs. V. R. Kamath	Ph.D. Applied Biology	Studies of Immune response due to <i>Ancylostoma ceylanicum</i> .
2. Mrs. S. R. Nair	Ph.D. Applied Biology	Studies on the Development of Co ⁶⁰ irradiated antirabies vaccine.
3. Mrs. P. S. Rane	Ph.D. Applied Biology	Immunological studies in Malnutrition.
4. Mrs. Shaila Narayan	Ph.D. Microbiology	Study of some immunity parameters in experimental rabies.
5. Kum. N. D'Souza	Ph.D. Biochemistry	Studies on the effects of under-nutrition and Hepatotoxic Agents on lipid peroxidation.
6. Shri S. V. Gharat	Ph.D. Biochemistry	Immuno-Biochemical studies of hepatitis.
7. Shri R. B. Ghooi	Ph.D. Pharmacology	Studies on Anaphylaxis Mechanism and control.
8. Kum. S. S. Mule	M.Sc. Microbiology	Studies on Antibody response of human subjects.
9. Kum. S. Menon	M.Sc. Zoology	Immunity in Experimental Ancylostomiasis
10. Dr. (Mrs.) R. A. Deshmukh	M D. Pathology	—

APPENDIX XV
Post-Graduate Teachers

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

Name	Qualifications
Dr. K. D. Sharma	M.D., M.Sc. (Medical)
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.
Dr. V. R. Rao	M.V.Sc., Ph.D.
Dr. F. L. Saldanha	M.Sc., Ph.D., F.I.C. (Cal)
Dr. A. J. Baxi	M.Sc., Ph.D.
Dr. F. Fernandes	M.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. H. J. Miansharamani	M.B.B.S., Ph.D.

APPENDIX XVI

Papers presented at National and International Conferences

Fourth National Seminar on Zoonoses and their Control, held in January, 1984.

A. K. Patil and D. M. Renapurkar: Studies on halophilic vibrios in deep sea off Maharashtra coast.

B. D. Sathe and D. M. Renapurkar: Study of experimental infection of laboratory animals with schistosome cercariae and prevalence of animal schistosomiasis in village Gimvi.

D. M. Renapurkar: Current status of enzootic rodent plague in India.

D. M. Renapurkar, R. P. Bhamaria and R. P. Hemkar: Studies on susceptibility of commensal rats to experimental plague infection in Greater Bombay.

'D. R. Ashtekar, B. G. Khadse, F. Fernandes, M. N. Deodhar, R. N. Kale, R. D. Ganatra, M. V. N. Shirodkar, and V. R. Deshpande: Rapid Drug Screening Techniques in Mycobacteriology.

— Abstract No. B-16, Page No. 38.

Gandhi, A. R. and Shirodkar, M. V. N.: Plasma factor, an antiviral antibiotic distinct from interferon.

— Abstract 14-15, Bombay, India, January 9-11, 1984.

Lalita S. Limaye, M. K. Bhopale, D. M. Renapurkar, V. R. Pradhan and K. D. Sharma: A study of angiostrongyliasis in Bombay.

R. P. Hemkar and D. M. Renapurkar: Observation on distribution of commensal rats in Greater Bombay area.

Shirodkar, M. V. N.; Chanderkar, N. G.; Deshmukh, R. A.; Kinare, S. G. and Bapat, R. D.: A new non-human primate model for viral hepatitis B infection and its possible implication for viral zoonosis.

— Abstracts, 21.

Shirodkar, M. V. N.: The Zoonoses: epitome of unity in diversity in biology — a tribute.

— Souvenir.

Sandhya S. Mule and H. J. Mansharamani: Rapid screening of rabies antibodies in human and animals sera by CIEP technique.

Shaila Narayan, H. J. Mansharamani and K. D. Sharma: Vaccine-induced factors contributing to protection in experimental rabies.

H. J. Mansharamani, C. N. Dandawate and B. G. Krishnamurthy: Experimental vaccine against Kyasanur Forest Disease (KFD) — Some laboratory data on its preparation and immunogenicity.

Sushila Menon and M. K. Bhopale: Evaluation of UV-irradiated larval vaccine *Ancylostoma ceylanicum* in experimental host.

Vijayalaxmi R. Kamath, M. K. Bhopale and M. B. Bhide: Immunological evidence of chemotherapeutic action of mebendazole against *Ancylostoma ceylanicum* in hamster (*M. auratus*).

Second International Conference, Germany (West) May, 1983

S. R. Chavan: Chemistry of Alkanes separated from leaves of *Azadirachta indica* and their Mosquito Larvicidal/insecticidal activity as Vector Control Agents.

APPENDIX XVII

Papers Published 1983-84

A. B. Kulkarni, M. K. Bhopale and K. D. Sharma : Pulmonary superoxide dismutase activity in mice infected with *Ancylostoma caninum*.

— J. of Helminthology 57, 237-239, 1983.

A. S. Bobade, S. R. Lokhande, Mrs. M. R. Patel and B. G. Khadse : Synthesis and antitubercular studies of 2-substituted alkyl or arylamino-6-methoxy benzothiazoles.

— Bull. Haff. Inst., 11 (2), Aug. 1983.

A. V. Pande, S. R. Lokhande and B. G. Khadse : Synthesis of 2-substituted-phenyl amino thiazoles and substituted phenyl isothiocyanates as antituberculosis agents.

— Indian J. Chem., Sec. B., March, 1983.

C. R. Savitri and D. M. Renapurkar : Iodine uptake studies in cotton rat filariasis.

— Folia Parasitologica (Praha) 30, 43-46, 1983.

L. S. Limaye, M. K. Bhopale, D. M. Renapurkar and K. D. Sharma : The distribution of *Angiostrongylus cantonensis* worms in central nervous system of rats.

— Folia Parasitologica (Praha) 30 (3) 281-284, 1983.

M. Kishore and P. Ramakrishnan : Comparison of the hypoglycemic effects of three different extracts from *Bordetella pertussis*.

— Bull. Haff. Inst. 11 (1), 7-10, 1983.

M. Kishore and P. Ramakrishnan : Purification of blood sugar lowering (Hypoglycemic) factor from *Bordetella pertussis*.

Proc. 1st Annual Conf. Assoc. of Clinical Biochemists of India, Bombay 30-31, 1984.

R. Mirchandani, K. D. Sharma, D. S. Chitnis, R. S. Kamat : Colonisation factor antigen and serogroups of enterotoxigenic *Escherichia Coli*.

— Current Science. 57, 308, 1983.

R. S. Kamat and Vineeta Bal : Immunity in Salmonellosis.

— Bull. Haff. Inst. 11, 1, 1983.

Sandhya S. Mule, H. J. Mansharamani : Suitability of Counter-Immuno-electrophoresis for detection of Antibodies to rabies virus.

— Bull. Haff. Inst. 11 (2), 41-48, 1983.

S. Bhogale, K. D. Sharma and R. S. Kamat : Role of Heat labile antigens of *S. flexneri* in HeLa cell invasion.

— J. Med. Microbiology 16, 37, 1983.

Shaila Narayan, H. J. Mansharamani, K. D. Sharma : Immune Status of experimental mice following different dose-schedules of Rabies Vaccination.

— Bull. Haff. Inst. 11 (2), 33-40, 1983.

S. Hattikudur and R. S. Kamat : Characterisation of a target antigen of Cellular Immunity in the sonic extract of *M. tuberculosis* H37Rv.

— J. Med. Microbiology 18, 17, 1984.

S. R. Chavan, Neena P. Shah and S. T. Nikam : Individual and synergistic activity of some essential oils as Mosquito Larvicidal Agents.

— Bull. Haff. Inst. 11 (1), 18-21, 1983.

Vineeta Bal and R. S. Kamat : Enzyme-linked immunosorbent assay for the diagnosis of tuberculous meningitis.

— Ind. J. Med. Research 78, 477, 1983.

V. M. Rangnekar, S. R. Lokhande, R. P. Bhamaria and B. G. Khadse : Synthesis of N-aryl/substituted methyl/heteroaryl-x-pyrrolidino/piperidino succinimides as antituberculosis agents.

— Indian J. Chem., 22 b, Oct. (1983), 1070-71.

V. R. Kamath and D. M. Renapurkar : Laboratory trials of pirimiphos methyl a new organophosphorus insecticide against insects of medical importance.

— Pestology 8 (3) 28-30, 1984.

APPENDIX XVIII

Papers sent for Publication

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

— Bull. Haff., 1984.

A. V. Panda, P. A. Kirke and B. G. Khadse : Synthesis and study of 2-benzyl-thiobenzothiazoles and 2-benzylthiobenzimidazoles as antituberculosis agents.

— Bull., Haff. 1983.

B. D. Sathe and D. M. Renapurkar : Studies on snails in village Gimvi, a focus of human schistosomiasis in India.

— Bull. Haff., 1983.

B. D. Sathe and D. M. Renapurkar : Periodicity of output of schistosome eggs in the urine samples of infected persons in an endemic area of schistosomiasis in village Gimvi, India.

— Bull. Haff. Instt. 1983.

M. K. Bhopale, L. S. Limaye, V. R. Pradhan, D. M. Renapurkar and K. D. Sharma : (1984) Studies on suspected clinical and experimental angiostrongyliasis : serological responses.

— J. Hyg. Epid. Microbiol. Immunol.

M. K. Bhopale, Vijayalaxmi R. Kamath and Sushila Menon : *Necator americanus* in infant rabbit model and its infectivity in other hosts.

— Helminthologia (1984).

Sandhya Hattikudur and R. S. Kamat : Polymorphism of a mycobacterial antigen participating in cell mediated immunity.

— J. Med. Microbiology (1984).

Sushila Menon and M. K. Bhopale: *Ancylostoma ceylanicum* (Zooss, 1911) in golden hamsters (*Mesocricetus auratus*): Pathogenicity and humoral immune response to a primary infection.

— J. Helminthology (1984).

Swati Kulkarni and R. S. Kamat: Cross-reactivity in Cell Mediated Immunity of a typical mycobacteria.

— Jour. Med. Microbiology.

Vidya Pradhan and D. M. Renapurkar: Antimalarial activity of Sudarshan Ghanvati — an Ayurvedic preparation.

— Bull. Haff. Instt. (1983).

Vidya R. Pradhan and D. M. Renapurkar: Protection against *P. b. yoelii* in chloroquine and Pyrimethamine treated mice.

— Folia Parasitologica.

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

— Indian J. Pharm. Sci.

V. R. Kamath, Sushila Menon, M. K. Bhopale and D. M. Renapurkar: Experimental chemotherapy of *Angiostrongylus cantonensis* infection in mice

— Annals of Trop. Med. Parasit (1984).

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

— Ind. J. Med. Research (1984).

Published by :
Director, Haffkine Institute
Parel, Bombay-400 012

for the year
April 1983 to March 1984
