

Annual Report of the Haffkine Institute

1980 - 1981



CONTENTS

	Pages
Administration :	
Governing Council 	1
Academic Council 	3
Advisory Council for Research 	3
Executive Committee 	3
Staff of the Institute 	4
 Introduction : Highlights of Activities of Departments 	 7
Departments 	11
Development of Teaching 	19
 Research Progress :—	
Bacteriology 	20
Biochemistry 	20
Chemotherapy 	23
Clinical Pathology 	26
Parasitic Diseases and Zoonoses 	27
Pharmacology 	30
Rabies Research Unit 	32
Phyto Chemistry 	37
Radiation Biology 	39
Virology 	40
 General Activities	 43
 Appendices :—	
I Bacteriology Department 	44
II Clinical Pathology, Routine 	44
III Biochemical Tests 	45

	Pages
IV Clinical Pathology	45
V Human Pharmacology and Bioavailability Unit	45
VI Routine Diagnostic Work (Rabies Research Unit)	46
VII Radiation Biology Unit	46
VIII Testing Department	47
IX Testing Department	47
X Samples Tested for Toxicity	48
XI Acute Oral Toxicity of Some Pesticides	49
XII Acute Dermal Toxicity Studies of Some Pesticides	50
XIII Courses Offered	51
XIV Post-Graduate Teachers	51
XV Statistics Relating to Students	52
XVI Short Term Training Courses	52
XVII Trainees and Research Fellowships and Scholarships	52
XVIII Degree Awards during the Year	53
XIX Venom produced and supplied during the year 1980-81	54
XX Papers Published 1980-81	55
XXI Papers Submitted for Publication	56
XXII Papers Presented in Conferences	59

**HAFFKINE INSTITUTE FOR TRAINING
RESEARCH AND TESTING**

GOVERNING COUNCIL

President

Dr. BALIRAM W. HIRE,
Honourable Minister for Public Health, Government of Maharashtra,
Mantralaya, Bombay-32.

Vice-President

Shri SATISH CHATURVEDI,
Honourable Minister of State for Public Health,
Government of Maharashtra,
Mantralaya, Bombay-32.

Member-Secretary

Dr. K. D. SHARMA,
Director, Haffkine Institute, Parel, Bombay-32.

Members

Secretary to Government of Maharashtra, Urban Development
and Public Health Department, Mantralaya, Bombay-32.

Shri N. V. SUNDARARAMAN

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

SHRI A. S. RAO

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

Dr. RANJIT SEN

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

SHRI V. C. SANE

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

Dr. V. G. GANLA.

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

Prof. V. RAMLINGSWAMI.

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

Prof. RAM JOSHI.

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

Dr. C. G. PANDIT.

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

Dr. (Smt.) M. R. CHANDRIKAPURE.

ACADEMIC COUNCIL

Chairman :

Dr. K. D. SHARMA

Members :

Dr. V. D. DESHMUKH
Dr. S. S. GOTHOSKAR
Dr. (Smt.) S. M. VACHHA
Dr. M. B. BHIDE
Dr. V. V. KAYARKAR upto 4-11-1979
Dr. (Smt.) M. R. CHANDRIKAPURE

Convener :

Dr. S. MUKERJI

ADVISORY COUNCIL FOR RESEARCH

Chairman :

Dr. K. D. SHARMA

Members :

Dr. C. G. PANDIT
Dr. A. B. CHOWDHURY
Dr. RANJIT SEN
Dr. K. K. G. MENON
Dr. NITYA NAND
Dr. T. H. RINDANI
Dr. R. D. GANATRA

Convener :

Dr. D. M. RENAPURKAR

EXECUTIVE COMMITTEE

Chairman :

Dr. K. D. SHARMA

Members :

Dr. M. V. N. SHIRODKAR
Dr. R. S. KAMAT
Dr. S. T. VANJARI

Convener :

Dr. M. H. SHAH

**STAFF OF THE HAFKINE INSTITUTE
ADMINISTRATION**

Director :

K. D. SHARMA, MBBS, MSc, MD

Chief Administrative Officer :

S. T. VANJARI

DIRECTOR'S STAFF

Scientific Secretary :

R. B. GHOOI, MSc

Accounts Officer :

S. G. MORE

BACTERIOLOGY

Senior Scientific Officer Class I :

S. V. GADRE, MSc, PhD

Junior Scientific Officer :

R. P. BHAMARIA, MSc, PhD

BIOCHEMISTRY

Senior Scientific Officer Class I :

P. RAMKRISHNAN, MSc, PhD

R. N. JOSHI, MSc

Junior Scientific Officer :

A. B. KULKARNI, MSc

INSTRUMENTATION CELL

Junior Scientific Officer :

J. V. SAWANT, MSc

IMMUNOLOGY

Senior Scientific Officer Class II :

Smt. S. N. HATTIKUDUR, MSc

CHEMOTHERAPY

Assistant Director :

M. H. SHAH, MSc, PhD

Senior Scientific Officer Class I :

D. T. CHAUDHARI, MSc, PhD

B. G. KHADSE, MSc, PhD

Senior Scientific Officer Class II :

P. P. DALVI, MSc

Junior Scientific Officer :

M. R. PATEL, MSc

N. T. JAOKAR, MSc

CLINICAL PATHOLOGY

Assistant Director :

R. S. KAMAT, MBBS, MD

Senior Scientific Officer Class I :

D. S. CHITNIS, MSc

Junior Scientific Officer :

J. P. MEHTA, MSc

Medical Officer :

Y. FERNANDES, MBBS, DPB

RANJANA A. DESHMUKH, MBBS

IMMUNOHAEMATOLOGY

Senior Scientific Officer Class I :

D. CHENGAPPA, MBBS

PHARMACOLOGY

Assistant Director :

M. B. BHIDE, MBBS, MD

Senior Scientific Officer Class II :

R. S. JOSHI, MSc

TRAINING

Senior Scientific Officer Class I :

S. MUKERJI, MBBS, MSc, PhD

VIROLOGY

Assistant Director :

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

Medical Officer :

CHHAYA H. PANDIT, MBBS

ZOOONOSIS

Assistant Director :

D. M. RENAPURKAR, MSc, PhD

Senior Scientific Officer Class II :

M. K. BHOPALE, MSc, PhD

Junior Scientific Officer :

T. G. TARE, BSc
B. D. SATHE, MSc
V. R. KAMATH, MSc
N. K. SUTAR, BSc

STATISTICAL SECTION

A. S. AREKAR, MSc

PHYTOCHEMISTRY

Senior Scientific Officer Class I :

S. R. CHAVAN, MSc

Junior Scientific Officer :

S. T. NIKAM, MSc
R. P. SUBHEDAR, MSc

HUMAN PHARMACOLOGY AND BIO-AVAILABILITY

Senior Scientific Officer Class I :

N. G. CHANDERKAR, MBBS, BSc, PhD

Junior Scientific Officer Class II :

S. S. SATHE, MSc

RADIATION BIOLOGY

Senior Scientific Officer Class I :

N. G. CHANDERKAR, MBBS, BSc, PhD

RABIES RESEARCH UNIT

Senior Scientific Officer Class I :

H. J. MANSHARAMANI, MBBS, MSc, PhD

TESTING LABORATORY

Senior Scientific Officer Class I :

H. K. SONI, MSc

Junior Scientific Officer :

H. K. DALAL, BSc
S. S. JAIN, MPharm
S. B. DHOLAM, MSc

TOXICOLOGY

Assistant Director :

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

Senior Scientific Officer Class II :

M. G. PATHAK, MSc

CLINICAL RESEARCH DIVISION

Senior Scientific Officer Class II :

A. P. KULKARNI, MSc

CHAPTER I

INTRODUCTION

The year 1980-81 proved to be a year of extreme financial stringency. Rising costs of chemicals, equipment and experimental animals coupled with limited resources available at the disposal of the Institute seriously affected various research programmes. It was not possible to fill up a number of vacant positions for similar reasons. In spite of financial problems significant strides were made on various fronts and it is a matter of pride for us to highlight our achievements in the present narrative.

To begin with, this year marked the beginning of a new project on the development of an invasive strain of **Salmonella enteritidis** carrying V. cholerae 'O' antigens at the Department of Bacteriology. The methodology for this enterprise consists of employing primary techniques in genetic engineering such as protoplast fusion to obtain recombinant strains. The object of this study is to select out a strain of vibrio with invasive properties. A strain of this type could be expected to evoke a better and sustained immune response and hence could be a candidate for improved cholera vaccine at a later stage. This project marks the entry of the Haffkine Institute in the field of genetic engineering.

In our studies on the blood sugar lowering factor from **Bordetella pertussis** attempt to obtain purified preparations from cultivated pertussis cells was pursued. The reversed passive haemagglutination assay (RPHA) was indigenously developed and standardised for the detection of hepatitis B surface antigen (HB_sAg), the marker for hepatitis B virus. This RPHA was found to be about 400 times more sensitive than counter-immuno-electrophoresis.

Synthesis of chemical compounds and their screening for biomedical activities like anthelmintic, antimalarial, antifungal and antituberculosis properties was vigorously pursued at the Dept. of Chemotherapy. In all 296 compounds were synthesised and screened. About 30 drugs were observed to exhibit significant antimalarial properties. Further studies are continued with promising leads to explore their possible use in malaria chemotherapy.

At the Director's Laboratory pharmacological studies were carried out on the active fractions from extracts of **Semicarpus anacardium** to identify anticancer principles. These studies have demonstrated potent analgesic, anticonvulsant and anti-inflammatory activities in various fractions.

In an epidemiological study of enterotoxigenic **E. coli** diarrhoeas at the Department of Clinical Pathology very interesting features were observed. A large number of isolates were found

to have atypical features, namely, they had no fimbrial antigens and they did not belong to classical serogroups. In another study, relative importance of cellular and humoral immunity in salmonellosis was investigated and the role of both the functions was confirmed.

In a study of mycobacteria the antigen of *M. tuberculosis* H₃₇Rv responsible for delayed hypersensitivity was identified to be a lipoprotein with its apoprotein moiety having the approximate molecular weight of 150,000 daltons.

In the leprosy research programme in addition to screening of indigenous and synthetic drugs against leprosy using Shepard's mouse foot pad technique, a study was initiated in the department of Pharmacology to explore the possibility of using a vaccine prepared from ICRC bacilli against leprosy. The vaccine was found to be promising and has been sent for phase I and II clinical trials.

In order to develop a suitable tissue culture vaccine against rabies, a cell strain from an aborted fetus obtained from KEM Hospital has been established at the Rabies Research Unit. A purified and concentrated rabies virus antigen has been prepared for the detection of rabies antibody by counterimmuno-electrophoresis. This method eliminates the need to employ laboratory mice and yields results within a few hours.

The problem of developing alternative insecticides for vector control has assumed special significance with the resurgence of malaria and increase in the population at risk of filariasis and other vector borne diseases. With the realisation that plants could be a potential source of vector control agents, a programme is being vigorously pursued at the Phytochemistry Unit to screen various indigenous plants for insecticidal activity. Among the plants examined, *O. sanctum*, *O. basilicum* and neem plants are observed to have insecticidal properties. Further studies are being pursued to identify the insecticidal principle and explore the possibility of using the material for vector control programmes.

The story of success achieved at the Department of Virology in experiments directed towards further developing a new, cheaper animal model system for the cultivation of human hepatitis B virus has been documented in the earlier report. The studies show that in langur monkeys we have a suitable model for investigating various aspects of human hepatitis B.

A procedure yielding human leucocyte interferon from fresh buffy coat has been standardised and about 2.5 litres of interferon has been obtained from leucocytes from fresh human blood.

In the study of endemic focus of schistomiasis situated in the village Gimvi, more attention now is focussed on snail reservoirs

of infection and the extent of distribution of cercariae in various snail species.

One of the major achievements of the department is the detection of a focus of **Angiostrongylus cantonensis** infection in rats and slugs (molluscs) in Greater Bombay area for the first time.

This is a nematode parasite and the infection in man leads to eosinophilic meningitis. The infection is fairly common in South East Asia and the Pacific Islands, but is probably being reported from India for the first time. The notable feature of the study is the demonstration of invertebrate and vertebrate reservoir hosts in the area.

In filariasis research programme several attempts were made to infect various laboratory animals including infant rabbits with human filarial parasite **W. bancrofti**. Although our attempts did not prove fruitful, we propose to pursue our efforts in future.

Apart from several projects highlighted above, the Institute continued its research activities on various aspects of other communicable diseases. There was active participation by our Institute in several health programmes undertaken by Health Dept. of Govt. of Maharashtra, I.C.M.R. and Director General of Health Services.

Venomous animals research Unit

The institute is bestowed with a unique venomous animals research unit, a part of Zoonosis Department. The unit maintains four types of poisonous snakes viz., cobra, viper, krait and sawscaled vipers and two types of scorpions viz. **Buthus** sp. and **Palamnaeus**. The major purpose of this unit is to obtain venom for research and antivenin production. The unit also undertakes to educate masses in matters relating to identification of snakes and 'First aid' steps in the event of a snake bite.

Toxicology

This recently started department has assumed a prominent position among toxicology departments existing in India. The department has been actively involved in toxicological evaluation of different types of pesticides, drugs, chemicals and plastics, referred by Haffkine Bio-pharmaceutical Corporation Ltd. and other Government and non governmental agencies and has become a centre for toxicological research.

Testing

A large number of drugs, chemicals, antibiotics and biological products were tested in Testing, Toxicology and Bio-availability units. Besides, several commercial insecticide and rodenticide

preparations were evaluated for bio-efficacy in the Dept. of Zoonosis. The Testing Unit examined a number of pharmaceutical and biological products as per specifications laid down by I P C.

At the Bio-availability Unit assays were undertaken to estimate the levels of various drugs and antibiotics in subjects receiving these substances.

Blood Transfusion

The Institute continued its activity to provide transfusion facilities to the needy and poor and help alleviate the misery of patients suffering from various blood disorders. Research on hemolytic anemias was pursued by ICMR sponsored Blood Group Reference Centre attached to this Unit.

Sponsored Research Projects

The Institute received support from national and international agencies to carry out research projects sponsored by these agencies. Prominent organisations who lent their support for these programmes include Indian Council of Medical Research, Department of Science and Technology, Ministry of Defence, CCRASSI Dept. of Science and Technology, Govt. of Maharashtra and ICAR. The Institute is indeed grateful to these organisations for their support at this critical juncture.

Advisory Council and Committees

Various members of the Governing Council along with its Chairman, The Hon'ble Minister for Health, have all contributed towards the successful march of the Institute for which they deserve all praise. Their task was ably supported by organisations like Academic Council, Executive Committee, Advisory Council for Research, etc. and other similar bodies. The Director thanks members of various advisory bodies for their assistance.

CHAPTER II

DEPARTMENTS

BACTERIOLOGY

All the three students registered for M.Sc. (Part II) by papers in Microbiology secured first class, one of them topping the University with 72% marks. Of the five students registered for M.Sc. (Part I), four secured first class and one secured a higher second class. The dissertation titles of M.Sc. (Part II) students working under Dr. S. V. Gadre are: (1) Microbial detoxification of meta-dinitrobenzene in effluent; (2) Utilization of diphtheria cell-waste.

Training programmes were also conducted for D.M.L.T., Bio-assay students and short-term trainees.

BIOCHEMISTRY

The D.M.L.T., Short-term and M.Sc. Biochemistry (by papers) courses were actively conducted. The D.M.L.T. candidates who opted for advanced chemical pathology (Biochemistry) gave a creditable performance at the University final examinations held in April — May 1981.

CHEMOTHERAPY

The programme of synthesis of organic compounds for their possible use as therapeutic agents was much affected due to inadequate funds this year. There are 10 students working for their degrees in the department. A series of compounds were synthesised for evaluation of biological activities like anthelmintic, antimalarial, antifungal and antibacterial including anti-tuberculosis activity.

In all, 296 new compounds synthesized were analysed for elemental analysis and screened for antibacterial and antifungal activity in Chemotherapy Department and many compounds remained untested for antiparasitic screening, especially for anthelmintic screening due to various problems.

During the year quite a few of the compounds synthesized showed promising activities, especially *in vitro* antituberculosis activity. Few compounds showed antimalarial activity in mice.

The departmental scientific meetings of journal club were held from time to time for scientific discussion to give a thought to scientific problems, if any.

Dr. B. G. Khadse presented papers in the meeting of Indian Pharmaceutical Science Congress Association held in Nagpur. Dr. D. T. Chaudhari and Dr. B. G. Khadse attended conference on Natural Products and Medicinal Chemistry held at Institute of Science, Bombay. One paper was accepted for

presentation at International Conference on Tropical diseases and malaria, held at Manila, Philippines.

Dr. M. H. Shah, Asstt. Director, Department of Chemotherapy retired on 30th September 1980 after his long association of 36 years with this Department and Dr. D. T. Chaudhari, Sr. Scientific Officer, Class I took over the additional charge of the vacant post of Asstt. Director, Department of Chemotherapy.

One senior Research Fellow was appointed for *in vivo* anti-tuberculosis screening of organic compounds. One Research student and one senior Research Fellow continued to work on the problems of synthesis of anthelmintic and antifungal agents. Similarly one Govt. of Maharashtra Scholar also worked on this problem.

Dr. D. T. Chaudhari and Dr. B. G. Khadse were recognised as post-graduate teachers in Organic Chemistry for Ph.D degree by the University of Bombay. During the year under review, one student obtained his Ph.D. degree and two students were awarded M.Sc. degrees of the University of Bombay in Organic Chemistry.

The micro-analytical section carried out 1132 estimations of organic compounds. Out of this 1064 estimations were made from compounds synthesised in department and the remaining from outside laboratories. There were 532 estimations of Nitrogen and 600 were of Carbon and Hydrogen.

The microbiological *in vitro* screening section of the Department screened 507 compounds for antifungal activity. About 211 compounds were received from other research laboratories and 296 compounds were from the Department. Some of these compounds have shown very promising results *in vitro* (specially antituberculosis) activity.

RADIATION BIOLOGY UNIT

The unit continued to function with the core staff for the benefit of public utility and propagation of applied research.

Routine Activity

No.

No. of Total cases referred	: 87
Detected Hyperthyroid	: 22 (7 Toxic Goitres)
Detected Hypothyroid	: 1 (Myxoedema)
Detected Euthyroid	: 12 (3 Non toxic Goitre)
Thyroxine suppression	: 12
Advised	

Besides uptakes *in vivo*, the sera samples were tested for T_3 and T_4 levels which correlated with the uptake results (Radioimmunoassay). Depending on the allocation of the grants these facilities will also be extended to referred patients.

Research Activities

Irradiation Inactivated Tetanus and Venom Toxoids

The work was further continued in mice. Inactivated components of combined cobra venom and tetanus toxin, along with Freund's adjuvant were injected in mice at defined intervals. The total inactivation of above mentioned toxin was tested by injecting double the amount of lethal dose prior to beginning of immunization schedule.

The results of challenge dose given 8 weeks later following a single and multiple dose of venom toxin adjuvant combination, showed limited efficacy, in that the mice given minimum three doses of combination at weekly interval could withstand challenge with 2 M.L.D. while those with lesser doses succumbed.

Further work with reciprocal challenges is in progress.

Follow of titres of Tetanus antibodies in Individuals

The sera samples from Malgund, Ratnagiri Dist. were collected 3 years following a single dose of concentrated tetanus toxoid.

The neutralization test in mice to ascertain the antibody levels confirmed the limited efficacy of single dose vaccination for a long term immunity.

Binding of Tetanus Toxin to brain and spinal cord homogenate in Mice

I_{125} tetanus toxin was modified by oxidation with chloramine T, radio iodination and acetylation. The following properties were studied. Binding to brain homogenate, binding to whole spinal cord homogenate, development of generalized tetanus in mice and its correlation with above findings.

In conclusion it can be said that there is no correlation between binding to either brain or spinal cord *in vitro*, and fixation to spinal cord *in vivo* is necessary for its pharmacological activity. However mere fixation does not produce clinical toxicity.

The study proves that the limited efficacy of radioiodinated purified tetanus toxin is due to its fast decay from blood circulation.

CLINICAL PATHOLOGY

Clinico Pathological Investigation

The Department undertook investigations from various hospitals and agencies (Appendix IV). The services offered cover the following general areas (Appendix II).

- (a) Cytology of various body fluids.
- (b) Routine Histological examination.
- (c) Diagnostic serology, bacteriology and mycology.

Training :

The department participated in the D.M.L.T. training course. It also offered short term training in Clinical Pathology. The staff participates in the comprehensive teaching programme for M.Sc. and Ph. D. students. Dr. Kamat also participates in the integrated Bombay University teaching programme for M.Sc. microbiology and M.D. pathology.

ZOONOSIS

Synthetic chemotherapeutic agents synthesised at the Department of Chemotherapy were screened for their activity against malarial parasites, intestinal and tissue nematodes and cestodes. Among synthetic agents thus screened anthelmintic agents constituted a major group. Apart from drugs, several plant extracts were examined for insecticidal/larvicidal properties with a view to develop alternative insecticides for vector control. This study is being carried out in collaboration with the Dept. of Phytochemistry.

It was for the first time possible to demonstrate the presence of a focus of angiostrongylus infection in Greater Bombay area. The disease commonly referred to as eosinophilic meningo encephalitis, has been reported to be widely distributed in the Pacific island and many Asian Countries. In India, except for the one suspected human case reported from Pune, the infection has not been recorded so far.

Studies on human hookworm, filariasis and schistosomiasis were continued with significant strides. Colonies of vector of medical importance, strains parasites viz., *L. carinii*, *A. ceylanicum*, *H. nana* and *Plasmodium berghei* are being maintained for testing and research activities. Commercial testing of insecticide and rodenticide formulations was undertaken. This year four products were submitted for testing.

Various poisonous and non-poisonous snakes were maintained for the extraction of their venom. Scorpions were also maintained

for the purpose of obtaining their venom. The quantity of venom extracted and supplied is shown in Appendix XIX.

The department participated in various training programmes undertaken by the Institute and helped several post-graduate students registered for M.Sc. and Ph.D. degree courses to pursue their research projects.

VIROLOGY

The activities of the Department of Virology during 1980-81 were as follows :

1. Academic.
2. Routine.
3. Research.

The Department conducted a postgraduate University teaching programme of one month duration for M.Sc. (Microbiology) and M. D. (Microbiology) students, in February, 1981. The course was appreciated by the students.

A paper entitled "The Necessity for Anti-rubella Vaccines" was presented at the Annual Conference of the Association of Microbiologists of India at Bombay in November, 1980, by Dr. Mrs. C. H. Pandit.

A lecture on the "Study of Exfoliative Cytology on Urine Samples for Cytomegalovirus Infection" was delivered on the 6th of February, 1981, by Dr. (Mrs.) C. H. Pandit, the Officer-in-charge of Immunization Clinic of the Department.

Dr. M. V. N. Shirodkar, Head of the Department, attended the Twenty-First Annual Conference of the Association of Microbiologists of India, from the 31st of October to 2nd November, 1980, at Bhavan's College Campus, Andheri, Bombay and organized a "Symposium on Viral Vaccines". He also delivered a lecture entitled "Vaccine against Hepatitis B Virus" at an AMI Meeting of the Pune Unit, in March, 1981.

The Immunization Clinic carried out the work of immunization viz. administration of injections of ARV, triple antigen, tetanus toxoid, DT, anti-cholera and oral polio doses. In all, seven hundred and seventy-eight patients were immunised with the various vaccines. One hundred-and-twenty staff members were also inoculated with the different vaccines.

For virological diagnostic tests, our Clinical Virology Unit receives blood, urine and CSF samples from several neighbouring general hospitals, as well as from private clinics. In brief, the following is the report of the work of this Unit during 1980-81.

Rubella : Serodiagnosis of rubella is carried out in this Unit. During 1980-81, one hundred and fifty sera were received for determination of rubella antibody titre. These were detected by the HAI test. Seventy samples were positive (1:10), while eighty were negative (1:10).

Hepatitis B surface antigen : Five sera were received from hepatitis patients for the detection of HBsAg. These were examined by the reversed passive haemagglutination test. Only one was positive for HBsAg.

Measles : Thirty-five serum samples were received from suspected SSPE patients for testing for measles antibody titre, utilizing the HAI test. Fifteen were positive and twenty negative.

Cytomegalovirus : Eleven urine samples were received for isolation of CMV. These were examined by light microscopy, following preparation of dry smears from the centrifuged sediment and Giemsa staining. The smears were observed for cytomegaloviral inclusion bodies (Owl's Eye cells). However, all eleven samples were negative.

TOXICOLOGY

The department was actively engaged in toxicological evaluation of different types of pesticides, drugs, chemicals and plastics. Appendix X shows types of products, number tested and toxicity parameters studied.

The total earnings of the department were Rs. 17,400.00. We had already accepted testing charges during the financial year of 1979-80 for tests to be conducted for the next two years. The department also undertook toxicity studies (Undue toxicity and depressor like substances tests) of samples referred by Testing Unit of the Institute. Besides the department also participated in training and teaching of drug analysis and bioassay courses.

The department was involved in two research projects.

(1) Acute toxicity studies of commonly used pesticides (continuation of last year's project).

(2) Protein deficiency and drug action.

A research paper from the department of Toxicology "Acute oral and dermal toxicity of some common pesticides" was presented by Mr. M. G. Phatak at the XII Annual conference of the Indian Pharmacological Society held in Jammu Tavi (September 30 — October 2, 1980). The authors of the paper were Dr. V. Ramakrishna Rao, M. G. Phatak and Dr. V. R. Dabholkar.

Toxicity Studies of Commonly used Pesticides :

Completed acute oral toxicity of Copper Oxychloride, Cythion Tech. and Household Insecticide in rats, and Malathion 50 EC,

Malathion Tech. Zinc phosphide, Metoxuron in mice. Zinc phosphide was more toxic (LD_{50} 42.5 mg/kg) than other pesticides tested in mice Appendix XI.

The Primary Skin Irritation effects of the following pesticides were studied — Malathion 50 EC and Malathion Tech. Malathion 50 EC produced mild skin irritation. Malathion Tech. did not produce any skin irritation or irritation to mucous membrane.

Acute toxicity to pigeon (M.L.D.) was conducted with Malathion Tech. and its 50 EC. Malathion Tech. was more toxic than Malathion 50 EC.

Protein Malnutrition and Drug Metabolism :

In continuation with the last year's report, in this year we carried out following studies and the results obtained are given below.

(1) **Urinary excretion of salicylate in control and protein deficient rats :** The excretion of Aspirin as salicylates was reduced in protein deficient animals than control.

(2) **Faecal excretion of salicylates in control and protein deficient rats :** Faecal salicylate levels were negligible in both the groups of animals indicating, it is not the chief route of excretion.

(3) **Tissue Salicylate Levels in Control and Protein Deficient rats :** The tissues (liver, kidney) salicylate levels were comparable with the blood levels and there was no statistical difference between them.

(4) **Studies on Protein Binding in normal and Protein Deficient Animals :** The protein binding of Salicylates and Aspirin were reduced in protein deficient animals than the normal. This could be due to the reduced albumin level in these animals.

Further work is in progress.

PHARMACOLOGY

The department regularly conducts journal clubs, seminars and research progress meetings.

The students of Drug Analysis course (Biological and Microbiological) are trained in this department.

Participation in conferences, symposia and seminars :

Dr. M. B. Bhide attended the following conferences :

1. 14th Annual Convention of I.C.A.I. at Simla in Sept. 80.
2. 13th Annual Conference of the I.P.S. at Jammu in Sept.-Oct. 80.

The department has several research projects either of the Institute or sponsored by C.C.R.A.S., and Ar. Ex. Laboratories Pvt. Ltd.

The following projects are in progress :

- I. Leprosy
- II. Indigenous Drug Research
- III. Antibodies to Tik 20

PHYTOCHEMISTRY

General activities :

- A. To survey and collect Indian plants from different parts of Maharashtra State for mosquito larvicidal insecticidal (*Culex p-fatigans*) activity.
- B. To screen various extracts of collected plant material for mosquito larvicidal activity (*Culex p-fatigans*).
- C. To study and purify the active principle and elucidate its structure.

To study hypoglycemic effect of *Pueraria tuberosa* (Marathi : Ghodkand).

Highlights of achievements :

During the course of our vector control studies very promising results were obtained from *Osimum basilicum* Linn. (Marathi Subja) essential oil for mosquito larvicidal activity. (*Culex p-fatigans*). Even steam distillate directly used showed 100 per cent mortality in a short period of time. Other plants studied for mosquito larvicidal activity also showed promising results.

A flavonoid obtained from *Pueraria tuberosa* tubers showed hypoglycemic activity comparable to a standard drug in laboratory animals. No mortality was observed even at the dose of 5 gm/kg in mice.

(I) To survey and collect Indian plants from different parts of Maharashtra State for larvicidal activity.

The following plants were collected from Satara and Pune districts.

1. *Ocimum basilicum* Linn (Leaves) Marathi — Subja (Bombay).
2. *Eucalyptus globulus labill* (Leaves) Marathi — Nilagiri (Haffkine Compound).
3. *Cuscuta reflexa* (Whole plant) Marathi — Amarvel.
4. *Scutia Indica* (Leaves and stems) Marathi — Chimat.
5. *Duranta Plumeri* Jacq (stems, leaves and fruits).

Vector control studies (*Culex p-fatigans* mosquito larvicidal activity) of plants collected.

CHAPTER III

DEVELOPMENT OF TEACHING

Various training courses carried out by the Institute attracted a large number of students. Some of these specialised postgraduate courses are of unique nature and are exclusively offered by the Haffkine Institute. Appendix XV provides a list of courses offered at the Institute. The students working for M.Sc., Ph.D. and M.D. degree courses continued their research work with enthusiasm and fervour. The students working for M.Sc. and M.D. degrees by papers acquitted creditably in their final examinations.

Postgraduate Courses (Research)

The list of students completing their research projects and obtaining their degrees along with titles of their projects is furnished in Appendix XVIII.

CHAPTER IV

RESEARCH PROGRESS

BACTERIOLOGY

The simplest technique to obtain recombination between species that do not yield to conjugation, transduction or transformation is "protoplast-fusion". The method consists of (1) Converting live young cells of the concerned species into "protoplasts" by stripping of the cell wall with the help of enzymes such as lysozyme. (2) fusing the protoplasts together with the help of agents such as polyethylene glycol. (3) regeneration of complete cells and (4) selection and characterization of the recombinants.

We have begun with *V. Cholerae* El tor Inaba and *Saimonella enteritidis* strains. One of the several procedures employed for protoplastization incubation in presence of 3% glycine in a buffered medium and treatment with lysozyme + EDTA at pH 9.0 has been found to be particularly successful.

(2) Before actual fusion, it is necessary to select suitable genetic marker that would help in the isolation of recombinants.

Spontaneous resistant mutants of these strains have been obtained. Efforts are being made to select some more easily recognisable markers on each of these strains.

BIOCHEMISTRY

Biochemical studies on *Bordetella pertussis* cell extracts.

1. The principal objective of this ICMR sponsored scheme (1979) is to isolate, purify and carry out physico-chemical studies on the hypoglycemia causing factor (HCF) from *B. pertussis* cell extracts.

Several lots of formalin treated *B. pertussis* cells were prepared with consistently improved yields (80-100 Billion cells/ml.). Initially a comparative assessment of the blood sugar lowering effect of live and formalin treated cell extracts indicated that there was no significant difference in their effect on mice. Blood sugar was estimated by the glucoseoxidase enzyme method. (Blood sugar for control = 80 ± 8.32 mg%; Formalin treated cells = 59.4 ± 7.56 mg% and live cell extract = 59.8 ± 10.56 mg%).

The cell wall proteins were next extracted by three different methods and these preparations were as below :—

(i) Alkaline saline extracts (SE)

- (ii) Triton X-100 treated extracts (TE)
- (iii) Enzyme treated extracts (EE) containing lysozyme, RNase and DNase.

A comparative study of the blood sugar lowering effects of these three preparations was next carried out. There was no significant difference in the effect between the three preparations (Blood sugar for control = 72.8 ± 3.96 mg%; SE = 54.4 ± 6.07 mg%; TE = 57.85 ± 7.5 mg%; EE = 63.1 ± 6.69 mg%). Enzyme treatment (method iii-EE) however removed most of the nucleic acids as indicated by extinction at 260 nm, and since this did not affect the blood sugar lowering effect, it was decided to follow this procedure for extraction of cells. Preliminary purification of the HCF was carried out by gel filtration using Sephadex G-100, Sephadex G-200 and Sepharose-4B. Maximum hypoglycemic action was found in the major chromatographic fraction obtained from Sepharose-4B purification. Further purification of this fraction is being carried out by ion-exchange chromatography with hydroxylapatite.

2. Biochemical studies on rodent malaria :

In view of the fact that malarial infection causes oxidative damage in liver and other tissues, it is suggested that as natural defence mechanism against oxidative damage, the activities of the enzymes which utilize the superoxide radicals and hydrogen peroxide are altered. Since there is a lack of information on these important species of malarial infection, it was considered worthwhile to study the lipid metabolism and the activities of the key enzymes superoxide dismutase and catalase, in different tissues of the animals infected with *P. berghei*.

Lipoprotein profiles revealed that with the increasing parasitaemia, alpha-lipoprotein levels decreased significantly. Additionally, an extra lipoprotein band between pre-B- lipoprotein and B-lipoprotein appeared which became distinct at the level of 50% parasitaemia. The significance of this observation is being looked into.

The activity of Catalase was found to be decreased by about 31, 32 and 30% in liver, kidney and brain respectively, whereas the same was found to be increased by 115% in the spleen. Superoxide dismutase activity was found to be decreased in liver but not in other tissues.

In order to ascertain whether the above changes were reflected in any other metabolic pathways, the enzymes glucose 6-phosphate dehydrogenase as representative of aerobic enzymes, and Lactate dehydrogenase as nonaerobic enzyme, were studied.

It was interesting to note that G-6PD was decreased in all the above tissues except spleen, where it tended to increase. LDH on the other hand, has either increased or remained unchanged. Thus these studies suggested that during *P. berghei* infection probably the overall oxidoreductive processes seem to be affected. Further studies with enzymes involved in other metabolic pathways along with isoenzymes of LDH, are in progress.

3. Effects of Vitamin D on Lipid Metabolism in Rat Brain

Overdosage of Vitamin D during pregnancy and early childhood is known to cause mental retardation besides other side effects. The mechanism/s underlying this phenomenon is/are not explicit. Since lipids are one of the important constituents of nerve tissue, the effects of hypervitaminosis D on brain lipids and on a representative lipogenic enzyme (G6PD) as well as on an enzyme involved in neurotransmitter metabolism (acetylcholine esterase-AChE) in brain, were investigated.

Hypervitaminosis was induced in weanling albino rats by feeding 5000 IU vitamin D₃/day/kg body weight. Hypervitaminosis D was assessed by a rise in the serum calcium level. The results were compared with pair fed group in order to minimise the effect of varied food intake.

The studies revealed that high doses of vitamin D decreased brain weight by 11%. The concentration of total lipids in brain increased by 21% and that of cholesterol by 12% whereas phospholipids decreased by 10%. Further the G6PD activity was significantly increased, whereas total LDH activity was not affected, suggesting the possibility of increased lipid synthesis. The results were in good agreement with our earlier findings that in liver, vitamin D stimulates the G6PD activity. Interestingly one of the key enzymes, AChE, involved in cholinergic neural transmission was found to be elevated in the hypervitaminotic rat brain. This along with the above data suggest that critical changes in the ultrastructure of the CNS component might have taken place resulting into possible aberration in brain function.

4. The purification of Hepatitis B surface antigen (HBsAg) and its use in the reversed passive haemagglutination assay (RPHA).

RPHA was standardized using simple physicochemical techniques, not involving ion exchange and affinity column chromatography.

The purification of HBsAg was achieved using Aerosil (colloidal silicic acid). The HBsAg-positive human serum was

used as the source of the HBsAg. The method standardized gave the (HBsAg) yield of 56%.

The problem of false positive interactions was tackled. Apparently the serum lipoproteins were found to be the cause of the false positive RPHA results. The method was therefore standardized to remove these false positive interactions to the extent of 80-90%, which has helped in increasing the credibility of the RPHA.

5. Studies on brain polyamines in undernourished rats

Since polyamines play a vital role in general metabolism and particularly in the formation of GABA — one of the key neurotransmitters, the present studies were initiated to investigate the effects of undernutrition on polyamine levels in the brain. The initial data suggest that the neonatal undernutrition induced by litter size manipulation results in significant decrease in the levels of all polyamines. The decrease was more in putrescine as compared to spermine and spermidine.

CHEMOTHERAPY

I. ANTHELMINTIC AGENTS

(a) Tricyclic imidazothiazolo pyridines

2-Substituted-aryl 6 and 7-nitroimidazo (2,1-b)-thiazolo (5,4-b) (4,5-b) pyridines as anthelmintic agents: The 6-phenylimidazo (2,1-b) thiazolo (5,4-b) pyridine, a tricyclic heterocycle reported from our laboratory was found to exhibit a significant anthelmintic activity against *N. dubius* in mice. So its isoster having 6-nitro group in the pyridine part was synthesized, since nitro group imparts antiparasitic and antiamebic activity. Thus, 6-nitro-2-substituted-phenylimidazo (2,1-b) thiazolo (5,4-b) pyridines were synthesized and evaluated for possible anthelmintic activity against *N. dubius* in mice. However, in this case, the introduction of nitro group did not give significant increase in anthelmintic activity as expected.

In another tricyclic series, 2-substituted-aryl-7-nitro imidazo (2,1-b) thiazolo (4,5-b) pyridines were synthesized. Few compounds from this series have shown anthelmintic activity against *N. dubius* in mice. Further testing in this series is on the way.

(b) Close isosters of benzimidazole carbamates:

From this carbamate series, 6-phenylthio-2-benzothiazolylebenzyl carbamate proved 69% effective against *N. dubius* & 6-phenoxy 2-benzothiazolyl-isobutyl-carbamate showed 68% activity against the same parasite. These two compounds are selected for further detailed studies (standard drug-Mebendazole. Dose = 20 mg/kg).

(c) 4-(N-Methyl-4-piperazinyl)-N-substituted-aryl succinimides

N-Methyl piperazine forms an important part of the antifilarial drug like DEC, hence, it was proposed to incorporate this moiety along with N-substituted-aryl succinimide and test these compounds against *L. carini* in mice. Synthesis of these types of compounds is in hand. Nine compounds in sufficient quantities have been sent to Central Drug Research Institute, Lucknow, for anthelmintic testing against various parasites.

(d) 3-Substituted-phenyl-6-phenyl/or methyl-5, 6-dihydroimidazo (2, 1-b) thiazoles

The preparation of organic compounds, analog to tetramisole, was done. In this type of compounds the various substitution or aryl groups at thiazole function has been made in the molecule. In this series of 3-substituted-phenyl-6-phenyl-5, 6-dihydroimidazo (2, 1-b)thiazoles one of the compounds, R-4325 showed the activity upto 90% against *N. dubius* in mice.

A little modification has been made in the above series, and 6-phenyl group was replaced by methyl group and the synthesis of the resulting 3-substituted 6-methyl-5, 6-dihydroimidazo (2, 1-B)thiazoles was undertaken starting from 2-methyl-5 mercapto imidazole.

II. ANTIFUNGAL AGENTS

(a) Synthesis of 1-(2'-aryl-2'-(5-aryl-2-(1, 3, 4-oxadiazolyl) thio) imidazoles :

Synthesis of 1- (2'-aryl-2'-(5-aryl-2-(1, 3, 4-oxadiazolyl) thio) ethyl) imidazoles was continued as analog to know antifungal drug 'Miconazole'. In this series two compounds -R-5076 and R-5081 inhibit the growth of *Tricophyton mentagrophytes* at the minimum concentration of 1.56 ug/ml in *vitro* test system. This activity is equal to the standard drug 'Miconazole' under identical conditions of *in vitro* testing.

(b) O-Substituted aryl-N-methyl or ethyl-N-phenylthio carbamates

Tolnaftate is a widely used compound as it has unusually high *in vitro* and *in vivo* activity against the dermatophytes and candidal lesions.

In the second series of O-Substituted-aryl-N-methyl or ethyl-N-phenylthiocarbamates compounds were prepared with structural analogy to well known antifungal drug 'tolnaftate'. This type of compounds, however, proved inactive, when tested against *T. mentagrophytes*, *Candida albicans* and *T. rubrum* in *in vitro* screening.

III. ANTIMALARIAL AGENTS

Dihydro-s-triazine derivatives (cycloguanil type of compounds) :

New 1, 2-dihydro-s-triazines were prepared from (a) 4-(p-aminophenyl) thiazoles and (b) 3, 5-dihalo-4-alkoxyanilines, as these thiazoles have given compounds active in antiparasitic screening and the above anilines afforded active compounds in antimalarial screening in pyrimidine series earlier from our laboratory. Twenty three compounds screened for antimalarial activity in mice against *Plasmodium berghei yoelii* using Rane's blood schizonticidal test. Dihydro-s-triazines derived from 3,5-dihalo-4-alkoxy anilines were more active than dihydro-s-triazines from 4-(p-aminophenyl) thiazoles. Nine compounds displayed the activity and one of the compounds, R-4956 recorded four cures out of five animals treated, at dose of 40 and 160 mg/kg.

IV. ANTITUBERCULOSIS AND ANTIMICROBIAL AGENTS

(a) N-(3',5'-Dihalo-4'-alkoxyphenyl)-pyrazinamides :

The parent drug pyrazinamide is highly potent but it requires strict medical supervision for clinical use due to its high toxic effects. In hope to get better compounds with minimum toxicity, N-substituted pyrazinamides were prepared. Two compounds, R-4615 and R-4651 in this series inhibited the growth of *Mycobacterium tuberculosis* at 0.25 ug/ml concentration (Pyrazinamide has minimum inhibitory concentration (MIC) at 12.5 ug/ml). One of the above two compounds R-4651 was submitted for *in vivo* antileprosy screening in animals.

(b) N-Substituted-arylamino succinimides and succinamide :

With the reported high order of *in vitro* and *in vivo* anti-tuberculosis activity of aspartic acid hydrazides derivatives, it was planned to synthesize new compounds in the series of N-substituted-phenyl-succinimides and succinamides, as analog to aspartic acid amide. Two compounds, R-4983 and R-4984 in this series displayed promising *in vitro* antituberculosis activity equal to isonicotinic acid hydrazid (INH) under the identical condition of *in vitro* testing, and have MIC = 0.06 ug/ml.

(c) 2-Substituted-arylamino-6-methoxybenzothiazoles and 5, 6-dichloro-2-substituted-benzylthiobenzimidazoles :

Considering the previously reported high anti tuberculosis activity of benzothiazole derivatives from our Laboratory, more compounds in the tilted benzothiazole series with different substitutions were prepared. These compounds, nearly 30 in number, were tested for antituberculosis activity *in vitro*. Some of them showed activity upto .06 ug/ml, to 1.56 ug/ml, compared to INH.

5, 6-Dichloro-2-mercaptobenzimidazole was prepared from 3, 4-dichloroaniline using various steps. Its sodium salt was

condensed with different substituted benzylhalides in alcohol to obtain the 5,6-dichlorobenzimidazole derivatives. Fifteen compounds of this series were synthesized and submitted for antituberculosis and other antimicrobial testing.

(d) 2-Substituted-arylamino-4-(3 pyrazinyl)-thiazole derivatives :

It is reported that 2-substituted-arylamino-4-(3-pyrazinyl) thiazoles exhibited activity against INH resistant strain of *M. tuberculosis* bacilli. Hence, it was proposed to synthesize the title compounds, and test against *M. tuberculosis* as well as against INH resistance strain if possible.

Nearly twenty compounds of this type were prepared and subjected for *in vitro* antituberculosis studies.

CLINICAL PATHOLOGY

The following are the salient research achievements of the department.

Epidemiology of Enterotoxigenic *E. coli* (ETEC) diarrhoeas.

309 strains of *E. coli* isolated from 274 cases of sporadic diarrhoea were studied. 61.16 per cent of these were enterotoxigenic *E. coli* (ETEC). 67 per cent of the ETEC were CFA negative and 58.73 per cent showed no haemagglutinating pili. The isolates were serogrouped for O-antigens. The predominant serogroups encountered amongst ETEC were 01, 02, 07, 017, 011 and 060. These appear to be different from those reported in the literature. The predominant serogroups amongst normal colonic *E. coli* were also the same. Isolation of a large number of strains of ETEC without CFA, indicates that the role of CFA in the adhesion of ETEC is overrated. Comparable high frequencies of a few serogroups amongst ETEC and non-toxigenic *E. coli* is highly significant from the point of view of epidemiology of ETEC diarrhoeas.

2. Role of surface antigens of *S. flexneri* in the invasion of HeLa cell monolayer.

HeLa cell monolayer invasion assay was standardised employing known invasive and non-invasive organisms. Results could be correlated with Sereny test.

Employing this assay, the role of surface antigens of *S. flexneri* in invasion of the HeLa Cells was investigated. Three types of antisera were generated by immunisation of rabbits (1) Anti-live *S. flexneri* antiserum (2) Anti-boiled *S. flexneri* antiserum (3) Anti live *S. flexneri* antiserum absorbed with boiled cells. The antisera (1) and (2) could inhibit adhesion *vis a vis* invasion of

the HeLa Cell monolayers at 1/200 dilution. However even on absorption of antilive cell antiserum with boiled cells to remove completely the antisomatic antibodies, the ability of the antiserum to inhibit adhesion was not completely absorbed out. Specific LPS of *S. flexneri* even at 1.0 mg/ml concentration could not inhibit adhesion of *S. flexneri* to HeLa Cells. Based on these data it was concluded that (a) Adhesion is a pre-requisite step prior to invasion (2) The bacterial surface molecule participating in adhesion is not somatic antigen. The hypothetical heat sensitive molecule is likely to be displayed on the bacterial cell surface in close proximity to somatic antigen because the antibody to latter could cause steric hindrance to the adhesion of *S. flexneri* to the HeLa cells. It is proposed that study be continued further with the purpose of identification of the specific surface molecule participating in adhesion.

3. Immune mechanisms in Salmonellosis.

The studies have resulted in the following conclusions.

1. Formalised vaccines give a good antibody response, and no cellular immune response.
2. The animals receiving formalised vaccines do show a very limited protection against homologous challenge.
3. Live cell immunisation raises excellent cellular immunity in the form of long lived effector T cells and activated macrophages. The latter activity is depleted as a function of time following immunisation. The former persist for long time and they can rapidly generate activated macrophages on antigenic challenge.
4. Animals receiving live cell immunisation show excellent protection on challenge with live organisms.
5. Serum antibody of formalised cell immunised mice does not seem to be capable of neutralising the effects of endotoxin.

PARASITIC DISEASES AND ZONOOSES

Malaria :

Morphological changes occurring in the blood stages of *Plasmodium berghei* yoelii following the administration of anti-malarial agents were studied by light microscopy. In addition to chloroquine, camoquin, pyrimethamine, erythromycin, dapson and sulphadiazine, some new drugs were also investigated. These were metakelfin (a combination of sulphamethapyrine and pyrimethamine), sulphamethapyrine, trimethoprin and erythromycin. Essentially three types of changes in parasite morphology were observed. In chloroquine pigments were observed to cluster in a clump. This pigment clump got extruded from the parasite body within 6 hours of drug administration. Disintegration of cytoplasm

occurred after 8 hours. In pyrimethamine nuclear division was observed to occur as in the normal schizogony, but these schizonts failed to produce merozoites. With erythromycin, no change could be detected during the first twenty four hours. Thereafter about 30 per cent parasites presented abnormal appearance.

An Ayurvedic preparation Sudarshan Ghanawati was investigated for its blood schizonticidal activity in *P. berghei*—mouse model. Various concentrations of the drug were employed and the drug was found to possess promising antimalarial activity at the dose 50 mg/kg. The drug was initially tried in malaria patients suffering from *P. vivax* infection by Podar Research Institute in Thane District and was found to be promising.

Filariasis :

Several attempts were made to infect infant rabbits with infective larvae of *W. bancrofti*. Infected mosquitoes were obtained from various filariasis endemic areas of Greater Bombay. These mosquitoes were dissected and infant rabbits were inoculated with the infective larvae recovered from mosquitoes. None of the rabbits however showed developing *Wucheraria bancrofti* worms.

Efforts were also made to establish *Litomosoides carinii* strains in laboratory strain of mice, infant rats and rabbits for chemotherapeutic studies. Transplantation of *L. carinii* worms in mice, infant rats and infant rabbits showed that although rabbits are susceptible to *L. carinii* infection, yield of microfilariae in blood is very low. Further studies on these lines are continued.

Schistosomiasis :

In our previous survey of schistosoma endemic focus situated in the village Gimvi, 42 individuals were observed to present haematuria. None of these individuals showed the presence of schistosome ova when examined further.

Morphological and biometric features of furcocercous cercariae liberated from two species of snails viz., *Paludomus obessa* and *Ferissia tenuis* were studied and compared with features of *Schistosoma haematobium* cercaria. It was observed that cercariae liberated by *F. tenuis* resembled very closely with *S. haematobium* cercariae. Efforts to infect experimental animals with schistosome cercariae are continued.

Hookworm :

Human hookworm (*N. americanus*) strain was established in infant rabbit model and successfully passaged upto the 7th generation (second time) without any difficulty. CIEP test was used for the immunodiagnosis of *N. americanus* infection in rabbit at different stages of infection, but this test was found of very limited use. In chemotherapy studies, mebendazole, Betoscanate,

Alcopar and Levamisole were used for in vitro test against *N. americanus* larvae (RSG 3). Later on in vivo test was developed in this *N. americanus* — rabbit system.

This was performed in infected rabbits using standard drugs : Bitoscante (200 mg/kg) and Mebendazole (50 mg/kg). Result showed that egg (eggs per gm of faeces) dropped from 61,000 (average) to 0 after 2 days treatment. Chemotherapeutic evaluation of the drug will be done on the basis of worm burden after standardising the dose of infection and worm load in this model.

Biochemical studies in hookworm infections :

A new line of research was proposed to study whether enzymes like SOD, Catalase, peroxidase influence the oxydoreductase system after the lung journey of 3rd stage larvae of hookworm in the host. These studies were carried out in three host systems : *N. americanus* in rabbits as principal model and *A. ceylanicum* in hamsters and *A. caninum* in mice for the comparative studies of lung and liver tissues. The tissues were collected 10 days post infection for the estimation of enzyme levels. For lipoprotein analysis, serum samples were collected from the rabbits infected with *N. americanus* at different intervals i.e. 20, 25, 35, 45, 50, 60, 70, 105, and 130 and 150 days post infection. These studies are under progress.

Angiostrongyliasis :

This study was undertaken following the accidental detection of a suspected case of human angiostrongyliasis in Greater Bombay. Although reported from other Asian countries, cases of human eosinophilic meningo encephalitis or angiostrongyliasis have not been reported from India except for one suspected case from Pune.

The patient developed symptoms of eosinophilic meningitis when he consumed a raw slug (*Laevicaulis altae*). When the slugs from the area from which the patient had consumed the slug were examined, they were found to harbour larvae of *Angiostrongylus cantonensis* : The infection was also found to be present in the vertebrate reservoir *Rattus norvegicus*. Complete life cycle of the parasite *A. cantonensis* in the experimental animal i.e. *R. norvegicus* (albino) was investigated. Identification of the nematode as *Angiostrongylus cantonensis* was confirmed. Studies are continued to assess the danger of human exposure to this infection by assessing the level of distribution of this infection in different vertebrate and invertebrate hosts.

Plague

In addition to rodents from Greater Bombay area, rodent and flea samples received from Pune, Marathwada area and other rural

parts of Maharashtra State were investigated for the presence of *Yersinia pestis* infection. In none of the isolations presence of *Yersinia pestis* was demonstrated.

Serological investigations with rodent sera from Greater Bombay also did not show any evidence of inapparent plague infection.

Leptospirosis :

A strain of *Leptospira javanica* obtained from IVRI Izzatnagar, is being maintained in the laboratory. A study has been initiated for the isolation and cultivation of strains of *Leptospira* from wild rodent populations and four strains have been isolated.

Venomous Animals :

A youngone of Russell's viper is being reared in captivity. It was fed on mice. It has thus been possible for this department to maintain this youngone for 17 months. Thirteen plant extracts were evaluated for snake repellent activity in the laboratory against Cobra snakes and were found to be inactive.

PHARMACOLOGY

The following projects are in progress at the Department of Pharmacology.

- I. Leprosy
- II. Indigenous Drug Research
- III. Antibodies to Tik 20

I. Leprosy :

(i) Antileprosy Vaccine :

In our earlier report, it has been shown that vaccine prepared from live ICRC bacilli shows better protection than the vaccine from dead ones and hence shows a better cell mediated immunity. This study was continued using different strains.

Non mycobacterial, non acid fast coccoid organisms IKS, its mycobacterial progeny strains IKS-P and another coccoid strain W.D. were tested for the protection against *M. Leprae* infection in mice, using Shephards foot pad technique. Strains IKS and IKS-P were able to give substantial degree of protection against foot pad multiplication of *M. leprae* whereas strain W.D. was less effective.

The splenic cells were harvested from mice vaccinated with IKS, IKS-P and W.D. and injected into the normal mice. The delayed hypersensitivity reactions were tested in these animals by using lepromin and vaccine under test in mouse foot pads. Strain IKS was found to be most effective in inducing the delayed type of hypersensitivity to *M. leprae*.

(ii) Anti leprotic drugs :

Four synthetic compounds synthesized by chemotherapy department and a plant extract have been screened for antileprotic activity using both *in vitro* and *in vivo* techniques.

Besides, drug conjugates synthesized with dapsone, and L-DOPA, di-DOPA and L-Tyrosine were tested for *in vitro* activity against ICRC bacilli and the *in vivo* testing is in progress.

II. Indigenous Drug Research (Since 1972) :

The studies on indigenous drugs is continued as before and the work in the following fields is in progress.

(i) Bone healing, wound healing and antibacterial activity of the Plants :

The plants with code names L and H have been tested for the above activities. The results are not very encouraging. Another plant with code S was tested against diphtheria toxin and it produced significant protection in skin erythema test in rabbits.

(ii) Antiamoebic activity :

The acetone extract of the plant *Orocylum indicum* was active *in vitro* and *in vivo* against caecal amoebiasis in infant rats. The extract was inactive against hepatic amoebiasis in golden hamsters. A flavonoid was isolated from this extract in a pure form which was active.

(iii) Neuromuscular blocking agents :

The isolation of an alkaloid from *Dioscorea hispida* having curare type of activity has already been reported last year. Now, another alkaloid has been isolated from the same plant in a pure crystalline form. It is sent for structural elucidation. This compound possesses a depolarising type of neuromuscular blocking activity both *in vitro* and *in vivo*. To the best of our knowledge, this is the first compound isolated from the plant, having depolarising type of activity.

Another plant called *Iris germanica* was also studied for neuromuscular blocking activity. A pure alkaloid was isolated from the petroleum ether extract of the rhizomes of this plant and sent for structural elucidation. It showed mixed type of activity i.e. depolarising and anti-depolarising.

(iv) Hypoglycemic activity :

A pure crystalline compound was isolated from the plant *Hamiltonia savenloens*. It showed hypoglycemic activity both in normal and alloxanized rats. The fall in blood sugar was found to be 28% and 34% in normal and alloxanized rats respectively. Studies on the mechanism of its action are in progress.

(v) Anti-asthmatic activity :

The plant *Butea monosperma* was tested for its anti-asthmatic activity. A semi pure compound was isolated from the methanolic extract of the bark of this plant. The compound produced relaxation of the bronchia^l smooth muscle which was blocked by propranolol. It also inhibited the release of histamine caused by compound 48/80 in mouse paw oedema. The compound seems to have the potential of being anti-asthmatic.

(vi) Studies on the steroids isolated from the plant :

The isolation of a steroid from the petroleum ether extract of the plant *Ipomea digitata* has already been reported. The isolation and identification of other steroids was continued. The petroleum ether extract of this plant contains about 5-6 steroids of which one is β -sitosterol. Fraction I has been shown to possess estrogonic activity. The isolation and purification of fraction II is in progress. The preliminary studies indicate that it possesses potent estrogenic activity in ovariectomized mice.

III. Antibodies to Tik-20 poison :

(This scheme is sponsored by ICMR, 1978).

The organophosphorous insecticide Fenitroth and active metabolite Fenitro-oxon were conjugated to BSA. Antibodies were raised against these conjugates in rabbits. The biological protection afforded by these antihodies was tested in mice using the method LD₅₀ and also on the ECG changes induced by the poison. This protection was found to be significant. (This scheme was discontinued from 1st February, 1981).

RABIES RESEARCH UNIT

(I) Project on the development of a tissue culture vaccine against rabies :

Work on this project sponsored by the Department of Science and Technology, Government of India, New Delhi was continued

A. Adaptation of Rabies virus in primary cultures of Baby Hamster Kidney (p BHK) :

Results of the kinetics of rabies virus replication in these cultures are reported earlier. The low yield of progeny virus in the fluid phase (medium) of the cultures did not permit sub passage of the virus. The proposed work on employing fresh seed virus mixed with the passaged progeny virus for sub-passage had to be shelved due to shortage of sterile working area.

B. Development of a strain of human diploid cells and its standardisation :

After the receipt of liquid nitrogen refrigerator, it was possible to attempt cultivation of human diploid cells from post-abortion

foetuses obtained from a neighbouring hospital. During the report period, we have successfully developed a cell strain of human foetal fibroblastic lung. The following studies have been done on the cell strain.

1. **Serial cultivation** : The cell line has been successfully subcultivated for 20 passages employing suitable growth medium and imported foetal bovine serum. A few ampoules at different passage levels have been stored in liquid nitrogen.

2. **Storage and revival** : Revival of the cell strain was attempted on a few ampoules of cells frozen in liquid nitrogen for periods varying from 10 days to 6 months. Good plating efficiency was noticed and the cultures readily formed a confluent sheet in 3 days after incubation at 37°C. These could be further sub-cultured like the other, unfrozen cultures.

3. **Screening of cultures** : Morphologically, the cells appeared to be spindle shaped (fibroblast-like). Growth was good with a split ratio of 1 : 2 and fair amount of acid production was noted as is known in normal diploid cells.

Sex chromatin was not found on suitable preparations and microscopic examination of a number of nuclei confirming the phenotypic sex (male) of the foetus.

Chromosome analysis was carried out on the coverslip preparations of the cultures. 21 out of the 23 metaphases examined were noted to be diploid in number representing the species.

Bacterial and fungal sterility tests were carried out in suitable media and the cultures were found to be free from these contaminants.

Detection of latent intrinsic and extrinsic agents was carried out in primary monkey kidney cell cultures. No agent was detected on culturing and blind passage of the material. No cytopathic effect (CPE) was detected and haemadsorption tests on the cultures, employing guinea pig erythrocytes were negative.

Mycoplasma detection is proposed to be carried out by arrangements with the All India Institute of Medical Sciences, New Delhi.

It is proposed to employ this cell strain for adaptation of rabies virus for vaccine developmental work.

C. Detection of rabies antigen (RbAg) by counter-immuno-electrophoresis (CIEP) :

Rapid detection of rabies virus antigen is an important requirement for monitoring viral presence and growth in tissue culture. In the previous report it was shown that a rabbit hyper-immune globulin after immunisation with virus of tissue culture (human diploid) origin gave non-specific reaction with normal human brain as well as serum.

Commercially available horse hyperimmune serum against rabies was tested for detection of rabies virus antigen and it was found to give positive results on infected mouse brain material. However, false negatives were found on tests with normal goat and sheep brains.

Hyperimmunisation is therefore being carried out in the laboratory in rabbits employing homologous infected brains as the antigen for immunisation.

(II) Non Project (Departmental) Research :

A. Rapid detection of rabies antibody by CIEP :

This test was successfully standardised by varying different parameters e.g. antigen processing, antiserum purification, changing of cavity sizes and distances between antigen and serum cavities, voltages and timings etc.

Optimum results were obtained when antigen was subjected to purification and concentration by ultrafiltration, employing serum cavity of 4 mm diameter and antigen cavity of 3 mm diameter arranged at 25° to each other and when a potential difference of 150 volts and 50 volts was applied for serum and antigen respectively, employing 30 mts. for the test serum and 5 mts. for the processed antigen.

Using these tests, no false positive results were seen against normal sera from goat, sheep, mice, rabbits and human subjects.

B. Antibody response of human subjects after different dosage schedules of antirabies vaccination by CIEP method.

During the report period, a total of 280 blood samples were collected from individuals undergoing antirabies vaccination at the K.E.M. Hospital, Bombay. 60 of the samples were before start of the treatment (controls) and the remaining samples from vaccinees at different time periods and after different number of doses.

The results of the CIEP test on the samples is given in the table below :

Category	No. tested	No. positive	% positive
Prevaccination (Control)	60	0	0
3 + 0	30	0	0
3 + 7	20	2	10
3 + 1 month	30	2	6.6
7 + 0	30	1	3.3
14 + 0	40	20	50
14 + 7	42	25	59.5
14 + 14	15	8	53.3
14 + 1 month	6	2	33.3
14 + 1B + 10	7	5	71.4

3 + 0 = 3 ARV injections & blood taken on 0 day of last injection.

3 + 7 = 3 ARV injections & blood taken on 7 days of last injection.

1B = One booster dose.

The results so far permit the following conclusions :

- (1) Better antibody responses were elicited in blood samples collected a week after the last vaccine dose as compared to the samples collected on the day of the last injection.
- (2) The response appeared to decline in samples collected at 1 month after the last dose of vaccine.
- (3) Increasingly higher proportion of the vaccinees elicited antibody response with the increasing number of injections.
- (4) Not all (59.5%) of the subjects responded to a full course (14 injections) of ARV.
- (5) Some proportion of subjects not responding to 14 injections of vaccine responded by virtue of booster injection given 10 days after the last dose.

The work is being further pursued.

C. Modification and improvement of the conventional Semple type (brain tissue) vaccine :

A few earlier experiments conducted in the department had shown that infectivity titres of the rabies infected mouse brain suspensions could be increased 4-10 fold by sonication of the

material. Also after centrifuging the material at 7550 x g, the sediment, on titration in mice was noted to contain more virus than was present in the supernate.

Based on these findings, an attempt was made to obtain, if possible, a partially purified and concentrated AR vaccine of brain origin.

In a series of experiments, rabies virus-infected mouse brain suspensions were subjected to sonication followed by a clarifying centrifugation at 1500 x g for 15 minutes. The supernates obtained were subjected to centrifugation at 7550 x g for 1 hour. The sediment obtained showed a five-fold decrease in protein content without appreciable decrease in its infectivity titre (tested in mice).

Two sets of potency tests were conducted on the vaccines prepared from the sediment suspension inactivated in a fashion similar to the Semple type vaccine. Unfortunately, the immunogenicity of the prepared vaccines was poor as compared to the control Semple vaccine.

It is likely that the actual antigenic material (component) of the rabies virus is either inactivated by the sonication process or else is diverted towards the supernate during centrifugation.

Further work is required to be done to clarify the matter.

PHYTOCHEMISTRY

1. **Ocimum basilicum** Linn (Leaves) Marathi — Subja. The essential oil from fresh leaves was obtained by steam distillation, and was screened for mosquito larvicidal activity. It was observed that 0.008 per cent of subja oil showed 100 per cent mortality within 24 hours. This was compared against different dilutions of house-hold insecticide (Finit, H.P. Products) products and it was found that 0.005 per cent of Finit showed 100 per cent mortality of mosquito larvae. One litre steam distillate obtained from 200 gms. of subja leaves showed 100 per cent mortality of mosquito larvae, within 3 hours. For all these studies, water control and solvent control (Acetone) were used and there was no mortality observed with the controls.

(II) Hypoglycemic activity of active principle isolated from **Pueraria tuberosa** Linn.

A flavonoid (m.p. 187-189 c) isolated from **Pueraria tuberosa** tubers. Its purity was confirmed by taking IR, UV and micro analysis. Hypoglycemic activity was studied in normal healthy rats, rabbits and mice at various dose levels. Maximum blood sugar lowering effect was observed at the dose level of 50 mg/kg. Oral administration of 50 mg/kg dose showed 35 to 40 mg per cent reduction in blood sugar level at the end of 7 hours which was comparable to that of tolbutamide.

The compound was found to be effective on hyperglycemic rats produced either by glucose meal or adrenaline injection. In glucose tolerance study, control group of rats showed rise in blood sugar level from 91.75 to 124.45 mg per cent whereas drug treated group increased blood sugar level from 87 to 104 mg per cent. Similarly in adrenaline induced hyperglycemic rats drug inhibits rise in blood sugar levels from 87 to 97.4 mg per cent whereas control group showed rise in blood sugar level from 91.75 to 144.4 mg per cent. The compound was also found effective in alloxan treated diabetic rats. It showed 22.2 mg per cent blood sugar reduction at the end of 7 hours with dose level of 50 mg/kg.

Acute toxicity study was carried out in normal mice and observed for one week. The flavonoid did not show mortality upto 5 gm/kg dose by oral administration. The compound was administered orally at the dose of 50 mg/kg day for the period of one month to see its toxicity effects and important biological parameters as listed below :

- (1) Liver glycogen
- (2) Inorganic phosphorus
- (3) Pyruvic acid
- (4) Free fatty acids

- (5) Triglyceride
- (6) Cholesterol
- (7) Total lipids
- (8) Liver function test
- (9) Kidney function test

During the experimental period food intake, body weight and drug resistance study was carried out. After 30 days experimental rats were killed, blood was collected and important organs such as, liver, adrenal gland, kidney were taken out for histopathological studies.

The compound did not show the resistance and loss of appetite. The gain in weight of drug treated group is equal to the control group at the (Drug treated rats = 154.3 ± 1.79 gm and control rats 156.9 ± 1.14 gm) end of one month. The prolonged feeding effect of the drug caused reduction in liver glycogen (2.7 to 1.6 mg/g wet weight of liver), serum cholesterol (120 to 92.1 mg%) and free fatty acids (249.5 to 210.5 μ mol/litre). Other biological parameters such as triglycerides, inorganic phosphorus, total lipids etc., produced some variation in comparison with control rats. Liver and kidney function tests did not reveal any significant alterations from normal. No abnormal changes were observed in different organs.

In conclusion a flavonoid isolated from *pureria tuberosa* is found to be a promising hypoglycemic drug in animals. Since it is available in vegetable sources and does not possess adverse effect, therefore this drug should be taken for toxicological and clinical study.

Eucalyptus globulus Labill (Leaves) Marathi — Nilagiri. The essential oil from the dried leaves of *Eucalyptus* was obtained by steam distillation and when tested for mosquito larvicidal activity, 0.08 per cent oil showed 100 per cent mortality.

Cuscuta reflexa (Whole plant) Marathi — Amarvel. **Cuscuta reflexa** was powdered to uniform mesh and extracted in Soxhlet serially with petroleum ether, acetone and alcohol. All these extracts were dried on water bath, and evaluated for mosquito larvicidal activity. It was found that petroleum ether extract showed 100 per cent mortality for 0.024 per cent concentration, whereas acetone extract showed 100 per cent mortality for 0.016 per cent. The chemical analysis showed that petroleum ether extract contained steroids and alkaloids, whereas acetone extract showed presence of flavonoids. Further work for purification and identification of active principle is in progress.

Scutia indica (Leaves) Marathi — Chimat.

Petroleum ether extract of **Scutia indica** leaves showed 100 per cent mortality for 0.24 per cent concentration, whereas

alcoholic extract showed 100 per cent mortality for 0.16 per cent concentration.

Petroleum ether extract was further fractionated using alumina column chromatography and eluting with different solvent systems. Petroleum ether elute showed 100 per cent mortality for 0.09 per cent concentration and Benzene : Chloroform (1 : 1) elute showed 100 per cent mortality for 0.028 per cent concentration. Further work of purification, identification of the active principle is in progress.

Duranta Plumeri, Jacq.

Isolation and purification of alkaloidal fraction of **Duranta Plumeri** fruits was undertaken in order to locate mosquito larvicidal active principle. The total alkaloids were chromatographed on silica gel thin layer chromatography using benzene : Methanol 8 : 2 mixture solvent system. Two fluorescent spots were observed. Work to separate the alkaloids by preparative TLC is in progress. Also work on leaves and stems for mosquito larvicidal activity is in progress.

RADIATION BIOLOGY

Irradiation Inactivated Tetanus and Venom Toxoids :

The work was further continued on mice. Inactivated components or combined cobra venom tetanus toxoid along with Freund's adjuvant were injected in mice at defined intervals. The total inactivation of above mentioned toxin was tested by injecting double the amount of lethal dose prior to beginning of immunization schedule.

The results of challenge dose given 8 weeks later following a single and multiple dose of venom toxin adjuvant combination, showed limited efficacy, in that the mice given minimum three doses of combination at weekly intervals could withstand challenge with 2 M.L.D. while those with lesser doses succumbed.

Further work with reciprocal challenges is in progress.

Follow up titres of Tetanus Antibodies in Individuals :

The sera samples from Malgund, Ratnagiri Dist. were collected 3 years following a single dose of concentrated tetanus toxoid.

The neutralization test in mice to ascertain the antibody levels confirmed the limited efficacy of single dose vaccination for a long term immunity.

Binding of Tetanus Toxin to Brain and spinal cord homogenate in Mice :

The tetanus toxin was modified by oxidation with chloramine T, radio iodination and acetylation. The following properties were

studied. Binding to brain homogenate, binding to whole spinal cord homogenate, development of generalised tetanus in mice and its correlation with above findings.

In conclusion it can be said that there is no correlation between binding to either brain or spinal cord *in vitro*, and fixation to spinal cord *in vivo* is necessary for its pharmacological activity, however mere fixation does not produce clinical toxicity.

The study however proves that the limited efficacy of radio-iodinate purified tetanus toxin is due to its fast decay from blood circulation.

Miscellaneous : Eight samples of blood and CSF were received from patients suffering from viral diseases of unknown origin. The sample fluids were inoculated into different host systems by different routes to study pathogenicity. None was observed and no viruses could be isolated.

VIROLOGY

Virus Research : The Department pursued its studies on the host-virus interaction with HIBOMA viruses. Human leucocyte interferon has now been produced in our laboratory. Studies have also been carried out towards the development of an ELISA test for viral diagnosis.

Development of the HIBOMA-Langur Model :

A multidisciplinary approach was made towards the *in vivo* development of a locally available, non-human primate model of human hepatitis B infection. The approach included basic studies of a virological, histopathological, biochemical and immunological nature.

Three types of control groups were utilized in the langur experiments : langur inoculated with a normal human serum pool ($n = 16$); langur inoculated with a 60 Co-irradiated HBsAg-positive human serum pool ($n = 4$) and uninoculated control langur ($n = 4$).

Experimental animals, classified on the basis of inoculum, were : langur inoculated with an HBsAg-positive human serum pool (RPHA titre = 1:25,000); ($n = 16$), and langur inoculated with an infected monkey liver homogenate pool ($n = 8$).

Langur in the control groups remained healthy throughout the length of the observational period of 28-32 weeks, there being neither clinical signs of disease, nor histopathological lesions in the liver, nor biochemical evidence of hepatocellular damage. Immunofluorescence studies aimed at demonstration of HBsAg in the control langur livers were negative. In the group inoculated with the HBsAg-positive human serum pool, antigen was detectable in the serum by RPHA in 5% of cases.

Of 16 langur inoculated with the HBsAg-positive human serum pool (HIBOMA-HSP), 65% exhibited a clinical syndrome characterised by anorexia and emaciation. SGPT elevations beyond the 3-sigma limits (3-45 IU) were shown by 85% of these animals. Histopathological lesions, characterised by portal inflammation and lobular infiltration by mononuclear cells, single cell necrosis and fatty change were seen in 90% of animals. Intracytoplasmic immunofluorescence, using FITC-labelled anti-HBs, was demonstrable in 44% of the animals.

When a pool from infected langur liver homogenates was inoculated into fresh langur, all 4 animals showed elevated SGPT levels, intermittently, during the 28-32 week observational period. Three out of four langur revealed clinical and histopathological evidence of liver disease. Only one animal revealed HBsAg-positive immunofluorescence. The liver homogenate of this langur was inoculated into 2 more animals. The liver homogenate from a langur from the HIBOMA-HSP group was also inoculated into 2 other animals.

At this second passage level, histopathological studies revealed evidence of classical viral hepatitis. The immunofluorescence test was positive in 2/4 animals, while SGPT elevation was exhibited by 2/4. Histopathological lesions were noted in subsequent biopsies, too, indicating that the virus was adapting itself towards the new host.

Thus, it was demonstrated that it was possible to infect langur with human HBV and that viral virulence increased with serial passage in the host. This is the first time that this locally readily available non-human primate has been shown to be a candidate model for human HBV infection.

Interferon :

Attempts have been put in by the Department towards the preparation of human leucocyte interferon from fresh buffy coats, (as per Cantell's method) obtained from the Blood Bank, Haffkine Institute.

After lysing erythrocytes with 0.83% NH_4Cl , the leucocytes were suspended in Eagle's MEM containing 4% human serum to a concentration of 10^7 cells/ml. One hundred HA units of Sendai virus per ml were used as inducer. Cells were incubated at 37.5°C on a magnetic stirrer for 20 h. This was followed by centrifugation at 2000 rpm for 40 min. The supernatant fluid was the crude interferon to be preliminarily tested for antiviral activity. The interferon pools from three such experiments were dialyzed at pH2 (glycine-HCL buffer) for 24 h, followed by back-dialysis with PBS (pH 7.2). The inducer, Sendai virus, was destroyed through dialysis at pH2. The interferon solution was centrifuged at 1200 rpm for 10 minutes to eliminate any possibility of precipitates.

being present. Interferon was filtered through millipore filter size 0.22 micrometre and was preserved at 20°C.

To eliminate the possibility of Sendai virus being still present 0.2 ml of the interferon solution (were inoculated) in 10-day embryonated hen's eggs by the intraallantoic route and allantoic fluids were collected after 72 h.

A titration experiment was performed using a standard haemagglutination method, using 0.5% chick erythrocytes, using 4 eggs/sample.

The following results were obtained :

Sample No.	Dose (ml.)/Egg.	Virus Titre
1	0.2	1 : 2
2	0.2	1 : 2
3	0.2	1 : 2
Virus Control	0.2	1 : 2048

The presence of interferon awaits future testing by its virus — inhibitory activity in a human amnion-vesicular stomatitis virus system. Human amnion cells have already been obtained from the Department of Biophysics, Cancer Research Institute, Bombay.

Development of ELISA Test :

Enzyme immunoassays have rapidly emerged, globally, in the field of diagnostic methods. These are easy to perform, speedier than other methods, highly sensitive and comparatively more economical, having a long shelf-life.

In our laboratory, an ELISA test was developed for the detection of anti-measles antibody. Measles antigen, obtained from M/s. Flow Laboratories and the NIV, Pune, was used for coating rhesus cells. One-in-fifty and 1 : 100 dilutions of human measles antibody (positive control from M/s. Flow Laboratories), Flow negative control serum, and normal human serum were used. It was found that 1 : 100 dilutions of the antigens were satisfactory for coating purposes. The human positive control serum yielded positive colour development at 1 : 50 to 1 : 100 dilutions. The negative controls, too yielded satisfactory results. However, insufficient numbers of samples from patients with measles have thus far been tested to indicate the reliability of this test in the clinical setting. We are planning to complete preliminary work of this nature by obtaining greater numbers of serum samples from the large general hospitals in our vicinity.

CHAPTER V

GENERAL ACTIVITIES

Administration :

Several steps were taken during the year to gear up the administration. The Director received immense help from the Executive Committee and other administrative committees in carrying out the administration of the Institute effectively.

Information and advice :

The Institute organised weekly demonstrations of methods used for the extraction of venom and first aid treatment in the cases of snake bite throughout the year. The Institute also provided expert assistance to different Institutions in matters regarding diagnosis of communicable diseases, identification of snakes, rodents, insects etc. Students from different colleges, including medical colleges, visited the Institute to gain first hand knowledge of the ongoing research projects at the Institute.

Library :

The library facilities of the Institute were made available to other Institutions as before. Despite want of adequate facilities like non-availability of adequate staff the library was kept open from 9.00 a.m. to 7.00 p.m. on week days and 9.00 a.m. to 5.00 p.m. on Saturdays. The library received generous donations of books and technical literature from WHO, British Council etc.

Artist Section :

A small unit attached to the Department of Pharmacology looks after the needs of the Institute concerning the preparation of photographic material etc.

APPENDIX I

Part III :

Bacteriology Department

Numbers

(1) Bacteriological analysis of chemicals, disinfectants and antibacterial agents		35
(2) Bacteriological analysis of water samples		8
(3) Testing water filters		2
(4) Lyophilization of bacterial cultures		69

APPENDIX II

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

	No. of samples
A. Histopathology	1,988
B. Haematology	791
C. Cytology for malignant cell	22
D. Clinical Pathology	
Urine for routine examination	177
Stool for routine examination	122
Semen examination	10
Immunological Pregnancy tests	958
Biological (Rat Test) Pregnancy Tests	81
Color Index	1
E. Bacteriology	
Culture for Tubercle Bacilli	168
Animal inoculation for tubercle bacilli	48
Smear & culture for c. diphtheriae	44
Urine culture	437
C.S.F. culture	9
Stool culture	44
Blood culture	61
Sputum culture	69
Miscellaneous body fluids & exudate culture	71
Antibiotic Sensitivity tests	566
Stool culture (Typhoid)	8
Urine culture (Typhoid)	7
Blood culture (Typhoid)	19
Pus culture	189
Enteropathogenicity test	56
Sputum for Gram staining	1
Culture of theatre swabs for anaerobes	300
Urine colony count	5
F. Serology	
Widal test	202
Weil Felix test	36
Brucellosis test	33
V.D.R.L. test	77
Kahn test	24
Paul Bunnel test	65
Casoni's test	7
Cold agglutinins	4
G. Mycology	
Isolation and identification of Pathogenic Fungi from clinical material	13

APPENDIX III

Biochemical tests of clinical samples were routinely carried out. A total number of 1150 samples were analysed (see Appendix).

Sr.	No.	Name of body fluid	Number of tests
1.	Blood		612
2.	Urine		116
3.	C.S.F. and other body fluids		3
4.	Special test		419
	Total :-		1,150

APPENDIX IV

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

Bombay Area	From Maharashtra
Cama And Albless Hospital	.. Civil Hospital, Thana
St. George's Hospital	.. Civil Hospital, Alibag, Kolaba
Jagjivanram Hospital	.. General Hospital, Sawantwadi
Central Railway Hospital	.. I.S.P. Hospital, Nasik
B.Y.L. Nair Charitable Hospital	.. Public Health Centres, Buldhana
K.E.M. Hospital	.. Civil Surgeon, Sangli
M.G.M. Hosnital	.. 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
G. T. Hospital	.. Civil Hospital, Nasik
J. J. Group of Hospitals	.. Hindustan Aeronautics Ltd. Nasik
Police Hospitals	..
M. A. Podar Hospitals	..
Greater Bombay Municipal Dispensaries	..

APPENDIX V

Human Pharmacology and Bioavailability Unit

The unit continued to function with the core staff in spite of flow of incoming pharmaceutical products. Besides analysing routine blood absorption some of the samples were of academic or applied interest and have been mentioned separately.

Routine Samples :

Manufacturers

- (1) Hindustan Antibiotics Pvt. Ltd.
- (2) Unique Pharmaceuticals
- (3) Alkem Laboratories
- (4) Nulife Pharmaceuticals
- (5) Haffkine Bio-Pharmaceuticals
- (6) Ethico Drug Mfg. Co.
- (7) Zandu Pharmaceuticals
- (8) Sterfil Laboratories
- (9) Fupharma Laboratories
- (10) Lyka Labs

Drug Preparations

Rifampicin — 2 samples
 Cephalixin — 2 samples : Caps. Tabs.
 Tetracycline — 3 samples
 Ampicillin — 3 samples
 Doxycycline
 Erythromycin
 Ampicillin : 3 preparations
 Tetracycline 2 preparations
 Erythromycin
 Ampicillin 2 preparations
 Doxycycline
 Tetracycline
 Rifampicin
 Doxycycline
 Phenoxymethyl Penicillin : 2
 Ampicillin Injections
 Ampicillin Injections
 Tetracycline

Proposals of Academic Interest:

- | | |
|---|---|
| (1) Bai Jerbai Wadia Hsp for Children, Bombay | Rifampicin levels in CSF and Blood in infants. |
| (2) Chemech Laboratories | HBsAg in Plasma, Fibrinogen and Gamma Globulin injections. |
| (3) Citadel Fine Pharmaceuticals | Cross over bio-availability of Doxy Tetra v/s. Tetra. alone |
| (4) Nicholas Lab. | Cross over bioavailability of Tab Disprin v/s. Micropyrin. |
| (5) Capsulation Services | Cross over Bioavailability of Tetra in Hard v/s. soft capsule. |
| (6) Indian Drugs and Pharmaceutical Ltd. | Cross over Bioavailability of New Synthetic antibiotic (SM37) v/s. Tetra & Doxy in animals (Rabbits). |

APPENDIX VI**II. Routine Diagnostic Work:**

The type and number of tissue specimens received for rabies diagnosis and the results of the laboratory tests are presented in the following table.

Sample	No. Recd.	(+) for Negri body	(+) by Mouse inoculation	(+) by histopathology
Human brain	4	Nil	Nil	—
*Human brain	1	—	—	—
Human CSF	2	Nil	Nil	—
Human Saliva	1	—	Nil	—
Dog brains	2	Nil	1	—
Buffalo brains	2	Nil	2	—

Note: — means not done

* Recd in formalin

APPENDIX VII**RADIATION BIOLOGY UNIT**

Routine Activity:	No.
No. of Total cases referred	: 87
Detected Hyperthyroid	: 22 (7 Toxic Goitres)
Detected Hypothyroid	: 1 (Myxoedema)
Detected Euthyroid	: 12 (3 Non toxic Goitres)
Thyroxine suppression	: 12

Besides uptakes in vivo, the sera samples were tested for T_3 and T_4 levels which correlated with the uptake results. Depending on the allocation of the grants these facilities will be extended to referred patients.

APPENDIX VIII
TESTING DEPARTMENT

Table showing number and nature of tests carried out on paid samples.

Nature of tests	No. of samples reported	No. of tests carried out
Pyrogen	272	272
Polymorph A	150	150
Sterility	15	15
Complete analysis	117	574
Adrenaline	61	61
Depressor substances	73	73
RWC & S.A.	60	115
Assay of Insulin	34	34
Toxoids for the content of merthiolate, formaldehyde etc.	59	137
Miscellaneous	36	105
Total	877	1536

APPENDIX IX
TESTING DEPARTMENT

Nature and number of samples received from Prohibition and Excise

Nature of samples received					No. of samples received
Liquors	..	..	..	..	1585
Beer	..	..	..	..	958
Toddy	..	..	..	..	370
Rectified Spirit	..	..	..	..	31
Denatured Spirit	..	..	..	..	44
French Polish	..	..	..	..	13
Bhang	..	..	..	..	17
Ganja	..	..	..	..	61
Opium	..	..	..	..	15
Charas	..	..	..	..	3
Total					3097

APPENDIX X
SAMPLES TESTED FOR TOXICITY

Sr. No.	Classification of Samples	No. of Samples tested	Toxicity Parameters
I.	Drugs	2	Acute oral toxicity
		9	Undue Toxicity
		75	Depressor substances test
II.	Chemicals	10	Acute oral toxicity
		3	Primary Skin Irritation
III.	Pesticides	8	Acute oral toxicity
		7	Acute dermal toxicity
		3	Primary skin Irritation
		1	Mucous Membrane Irritation
		1	Acute toxicity to fish
		2	Acute toxicity in Pigeon M.L.D.
IV.	Plastics	2	Toxicity of Plastic containers

APPENDIX XI
Acute Oral Toxicity of Some Pesticides

Sr. No.	Name of the compound	Species	LD ₅₀ value	Fiducial limits	Symptoms
1.	Copper Oxychloride	Rats	1300 mg/kg	930- 1820 mg/kg	Diarrhoea, emaciation, respiratory disturbances and death.
2.	Cythion (Tech)	Rats	2207 mg/kg	1765- 2759 mg/kg	Excitation, respiratory disturbances, diarrhoea, and death.
3.	Metoxuron	Mice	1300 mg/kg	1215- 1391 mg/kg	Sedation, respiratory disturbances and death.
4.	Household Insecticide	Mice	27460 mg/kg	20720-36380 mg/kg	Excitation, respiratory disturbances and death.
5.	Household Insecticide	Rats	15690 mg/kg	11620-21180 mg/kg	Excitation, respiratory disturbances and death.
6.	Malathion 50 E.C.	Mice	1720 mg/kg	1580- 1880 mg/kg	Excitation, tremors, respiratory disturbances and death.
7.	Malathion (Tech)	Mice	885.6 mg/kg	783.7- 1001 mg/kg	Excitation, respiratory disturbances, convulsion, tremors gasping and death.
8.	Zinc Phosphide	Mice	42.5 mg/kg	29.31-61.63 mg/kg	Excitation, respiratory disturbances, sedation, convulsion, loss of righting reflex death.

Primary Skin Irritation Study of some Pesticides

- | | | | |
|----|-----------------|---------|--|
| 1. | Cythion 50 EC | Rabbits | Cythion 50 EC produces mild irritation to skin when compared with control. |
| 2. | Malathion 50 EC | Rabbits | Malathion 50 EC produces mild irritation to skin when compared with control. |
| 3. | Malathion Tech. | Rabbits | Malathion Tech produces no irritation to skin. |

Irritation to Mucous Membrane Study of some Pesticides

- | | | | |
|----|-----------------|---------|---|
| 1. | Malathion Tech. | Rabbits | Malathion Tech produces no irritation to mucous membrane. |
|----|-----------------|---------|---|

Acute Toxicity to Fish of some Pesticides

- | | | | |
|----|-----------------|----------------|--|
| 1. | Malathion Tech. | Fish (Tilapia) | or 2.65 ppm Loss of equilibrium and death. |
|----|-----------------|----------------|--|

Acute Toxicity to Pigeon (M.L.D.) of some Pesticides

- | | | | |
|----|-----------------|--------|--|
| 1. | Malathion Tech. | Pigeon | 308 mg/kg M.L.D. Excitation, Loss of righting reflex, respiratory disturbances and death. |
| 2. | Malathion 50 EC | Pigeon | 2086 mg/kg M.L.D. Excitation, loss of righting reflex, respiratory disturbances and death. |

APPENDIX XII
Acute Dermal Toxicity Studies of Some Pesticides

Sr. No.	Name of the Compound	Species	LD₅₀ Value	Fiducial limits	Symptoms
1.	Dimethoate 30 EC	Rabbits	1708 mg/kg	1180-2474 mg/kg	Excitation, depression, respiratory disturbances and death.
2.	Dimethoate 40 EC	Rabbits	2052 mg/kg	1520-2770 mg/kg	Excitation, depression, respiratory disturbances and death.
3.	Copper Oxychloride	Rabbits	LD ₅₀ value is more than 10 gm/kg		Excitation, respiratory disturbances and death.
4.	2:4 D Amine salt	Rabbits	LD ₅₀ value is more than 10 ml/kg		Excitation, respiratory disturbances and death.
5.	2:4 D Sodium salt	Rabbits	LD ₅₀ value is more than 10 gm/kg		
6.	Household Insecticide	Rabbits	LD ₅₀ value is more than 10 ml/kg		
7.	Malathion 50 EC	Rabbits	2190 mg/kg	2028-2366 mg/kg	Excitation, tremors, lacrimation, diarrhoea, irritation to skin, and death.

APPENDIX XIII
COURSES OFFERED
DEGREE COURSES

M.Sc.	:	Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology.
M.Sc. (By Papers)	:	Microbiology, Biochemistry.
Ph.D.	:	Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology and Pharmacology.
M. Pharm	:	Pharmacology and Pharmaceutical Chemistry.
M.D.	:	Pathology, Pharmacology and Microbiology.

DIPLOMA COURSES

D.M.L.T.	:	Diploma in Medical Laboratory Technology.
----------	---	---

CERTIFICATE COURSES

Course in Drug Analysis and
Short-Term Training Courses

APPENDIX XIV

Post — Graduate Teachers

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

Name	Qualification
Dr. K. D. Sharma	M.D.
Dr. M. B. Bhide	M.D.
Dr. R. S. Kamat	M.D.
Dr. M. V. N. Shirodkar	M.B.B.S., A.B., Sc.D. (Johns Hopkins), P.B.K. (U.S.A.)
Dr. K. H. Dave	M.D., D.P.B.
Dr. D. M. Renapurkar	M.Sc., Ph.D. F.M.A.S.
Dr. V. R. Rao	M.VSc., Ph.D.
Dr. F. L. Saldhana	M.Sc., Ph.D., FLC (Col.)
Dr. A. J. Baxi	M.Sc., Ph.D.
Dr. M. H. Shah	M.Sc., Ph.D.
Dr. S. Mukerji	M.Sc., Ph.D.
Dr. P. Ramkrishnan	M.Sc., Ph.D.
Dr. S. V. Gadre	M.Sc., Ph.D.
Dr. D. T. Chaudhari	M.Sc., Ph.D.
Dr. B. G. Khadse	M.Sc., Ph.D.
Dr. M. K. Bhopale	M.Sc., Ph.D.
Dr. H. J. Mansharamani	M.B.B.S., Ph.D.
Dr. A. B. Kulkarni	M.Sc., Ph.D.
Dr. H. S. Ved	M.Sc., Ph.D.

APPENDIX XV

Statistics Relating to Students

Students enrolled at the Institute during the year 1980-81

M.D.											No. of Students
Pathology	..	..	..	..	..	..	..	..	..	..	1
Ph.D											
Pharmacology	..	..	..	..	..	..	..	..	..	..	2
Pharmacy	..	..	..	..	..	..	..	..	..	..	1
Applied Biology	..	..	..	..	..	..	..	..	..	..	2
Biochemistry	..	..	..	..	..	..	..	..	..	..	4
Organic Chemistry	..	..	..	..	..	..	..	..	..	..	3
Microbiology	..	..	..	..	..	..	..	..	..	..	5
M.Sc.											
Applied Biology	..	..	..	..	..	..	..	..	..	..	2
Microbiology	..	..	..	..	..	..	..	..	..	..	3
Organic Chemistry	..	..	..	..	..	..	..	..	..	..	2
Zoology	..	..	..	..	..	..	..	..	..	..	1

APPENDIX XVI

Short Term Training Courses

Thirty five students attended short-term training courses during the year. Details of subjects offered and the number of students who attended these courses are recorded here.

Clinical Pathology	..	..	..	..	..	..	..	..	15
Blood Banking	..	..	..	..	..	..	..	..	9
Bacteriology	..	..	..	..	..	..	..	..	1
Virology	..	..	..	..	..	..	..	..	1
Entomology and Parasitology	..	..	..	..	..	..	..	..	6
Testing	..	..	..	..	..	..	..	..	3
Total :-									<u>35</u>

APPENDIX XVII

Trainees and Research Fellowships and Scholarships

Trainees admitted :

Diploma in Medical Laboratory Technology (D.M.L.T.)	..	..	..	..	..	..	..	20
Course in Drug Analysis	..	..	..	..	..	..	..	14
Short Term Training Course	..	..	..	..	..	..	..	35
Number of Research Fellowships and Scholarships								
Senior Research Fellowships (Haffkine Institute)	..	..	..	..	..	..	..	2
Junior Research Scholarship (Haffkine Institute)	..	..	..	..	..	..	..	1
Senior Research Fellowship (Platinum Jubilee)	..	..	..	..	..	..	..	1

APPENDIX XVIII

Degree Awards during the year

Name of Candidate	Subject	Topic
Shri J. M. Deshpande	Ph.D. Applied Biology	Studies on the Characterization of HIBOMA viruses.
Shri A. B. Kulkarni	Ph.D. Biochemistry	Brain acetylcholine metabolism in malnourished rats".
Shri H. S. Ved	Ph.D. Biochemistry	Efforts of the change in the levels of dietary calcium and vitamin D on the lipid metabolism in albino rats.
Shri J. P. Bapat	Ph.D. Biochemistry	Studies on the intracellular and membrane markers in the cases of hematological disorders.
Shri H. K. Soni	Ph.D. Biochemistry	Mechanism of action of hemolytic drugs : <i>in vitro</i> studies
Shri A. V. Muthe	Ph.D. Biochemistry	Studies on the effect of snakes venom on blood coagulation factors.
Shri T. S. Malvankar	Ph.D. Org. Chemistry	Synthesis and study of organic compounds as possible "Antimicrobials".
Smt. S. S. Kantak	M.Sc. Applied Biology	Studies on Biological Standardization of Antivenin.
Kum. M. S. Vaidya	M.Sc. Biochemistry	Studies on red cell enzymes.
Kum. S. H. Bhadsavle	M.Sc. Biochemistry	Effect of snake venom on biochemistry of Red cells.
Shri D. S. Sanghvi	M.Sc. Org. Chemistry	Synthesis of organic compounds of possible therapeutic value. (Synthesis and study of Pyrimidine derivatives as possible anti malarial agents.)
Shri D. G. Kulkarni	M.Sc. Org. Chemistry	Synthesis of heterocyclic compounds as possible anthelmintic agents.
Shri A. S. Bobade	M.Sc. Org. Chemistry	Synthesis and study of benzothiazole derivatives as possible antituberculosis agents.
Shri G. R. Wader	M.Sc. Org. Chemistry	Synthesis and study of organic compounds of possible therapeutic value.
Shri P. P. Gudadhe	M.Sc. Org. Chemistry	Synthesis and study of dihydros-triazine derivatives as possible antimalarial agents.

Smt. E. R. D'souza	M.Sc. Microbiology	Studies in the antigenicity of diphtheria toxoid.
Smt. J. G. Nadkarni	M.Sc. Microbiology	Studies in typhoid vaccine.
Shri T. G. Tare	M.Sc. Zoology	Studies on scorpions.
Kum. V. R. Pradhan	M.Sc. Zoology	Host-parasite relationship and chemotherapeutic studies in the rodent malarial parasite, <i>Plasmodium berghei yoelii</i> .

APPENDIX XIX

Venom produced and supplied during the year 1980-81

	Venoms Produced	Venoms Supplied	Animals Procured
I.			
Cobra	89.33 gms.	86.89 gms.	125 Nos.
R. vipers	64.91 gms.	59.10 gms.	326 Nos.
Krait	15.97 gms.	10.82 gms.	29 Nos.
Total	170.21 gms.	167.89 gms.	600 Nos.
Red Scorpion	10.55 gms.	8.34 gms.	7742 Nos.
Black Scorpion	0.25 gms.	0.09 gms.	657 Nos.
TOTAL	10.80 gms.	8.43 gms.	8399 Nos.

II. Visitors to Snake Farm

(A) Students	1920 Nos.
(B) Staff of Scientific Institutions	85 Nos.
(C) Distinguished Visitors	118 Nos.
(D) Foreigners	79 Nos.
(E) Thursday Visitors	3575 Nos.
(F) Others	467 Nos.
Total	6244 Nos.

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

APPENDIX XX
PAPERS PUBLISHED 1980-81

- CHOWDHURY, A. B.: Conquest of Tropical Diseases.
Bulletin of Haffkine Institute, 1980(1), 1-14.
- GOSAVI, A. V.; RENAPURKAR, D. M.; KULKARNI, A. B.: Effect of Ascorbic acid on *in vitro* cultivation of *A. diesingi* eggs.
Bulletin of Haffkine Institute, 1980 (1), 15-20.
- KHADSE, B. G.; LOKHANDE, S. R.; CHAUDHARI, P. P.; BHIDE, M. B. and SHAILA R. GHOOI.: Synthesis and anthelmintic study of new 2-substituted phenyl hydrazino and amino pyrido (3, 2-d) thiazoles.
Bulletin of Haffkine Institute, 1980 (1), 21-26.
- NERURKAR, C. B.; CHAUDHARI, D. T.; MADHUBEN R. PATEL and SABNIS, S. S.: Synthesis and study of thiocarbamiides derived from 2-(4, aminophenyl) thiazoles and 4/(4 aminophenyl) thiazoles for *in vitro* antituberculosis activity -1-.
Bulletin of Haffkine Institute, 1980 (8) 1, 27-32.
- PENDALWAR, S. L.; CHAUDHARI, D. T.; and MADHUBEN R. PATEL.: Synthesis and study of 2-aryloxy thioisonicotinamides analogous to Ethionamide and thiazoles derived therefrom as antituberculosis agent-1.
Bulletin of Haffkine Institute, 1980, 8 (1) 33-40.
- ANITA, K. DAVE; S. MUKHERJI and GAITONDE, B. B.: Natural immunity to tetanus Part I. A study in adivasi population in Virar.
Bulletin of Haffkine Institute, 1980; Vol. 8 (2), 43-47.
- SAVITRI, S. R.; BHOPALE, M. K. and RENAPURKAR, D. M.: Serum antibody response in albino rats after the transfer of adult *Litomosoides carinii* worms.
Bulletin of Haffkine Institute, 1980; Vol. 8 (2) 48-52.
- KHAIRNAR, V. L.; LOKHANDE, S. R.; PATEL MADHUBEN R.; KHADSE, B. G.: Synthesis and study of 5(6) substituted-2 (substituted-benzyl) thio-sulfonyl benzimidiazoles as possible antituberculosis agents.
Bulletin of Haffkine Institute, 1980; Vol. 8 (2) 53-56.
- NERURKAR, C. B.; CHAUDHARI, D. T.; PATEL, MADHUBEN R. and SABNIS, S. S.: Synthesis and study of schiff bases derived from 2-(4-amino phenyl) thiazoles for *in vitro* antituberculosis activity-2.
Bulletin of Haffkine Institute.
- KHAIRNAR, V. L.; LOKHANDE, S. R.; PATEL, MADHUBEN R. and B. G. KHADSE: Synthesis and screening for antituberculosis activity of substituted -s-(pyrimidyl or quinolyl)-2-thio or sulfonyl).
Bulletin of Haffkine Institute, 1980, Vol. 8 (2) 65-70.
- KULKARNI, A. B. and GAITONDE, B. B.: Thiamine levels in the brain regions during onset of neurological symptoms of thiamine deficiency in rats.
Bulletin of Haffkine Institute, 1980, Vol. 8 (2) 71-74.
- GHOOI, R. B.: Proceedings of the Critics' Club.
Bulletin of Haffkine Institute, 1980, Vol. 8 (2) 75-76.
- CHITNIS, D. S.; SHARMA, K. D. and KAMAT, R. S.: Role of *V. cholerae* somatic antigens in the pathogenesis of cholera — A preliminary report.
Bulletin of Haffkine Institute, 1980, Vol. 8, No. 3, pg. 77-82.

RANGNEKAR, V. M.; GADRE, S. V.; MUKHERJI S. and CHITNIS, D. S. Prevalence of fertility inhibition negative type R. plasmids in faecal coliform. *Bulletin of Haffkine Institute*, 1980, Vol. 8, No. 8, pg. 83-86.

CHAUDHARI, P. P.; LOKHANDE, S. R.; KHADSE, B. G.; BHIDE, M. B. and GHOOI S. R.: Synthesis and anthelmintic activity of new pyridine (3, 2 d) thiozoles.

Bulletin of Haffkine Institute, 1980, Vol. 8, No. 3 pg. 87-94.

SANGHVI, D.S.; CHAUDHARI, D.T. and GUDADHE, P.P.; Synthesis and study of 2-aryl amino-4 (substituted amino) 6-methylpyrimidines as possible antimalarial agents.

Bulletin of Haffkine Institute, 1980, Vol. 8, No. 3 pg 95-101.

PENDALWAR, S. L.; CHAUDHARI, D. T. and PATEL, M. R. Anti-tuberculosis agents — II synthesis of some new N-substituted pyrazinamides.

Bulletin of Haffkine Institute, 1980, Vol. 8, No. 3, pg 102-106.

GOSAVI, A.V.; KULKARNI, A.B. and RENAPURKAR, D.M.: Effect of coconut milk on in vitro embryonic development of *Hammer schmittiella diesingi*.

Indian Journal of Parasitology.

RENAPURKAR, D.M.: Investigation of a suspected plague outbreak in 1975 in Tapni village, Maharashtra.

Indian Journal of Public Health, 25, 74-75 (1981).

RENAPURKAR, D.M.: Serological investigation of urban and rural commensal rodent plague in Maharashtra.

Journal of Communicable Diseases, 13, 110-114 (1981).

SAVITRI, C.R.; RENAPURKAR, D.M.: Iodine uptake studies in cotton rat filariasis.

Folia parasitologica, 1983.

GAITONDE, B.B.; SATHE, B.D.; MUKHERJI, S.; SUTAR, N.K.; ATHALYE, R.P.; KOTWAL, V.P.; and RENAPURKAR, D.M.: Studies on schistosomiasis in village Gimvi of Maharashtra.

Indian Journal of Medical Research, 1981, 74, 352-357.

APPENDIX XXI.

Papers submitted for publication

KHAIRNAR, V.L.; LOKHANDE, S.R.; PATEL, M.R. and KHADSE, B.G.: Synthesis and study of 5(6)-substituted-2 (Heterocyclic)-Thio sulfonyl Benzimidazoles as possible antituberculosis agents.

Bulletin of Haffkine Institute.

KHADSE, B.G.; LOKHANDE, S.R.; PATEL, M.R. and KHAIRNAR, V.L.: Synthesis and study of 2,4, diamino-6-substituted arylidene, heteroarylidene and benzimidazole quinazolines as possible antituberculosis agents.

Bulletin of Haffkine Institute.

NERURKAR, C.B.; CHAUDHARI, D.T.; PATEL, M.R. and SABNIS, S.S. : Synthesis and study of schiff derived from 2-(4'-Aminophenyl) Thiazoles for *in vitro* Antituberculosis activity II.

Bulletin of Haffkine Institute.

NERURKAR, C.B.; CHAUDHARI, D.T.; PATEL, M.R. and SABNIS, S.S. : Synthesis and study of Thiocarbanilides derived from 2-(4-Aminophenyl) Thiazoles and 4-(Aminophenyl)-Thiazoles for *in vitro* Antituberculosis activity-I.

Bulletin of Haffkine Institute.

DAVE, A.; MUKHERJEE, S.; GAITONDE, B. B. : Natural immunity against tetanus. Part I: A study in Adivasi population in Virar.

Bulletin of Haffkine Institute.

PENDALWAR, S.L.; CHAUDHARI, D.T.; PATEL, M.R. : Synthesis and study of 2-Aryloxy thioisonicotinamides analogous to ethionamide and thiazoles derived therefrom as antituberculosis agents — I.

Bulletin of Haffkine Institute.

PENDALWAR, S.L.; CHAUDHARI, D.T.; PATEL, M.R. : Synthesis and study of some new N-substituted pyrazinamides as antituberculosis agents — II.

Bulletin of Haffkine Institute.

CHAUDHARI, P.P.; LOKHANDE, S.R.; KHADSE, B.G.; BHIDE, M.B.; GHOOI SHAILA : Synthesis and anthelmintic activity of new pyrido(3,2-d) Thiazoles.

Bulletin of Haffkine Institute.

KHADSE, B.G.; LOKHANDE, S.R.; CHAUDHARI, P.P.; BHIDE, M.B. and GHOOI SHAILA : Synthesis and Anthelmintic study of new 2-substituted phenyl Hydrazino and Amino pyrido (3,2-d) Thiazoles.

Bulletin of Haffkine Institute.

SANGHAVI, D.S.; CHAUDHARI, D.T. and GUDADHE, P.P. : Synthesis and study of 2-arylamino-4-(Substituted amino)-6-methyl pyrimidines as possible antimalarial agents — I.

Bulletin of Haffkine Institute.

SANGHAVI, D.S.; CHAUDHARI, D.T. and GUDADHE, P.P. : Synthesis and study of 2-substituted-4-methyl-5-(B-hydroxyethyl)-6-hydroxy pyrimidines as possible antimalarial agents — II.

Bulletin of Haffkine Institute.

GOSAVI, A.V.; KULKARNI, A.B.; and RENAPURKAR, D.M. : Effect of coconut milk on *in vitro* embryonic development of *Hammerschmidtella diesingi*.

Indian Journal of Parasitology.

BHOPALE, M.K.; MENON, SUSHILA and KAMATH, VIJAYALAXMI, V.R. : A trial of counterimmunoelectrophoresis in hookworm infection.

Bulletin of Haffkine Institute.

SATHE, S.S.; CHANDERKAR, N.G.; AREKAR, A.S.; PANDIT, C.H.; BUDUK, D.P.; BAPAT, R.D.; KINARE, S.G. and SHIRODKAR, M.V.N. : Towards the development of new animal models of human hepatitis B. virus infection.

Indian Journal of Medical Research.

DAVE, A. K. and MUKHERJI, S.: Natural immunity against tetanus part II. Possible role of non-toxicogenic clostridium tetani in it.
Bulletin of Haffkine Institute.

RANGNEKAR, V. M.; GADRE, S. V.; MUKHERJI, S. and CHITNIS, D. S.: Prevalence of multi-drug resistant coliforma in human gut: Effect of antibiotic therapy.
Bulletin of Haffkine Institute.

MUKHERJI, S.: Motility and other properties of acinetobacter anitratus and acrinetobacter anitratus like bacterial strains.
Indian Journal of Medical Research.

SANGHAVI, D. S.; CHAUDHARI, D. T.; GUDADHE, P. P.: Synthesis and study of 2-substituted-4-methyl-5, 6-Duhydrofuro (22, 3-d-pyrimidines as possible antimalarial agents — III.
Bulletin of Haffkine Institute.

GHOOL, R. B.; RATH, S.; BHIDE, M. B.: 48/80 induced mouse pawedema — A model for screening antianaphylactic agents.
European Journal of Pharmacology.

KHADSE, B. G.; LOKHANDE, S. R.; KULKARNI, D. G.: Synthesis and study of 4-(Substituted — Aryl or Heteroaryl)-2-substituted phenyl) Amino thiazoles as antituberculosis agents.
Indian Journal of Chemistry.

RANGNEKAR V. M.; GADRE, S. V.; MUKHERJI, S. and CHITNIS, D. S.: Conjugal transferability of In cH and other incompatibility group R-plasmide to *S. typhi*.
Indian Journal of Microbiology.

RANGNEKAR, V. M.; GADRE, S. V.; MUKERJI, S. and CHITNIS, D. S.: Conjugate transferability of In cH, and other Incompatibility group R-plasmids to *S. typhi*.

KULKARNI, D. G.; LOKHANDE, S. R. and KHADSE, B. G.: Synthesis and study of new-2(Substituted Thiazoylphenylamino pyrino(3,2, d) thiazoles as possible anthelmintic agents.
Indian Journal of Pharmaceutical Science.

KHADSE, B. G.: Synthesis and anthelmintic activity of new substituted phenyl isothiocyanates.
Indian Drugs.

CHAVAN, S. R.; NIKAM, S. T.: Mosquito larvicidal activity of *Ocimum basilicum* Linn (labiate).
Indian Journal of Medical Research.

RENAPURKAR, D. M.: Investigation of a suspected plague out break in 1975 in Tapni village in Maharashtra.
Indian Journal of Public Health.

RENAPURKAR, D. M.: Serological investigation of urban and rural commensal rodent plague in Maharashtra.
Journal of Communicable Diseases.

RENAPURKAR, D. M.: Distribution of peridomestic and wild rodent fauna in Maharashtra state.
Pestology.

KULKARNI, A. B.; VED, H. S. and GAITONDE, B. B.: Effect of post-weaning prolonged protein deficiency of rat brain enzymes.
Bulletin of Haffkine Institute.

SAVITRI, C. R.; RENAPURKAR, D. M.: Iodine uptake studies in cotton rat filariasis.
Folia parasitologica.

APPENDIX XXII

PAPERS PRESENTED IN CONFERENCES

29th Annual Conference of Indian Association of Pathologists and Microbiologists held on 27th, 28th and 29th December 1980, Belgaum.

R. NAIR and R. S. KAMAT
Cellular Immunity in Salmonella Infection
Proceedings (Abst) 29th Annual Conference
Indian Association of Pathologists and Microbiologists 1980
M2 P. 33.

S. KHEDKAR and R. S. KAMAT
Mechanism of Immunity in Salmonellosis
Proceedings (Abst) 29th Annual Conference
Indian Association of Pathologists and Microbiologists 1980
M3 P. 34.

R. S. KAMAT and R. MIRCHANDANI
Relationship between colonisation factor antigen and serotypes of 'ETEC'
Proceedings (Abst) 29th Annual Conference
Indian Association of Pathologists and Microbiologists 1980
M8 P. 35.

B. BHOGALE and R. S. KAMAT
Hela cell invasion assay for enteric pathogens.
Proceedings (Abst) 29th Annual Conference
Indian Association of Pathologists and Microbiologists 1980
M9 P. 85.

D. S. CHITNIS and R. S. KAMAT
Role of somatic antigens in the adhesion of *V. cholerae* to the Intestinal epithelium.
Proceedings (Abst) 29th Annual Conference
Indian Association of Pathologists and Microbiologists 1980
M11 P. 86.

J. DESHPANDE and R. S. KAMAT
Lipoproteins — DH eliciting antigen/s of mycobacteria.
Proceedings (Abst) 29th Annual Conference.
Indian Association of Pathologists and Microbiologists 1980
M32 P. 96.

J. P. MEHTA and R. S. KAMAT
An antigen/s specific to the mycelial phase of *C. albicans*.
Proceedings (Abst) 29th Annual Conference.
Indian Association of Pathologists and Microbiologists 1980
M47 P. 101.

"Dr. P. V. Gharpure Prize" for 1980-81 of Teaching Pathologists and Microbiologists of Bombay was awarded to Miss Rohini Nair and Shri Sharad Khedkar.

