

Annual Report of the Haffkine Institute

1978 – 1979



HAFFKINE INSTITUTE
PAREL, BOMBAY-400 012
INDIA

CONTENTS

Pages

ADMINISTRATION—

Governing Council	1
Academic Council	3
Advisory Council for Research	3
Executive Committee (Administration)	3
Staff of the Institute	4

INTRODUCTION—

Activities : Highlights of Achievements : Departments	8
---	---

DEVELOPMENT IN TEACHING

17

RESEARCH PROGRESS—

Bacteriology	19
Biochemistry	21
Chemotherapy	24
Clinical Pathology	26
Director's Research Cell	29
Immunology	33
Parasitic Diseases and Zoonosis	35
Pharmacology	37
Phytochemistry	39
Radiation Biology Unit	41
Toxicology	43
Virology	44

TESTING

49

GENERAL ACTIVITIES

51

APPENDICES—

I Department of Biochemistry	52
II-A Department of Clinical Pathology	53
II-B Isolation of <i>C. albicans</i> from sputum	55
III Rabies Research Unit	56
IV Radiation Biology Unit	56
V Samples for Toxicity	57

(ii)

CONTENTS

	PAGE
VI Acute oral Toxicity	58
VII Acute Dermal Toxicity	59
VIII Snake and Scorpion Venom processing	60
IX Postgraduate Courses	61
X Postgraduate Teachers	61
XI Statistics relating to postgraduate students	62
XII Degree awarded and Research Topics	62
XIII Short term Training Courses	63
XIV Trainees, Research Fellowships and Scholarships	64
XV Prohibition and Excise Samples	64
XVI Biological and Microbiological Tests	65
XVII Chemical Tests	65
XVIII Critic's Club Lectures	66
XIX Working of the Library	67
XX Papers sent for publication	68
XXI List of Papers published	70

HAFFKINE INSTITUTE FOR TRAINING RESEARCH AND TESTING

GOVERNING COUNCIL

President

- (1) SHRI SHIVAJIRAO PATIL (NILANGEKAR), upto 14th September 1978.
- (2) DR. (SMT.) PRAMILA TOPLE, w.e.f. 15th September 1978.

Honourable Ministers for Public Health, Government of Maharashtra,
Mantralaya, Bombay-32.

Vice-President

- (1) SHRI SUSHILKUMAR SHINDE, upto 14th September 1978.
- (2) SHRI N. B. GADEKAR, w.e.f. 15th September 1978.

Honourable Ministers for State for Public Health, Government of
Maharashtra, Mantralaya, Bombay 32.

Member-Secretary

DR. B. B. GAITONDE, upto 17th February 1979.
Director, Haffkine Institute, Parel, Bombay 12.

Members:

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

- (1) Shri M. S. PALNITKAR, upto 13th June 1978.
- (2) Shri N. V. SUNDARARAMAN, w.e.f., 14th June 1978.

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

Shri V. PRABHAKAR upto 1st February 1979.

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

Shri B. D. Joshi, w.e.f., 2nd February 1979.

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. D. DESHMUKH upto 16th June 1978.

Shri N. C. VENKATCHILAN, w.e.f., 2nd October 1978.

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

Dr. B. B. GAITONDE upto 15th February 1979.

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

(1) Dr. C. GOPALAN upto 18th February 1979.

(2) Prof. V. RAMALINGASWAMI, w.e.p. 19th February 1979.

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

Prof. RAM JOSHI.

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

Dr. C. G. PANDIT.

Trustee, Dorabji Tata Trust, Bombay House, Homi Modi Street, Fort
Bombay-1.

Prof. R. CHOKSI upto 30th March 1979.

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

Dr. V. V. KAYARKAR.

ACADEMIC COUNCIL

Chairman :

Dr. B. B. GAITONDE upto 17th February 1979.

Members :

Dr. V. D. DESHMUKH
Dr. S. S. GOTHOSKAR
Dr. (Smt.) S. M. VACHHA
Dr. M. B. BHIDE
Dr. V. V. KAYARKAR

Convener :

Dr. S. MUKERJI

ADVISORY COUNCIL FOR RESEARCH

Chairman :

Dr. B. B. GAITONDE upto 17th February 1979.

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. B. B. GAITONDE upto 17th February 1979.

Members :

Dr. M. V. N. SHIRODKAR
Shri Y. V. THAKRE upto 11th July 1978
Shri S. T. Vanjari w.e.f. 12th July 1978.

Convener :

Dr. M. H. SHAH

STAFF OF THE HAFFKINE INSTITUTE

ADMINISTRATION

Director :

B. B. GAITONDE, MD., MSc., (Med.), FASc. upto 17th February 1979.

Honorary Administrative Officer :

Y. V. THAKRE, MA., LL.B., upto 11th July 1978,

S. T. VANJARI, w.e.f., 12th July 1978,

Scientific Secretary :

R. B. GHOOI, MSc.

Accounts Officer :

P. D. SATHE upto 30th November 1978.

S. G. MORE w. e. f. 1st December 1978.

BIOCHEMISTRY

Senior Scientific Officer Class I :

P. RAMKRISHAN, MSc., Phd.

R. N. JOSHI, MSc.

Junior Scientific Officer :

A. B. KULKARNI, MSc.

H. S. VED, MSc.

BACTERIOLOGY

Senior Scientific Officer Class I :

S. V. GADRE, MSc., Ph.D.

Senior Scientific Officer Class II :

D. S. CHITNIS, MSc., upto 15th June 1978.

Junior Scientific Officer :

R. P. BHAMARIA, MSc., Ph.D.

CLINICAL PATHOLOGY

Assistant Director :

R. S. KAMAT, MBBS., MD.

Senior Scientific Officer Class I :

S. MUKERJI, MBBS., MSc., Ph.D., upto 15th June 1978

D. S. CHITNIS w. e. f. 16th June 1978.

Junior Scientific Officer :

J. P. MEHTA, MSc.

Medical Officer :

Y. FERNANDES, MBBS., DPB.

RANJANA A. DESHMUKH, MBBS.

CLINICAL RESEARCH DIVISION

Senior Scientific Officer Class II :

A. P. KULKARNI, MSc., w.e.f., 1st November 1978.

CHEMOTHERAPY

Assistant Director :

M. H. SHAH, MSc., PhD.

Senior Scientific Officer Class I :

D. T. CHAUDHARI, MSc., PhD.

B. G. KHADSE, MS., PhD.

Senior Scientific Officer Class II :

P. P. DALVI, MSc. w.e.f. 2nd November 1978.

Junior Scientific Officer :

M. R. PATEL, MSc.

N. T. JAOKAR, MSc.

DIRECTOR'S RESEARCH CELL

Junior Scientific Officer :

S. S. JADHAV, M Pharm.

HUMAN PHARMACOLOGY AND BIOAVAILABILITY

Senior Scientific Officer Class I :

P. S. WORLIKAR, MBBS., MD., 31st May 1978.

Senior Scientific Officer Class II :

S. S. SATHE, MSc.

INSTRUMENTATION CELL

Junior Scientific Officer :

J. V. SAWANT, MSc.

IMMUNOHAEMATOLOGY

Senior Scientific Officer Class I :

D. CHENGAPPA, MBBS.

IMMUNOLOGY

Senior Scientific Officer Class II :

Smt. S. N. HATTIKUDUR, MSc.

PHARMACOLOGY

Assistant Director :

M. B. BHIDE, MBBS, MD.

Senior Scientific Officer Class II :

R. N. JOSHI, MSc.

Junior Scientific Officer :

S. T. NIKAM, MSc., upto 6th November 1978.

PHYTOCHEMISTRY

Senior Scientific Officer Class I :

S. R. CHAVAN, MSc.

Junior Scientific Officer :

S. T. NIKAM, MSc, w. e. f. 7th November 1978.

R. P. SUBHEDAR, MSc.

RABIES UNIT

Senior Scientific Officer Class I :

H. J. MANSHARAMANI, MBBS., MSc., PhD.

RADIATION BIOLOGY

Senior Scientific Officer Class I :

N. G. CHANDERKAR, MBBS., BSc., (Med.), PhD.

Junior Scientific Officer :

S. C. TALWALKAR, MSc., upto 30th June 1979.

STATISTICAL SECTION

Statistician :

A. S. AREKAR, MSc.

TESTING LABORATORY

Senior Scientific Officer Class I :

V. P. SHINDE, MSc., PhD., upto 4th July 1978.

H. K. SONI, MSc., w.e.f. 5th July 1978.

Junior Scientific Officer :

H. K. SONI, MSc., upto 4th July 1978.

H. K. DALAL, BSc.,

S. S. JAIN, M Pharm.

S. B. DHOLAM, MSc., w.e.f. 10th July 1978.

TOXICOLOGY

Senior Scientific Officer Class I :

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

Senior Scientific Officer Class II :

M. G. PHATAK, MSc.

TRAINING

Senior Scientific Officer Class I :

S. MUKERJI, MBBS., MSc., PhD.

VIROLOGY

Assistant Director :

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

Senior Scientific Officer Class II :

A. P. KULKARNI, MSc. upto 31st October 1979.

Medical Officer :

CHHAYA H. PANDIT, MBBS.

ZOOONOSIS

Assistant Director

D. M. RENAPURKAR, MSc., Ph.D.

Senior Scientific Officer Class I :

V. H. APTE, MVSc., upto 18th October 1978.

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. w.e.f., 6th November 1978.

N. K. SUTAR, BSc., w.e.f. 8th December 1978.

CHAPTER I

INTRODUCTION

Haffkine Institute is mostly engaged and concerned with research and control of communicable diseases. Keeping this in view, the institute organized one seminar and a workshop pertaining to the control and laboratory diagnosis of acute diarrhoeas during April 1978. The seminar was held from 14th April 1978 to 18th April 1978 and was organized in collaboration with Director of Health Services, Government of India. The seminar dealt with "Oral rehydration therapy in cholera and other diarrhoeas". The participants included Assistant Director of Health Services (Cholera) of India, prominent clinicians attached to local hospitals and medical colleges, professor of preventive medicine and professor of physiology of local medical colleges, one engineer from Environmental Engineering Research Institute, Government of India, scientists of the institute and of Kasturba Hospital of Infectious Diseases, Bombay and public health department personale from Gujarat, Maharashtra, Rajasthan and M.P. States. The workshop was held from 24th April 1978 to 29th April 1978 and its collaborators were WHO Regional Advisor (S. E. Asia) in health Laboratory Services, New Delhi, Dr. Ivanov, WHO Medical Bacteriologist and Public Health Laboratory Services of Maharashtra State. The subject of workshop was "Applied course in microbiology and its quality control". It proved useful to laboratory technicians attached to various public health and district hospital laboratories of the State.

RESEARCH HIGHLIGHTS

Diagnostic reagent kits

Diagnostic kits for pregnancy (haemagglutination inhibition test) and for Australia Antigen (reverse passive haemagglutination test) have been standardized. These have to be tested by other laboratories with an adequate number of clinical samples before they can go for production.

Drugs

A large number of chemical compounds have been synthesized for anti-helminthic-tuberculosis-amoebic activities. Among these, pyriodothiazole (one), isothiocyanatobenzophenone (one), and N-substituted-N-(2-benzimidazoacetyl) piperazine (two) derivatives showed good anthelmintic activity *in vivo* against *H. nana*. Three compounds of benzimidazolylbenzyl sulphides have shown promising antituberculosis activity *in vitro*. The process of standardization of phenytoin from benzaldehyde has been completed for production purposes.

Among indigenous drugs, extracts of two plants have shown equivalent results to antidiabetic drug tolbutamide in animal experiments. Another plant extract has shown curare-like neuromuscular blocking activity in animal experiments.

Immunology

Several interesting findings have come to light regarding immunological aspects of *V.cholera*, *Cl. tetani* and *C. albicans*. In *V.cholerae* employing rabbit ileal loop technique, it has been observed that somatic antigen or LPS of the organism produced their adhesion to the gut-wall and its anti-LPS antiserum protected the animal against challenge of live *V. cholerae*. These facts suggest that a vaccine containing LPS of the bacteria might provide an effective control against cholera.

People living in contact with a highly polluted *Cl. tetani* environment develop antitoxin in sera because of immunogenic effect of injected bacilli from the small intestine. This fact could be proved in a study of non-immunized Adivasi Population of Virar. Sixteen out of twenty-nine sera samples of the population showed presence of antitoxin in sera and toxigenic and non-toxic *Cl. tetani* strains were isolated from stool samples of 14 individuals. Extra-cellular (a), Cellular (b) and Protoplasmic (c) fractions of a non-toxigenic strain were injected S.C. in guinea-pigs and the immunized animals with (a & c) survived when challenged with one MLD of the toxin. These facts suggest that even a non-toxigenic *Cl. tetani* can provide an adequate protection against tetanus.

Nowadays, *C. albicans* has been reported to spread by blood and infect other tissues of the body by its mycelial form. Under these circumstances, a serodiagnosis of the disease can be best done by using a specific mycelial antigen. This led to the preparation of a mycelial antigen and it has shown specificity in serological tests.

Parasitic infection

Previously infant rabbit model was found to be suitable for the maintenance and production of complete life-cycle of *N. americanus*. The same model proved successful for *A. duodenale*. This will be used for testing of antihelmintic effect of drugs.

While studying the problem of human schistosomiasis in village Gimvi, bifurcated cercariae were seen to be liberated twice a year (April-May and October-December) from *F. tenuis* and *P. obesa* snails. Efforts are being made to produce adults of the parasite by infecting these cercariae in suitable animal models.

Vaccines

A polyvalent pseudomonas vaccine containing LPS of prevalent eight serotypes of *P. aeruginosa* in Bombay hospitals has been prepared. Its protective effect in immunized animals and lack of toxicity suggest that a good vaccine can be available against *P. aeruginosa* infection, specially of burn cases.

A vaccine of *M. leprae* prepared from ICRC bacilli of Cancer Hospital, Bombay is ready for human trial.

Venoms

Venoms of four poisonous snakes prevailing in India e.g. Cobra, Krait, Echis and Viper were extracted and their biochemical, blood coagulation and pharmacological properties were studied. Next, a toxoid of the 4 venoms has been prepared. This can be used for prophylactic purpose. A mono-valent antivenin against *E. schistosa* (Sea-snake) is also ready for use.

Virus disease

After years of sustained and dedicated efforts, it has been possible to maintain and replicate HIBOMA/HBV in langu (*Presbytis entellus*). This may well have the way towards the development of an anti-hepatitis B vaccine in the future. Other research activities are shown in Chapter III.

Training and Teaching

The institute runs various short and long term training courses for B.Sc. students. The state requires well trained technicians to look after the public health, district hospital and other biomedical laboratories. To serve that purpose the institute runs D.M.L.T. (Diploma in Medical Laboratory Technology) and short term (3 months) courses in Clinical Pathology. Students not getting admission for the former, are benefitted by joining the latter course. Students who wish to join some pharmaceutical concerns as a pharmacist can join two Drug Courses run by the institute. Besides the institute runs specialized courses in various subjects which are meant for candidates sponsored by government and commercial organizations. WHO also sends trainees from S.E. Asia for these courses. The first batches of M.Sc. (paper) in Microbiology and Biochemistry admitted in years 1976-77, appeared for the final examination in May 1978.

Testing

A large number of drugs, chemicals and biological products are tested in Testing, Toxicology and Bio-availability units. The former Unit examines a number of Pharmaceutical and Biological products as per standard specifications. The Toxicology Unit determines toxic effects of various pesticides, plastic paints and other chemicals by animal experiments. The Bio-availability Unit assays the level of various drugs and antibiotics in sera samples of humans taking these medicines.

Blood Transfusion Unit

This Unit provides transfusion facilities to the needy and poor. A good number of patients suffering from various blood disorders are benefitted by this service. I.C.M.R. sponsored Blood Group Reference Centre attached to this unit is actively engaged in research of various haemolytic enemies in human beings. Medical officers attached to various blood banks of the State are given a refresher's course for 4-6 weeks in this unit.

Administrative Councils and Committees

Governing Council headed by Minister of Health of the State is the supreme body which controls all the affairs of the institute. An Executive Committee approved by the Council helps the Director in implementation of decisions of the latter body.

An Academic Council advises the organization for running various training and teaching programmes of the institute. A Research Advisory Board serves the same purpose as regards the various research activities of the institute. These two bodies met once this year and expressed satisfaction over the progress of both the activities of the institute.

Sponserrng Research Agents

These include Indian Council of Medical Research, Department of Science and Technology, Ministry of Defence and Central Council of Research in Ayurvedic and Siddha System of India. The institute owes a great deal for the interest and financial support of these bodies for various research projects provided for a short or long period.

Oration and other Academic activities

Dr. T. Jacob John, Professor of Microbiology and Chief of Enterovirus Laboratory, Vellore Medical College delivered the fourth Dr. P. V. Gharpure Oration on 31st January 1978 on "the epidemiology of enterovirus infections and disease in India".

Dr. M.V. N. Shirodkar, of the Department of Virology, participated in a Symposium on "Hepatocellular Jaundice" at the 35 the Bombay Medical Congress. He Spoke on "The Utility of a Vaccine against Hepatitis B and its Preparation". During January-March, 1979, he lectured at the B. J. Medical College, Pune, M. S. University, Baroda and Osmania University, Hyderabad, on hepatitis, interference phenomena and interferon.

Dr. M. B. Bhide of Department of Pharmacology attended Annual Conference of Indian Microbiological Association of India at Baroda and Leprosy Symposia held at Solapur and Delhi. Miss Utagi and Shri Rangnekar of Department of Bacteriology also presented papers on "Poly-valent Pseudomonas vaccine" and "Drug Resistance of Enterobacteriaceae" in the Indian Microbiological Association conference at Baroda.

Shri A. B. Kulkarni of Department of Biochemistry read a paper on "Effects of thiamine deficiency on cholinergic mechanism in rat brain" in Annual meeting of S.B.C. held at New Delhi. He was given grant-in-aid award for his research on "Under nutrition and acetylcholine metabolism" by Nutrition Society of India.

Shri D. S. Chitnis of Department of Clinical Pathology and Miss. S. W. Awachat of Department of Immunology participated in conference of Bombay Chapter of Immunological Society held in Bombay. Their subjects of

presentation were "Role of bacterial surface antigens on adhesion of non-invasive enteropathogens in health and diseases" and "Fractionation of mycobacterial antigen capable of eliciting delayed type of hypersensitivity from human tuberculosis H₃₇ Rv.

Mrs. S. N. Hattikudur, Jr. Scientific Officer of Immunology department from 19th November 1978 to 2nd December 1978, was deputed to attend WHO Sponsored Immunology Workshop at Bangkok. Dr. S. C. Talkalkar, Jr. Scientific Officer of Radiation Biology Unit participated in 4 weeks Regional Training Course on "Radiationimmunoassay and related procedures" organised by I.A.E.A. at Sri Lanka in March 1979.

Honours and Awards

Dr. B. B. Gaitonde, Director of the Institute joined as a advisor to Regional Director, WHO, S. E. Asia from March 1979.

Dr. D. M. Renapurkar, Assistant Director of Department of Zoonosis was awarded one year WHO fellowship for training in Parasitology and Zoonosis at Tropical School of Medicine, Liverpool from October 1978.

Dr. M. V. N. Shirodkar, Head, Department of Virology was invited to attend a meeting of the Expert Group at Hazardous Chemicals and Microorganisms of the the DST at New Delhi. He served as a member of a selection committee at the Department of Microbiology, Osmania University Hyderabad. He was elected a Fellow of the Maharashtra Academy of Sciences, Pune.

Shri Sami Y. Guirges, a Government of India Scholar from Iraq was given the Haffkine award for his research work on "The rabbit as a New Laboratory Model for Experimental Hepatic Amoebiosis". Shri Jagdish M. Deshpande was the recipient of the Dr. P. V. Garpure award for the best paper presented at the meetings of the Teaching Pathologists of Bombay on "The Purification of Hepatitis B Surface Antigen.

DEPARTMENTS

Bacteriology

The department has developed a polyvalent pseudomonas vaccine that has yet to be evaluated as a prophylactic vaccine against rising trends of post-operative and burn cases admitted to hospitals. The department is carrying out other research activities and engaged in teaching of M.Sc. (by paper) in Microbiology.

Biochemistry

It carried out clinical biochemistry investigations referred by various city hospitals, private practitioners and Medical Officer of the Institute. The total number of investigations are shown in Appendix I. This department has

been busy in preparation of diagnostic kits for pregnancy and Australia antigen. These have been tested with clinical specimens and found to give satisfactory results. Other research activities are shown in Chapter III. The department takes part in M.Sc. (paper), D.M.L.T. and short term training programmes.

Chemotherapy

This department continued to synthesize compounds having specific anti-tuberculosis, amebic, helmenthic, malarial and fungal activities. A number of compounds having promising anti-tuberculosis activity *in vitro* have been selected for further testing *in vivo*.

This year Dapsone injection was formulated on request from Superintendent, Acworth Hospital, Leprosy Hospital, Bombay for clinical trial in the hospital.

The micro-analytical section carried out 1,101 examinations of Organic compounds and screening section screened 750 compounds for anti-bacterial or anti-tuberculosis activity. Most of the samples were received from other research laboratories.

Clinical Pathology

This department carries routine clinico-pathological investigations (Appendix II) referred by various hospitals and agencies. The investigations pertain to Diagnostic Bacteriology, Mycology, Serology and Histology. Its research activities pertain to immunological aspect of candida, *V. cholera* and salmonella. This department takes active part in post-graduate training and teaching programme.

Immunology

The following research activities are in progress :

1. Immunological aspect of leprosy and *Mycobacterium tuberculosis*.
2. Preparation of anti-human gamma, alpha mu and light chain reagents.

Pharmacology

This department is engaged in indigenous drugs which may prove useful for treatment of various diseases. For this, close contacts have been established with practitioners at remote areas of the state. The highlights of achievements are—

1. A vaccine for leprosy. The Drug Controller of India has permitted collaborators—Cancer Research Institute to manufacture the vaccine for Phase I and II clinical trials.
2. A new glucosteroid with N-M blocking activity was isolated in pure crystalline form. Its structural elucidation will be attempted this year with the help of Dr. T. Ravindranathan of National Chemical Laboratories.

The department takes interest in running of Drug Analysis course run by the Institute.

Phytochemistry

The department is mainly concerned in screening of Indian plants for larvicidal/insecticidal and biological activities. The active principle showing the above activity or activities is isolated, purified and structure established.

Rabies Unit

This unit screens post-mortem brain specimen of human beings and of animals for confirmation of diagnosis of rabies (Appendix III). It is at present engaged in the development of a safe and effective vaccine against rabies using tissue culture method.

Radiation Biology

This department routinely employs radioactive ^{131}I for the evaluation of thyroid function for thyroid disorder cases referred by various hospitals and practitioners (Appendix IV). This department is also labelling tetanus toxin and *M. tuberculosis* antigen for radioimmunoassay studies.

Toxicology

The department has been actively engaged in toxicological evaluation of different types of drugs, pesticides, chemicals, food additives, plastics and paints (Appendix V). The department also offers expert advice on these subjects and takes part in short term training programme.

Virology

The department pursued its studies on the host-virus interaction, with HIBOMA, Rous sarcoma (RSV) and influenza viruses. Intensive studies continued on the virological, oncological, biochemical, radiobiological and pharmacotherapeutic aspects of the HIBOMA problem. In particular, active effort continued, in species heterologous to man, of developing vaccines against HIBOMA/hepatitis B(HBV) viruses.

The earlier decision to embark upon Phase II (Non-human Primate Studies) of the Project generously sponsored by the Department of Science and Technology (DST), New Delhi proved not unwise. It became clear that some suitable non-human primate model of human hepatitis B, would become mandatory for the local and national testing/development of vaccines and also for carrying out basic and applied studies on the host-virus interaction. Thus far, the only animal model available is the chimpanzee. This species, however, is available only to a few elite laboratories around the globe. The rhesus monkey is believed to unsuitable. Furthermore, even the chimpanzee does not show any clinical signs of infection with HBV. Hence, the scientists of this Department have ever been alert to the possible detection of clinically *readily recognizable signs* in animal models under development in this laboratory. Data accruing from the rabbit and langur experimental work during 1978-1979 appear most interesting, from the above point of view.

The methodology and reagents developed in this department (Annual Report, 1977-78) have led to the development and routine use of sensitive, third generation techniques for detection of HBsAg in humans, rabbits and monkeys, has been in routine use for screening sera not only from our Institute and the Kasturba Infectious Disease Hospital, Bombay, but also from several general and private hospitals from all over the City of Bombay. The practical application of the indigenous development of such economically and readily available diagnostic reagents was demonstrated during the non-epidemic and epidemic (non-B hepatitis) periods in Bombay City during 1977-78. Furthermore, it is of great interest that such reagents are now in active use for the development of sophisticated methods (for HBsAg and HBsAb detection), such as the immunoperoxidase and ELISA tests.

The work on the rabies-RSV blockade system, also originally developed in this department, continued and important results were published in November 1978. Further work is in progress.

During the epidemic of non-B hepatitis, which swept through Bombay City and suburbs in 1977-78, the Head of the Department of Virology served as Consultant to the Mayor and Commissioner of the Municipal Corporation of Greater Bombay, as to the ways and means of combating the epidemic. Throughout the outbreak and until its abatement, he served, along with the Director of Medical Education and Research, Maharashtra State and the Deans of the Medical Colleges of Bombay City on the Expert Committee set up for the purpose.

The Clinical Virology Section of this Department continued its valuable Immunization Clinic and diagnostic virological services for the staff of our Institute, those of the general and private hospitals of Bombay and for the public, at large.

The clinic is conducted daily between 10-30 and 12-00 hours. The total number of patients who underwent immunization during 1978-79 was 597, the figure including those administered oral polio, antirabic, triple, TT, DT, anti-cholera anti-typhoid vaccines.

The Diagnostic Virology Unit of the Clinical Virology Section conducted tests on virus isolation/identification on a total of 205 specimens from patients with diseases of suspected viral aetiology. The results were as follows of 36 sera from cases clinically diagnosed as SSPE when tested for antibody against rubella, six yielded titres of 1 : 20 or higher, sixteen of 1 : 10 and 14 of less than 1 : 10. The total number of sera tested for the presence of antirubella antibody was 93, of these, 23 showed titres of 1 : 20 or more, 36 of 1 : 10 and 34 of 1 : 10. Of 42 samples tested for isolation of influenza, Japanese encephalitis, herpes simplex and cytomegalovirus *in vivo* (mice), *in ovo* (hen's egg), or *in vitro* (chick embryo, or human amnion tissue cultures), none was positive. Of 19 sera tested for the presence of hepatitis B surface antigen by counterimmuno-electrophoresis, five were positive.

Zoonosis

Departments of this type are few in India. This department has four divisions under it viz. Parasitology, Entomology, Epidemiology and Herpetology. It involves itself in the investigation of important communicable diseases such as plague, filariasis, malaria, schistosomiasis and infestation with nematodes.

Six species of insect vectors of medically importance are being maintained in the laboratory for testing insecticidal activity of chemicals of commercial firms and Indian plant extracts of Phytochemistry Department.

The department received snakes, rodents and insects for identification. It maintains about 300-400 snakes and 150-200 scorpions for venom extraction. The number of snakes and scorpions purchased and quantity of venoms extracted are shown in Appendix VIII.

The department activity takes part in training and teaching programme.

CHAPTER II

DEVELOPMENT OF TEACHING

Haffkine Institute is one of the few research institutes in India which offers training in various bio-medical sciences. The Institute is recognised by Bombay, Poona, Nagpur, Baroda, Marathwada Universities and Konkan Krishi Vidyapeeth. WHO also sends trainees and post-graduate students for admission to various courses of Asian countries to this Institute. The Post-graduate research programme in Applied Biology, Microbiology, Bio-chemistry, Pharmacology, Pharmacy, Immunology, Virology, Organic chemistry, Pathology, Zoology are pursued with the help of well-qualified teachers (Appendix IX). A list of post-graduate teachers recognized by University of Bombay is provided in Appendix X. During this year, the following teachers were recognised by this University.

Dr. M. B. Bhide	...	Ph.D. (Pharmacology and Applied Biology).
Dr. D. T. Choudhary	...	M.Sc. (Organic chemistry)
Dr. B. G. Khadse	...	M.Sc. (Organic chemistry).
Dr. R. S. Kamat	...	M.Sc. (Microbiology).
Dr. S. Mukerji	...	M.D. (Microbiology).

Post-Graduate studies

During this year 72 students (Appendix XI) registered for various courses and six students were awarded post graduate degrees. The name and title of their thesis are shown in Appendix XII.

Other Training courses

D.M.L.T.

This year sixteen students were admitted out of which 15 students completed the course and twelve of them passed the final examination.

Drug Analysis

This included two certificate course—Drug Chemical and Drug Bio-Assay. Both of them ran concurrently and admitted eight and four students for the course respectively.

Appendix XIII provides data regarding the number of students admitted for various training courses.

Teachers Council

This council includes four committees to assess the progress and provide guidance to post-graduate students for their research problems. This year, fourteen meetings were held and approved the synopses of post graduate students.

Critics club

This club and Teacher's council provide valuable guidance for post-graduate research students in shaping and formulation of their research projects. This year, 22 lectures were arranged by the club, out of which 18 were delivered by the scientists of the Institute and 4 by guest scientists from abroad. A list of participants, along with the subject of the lectures are given in Appendix XXI. Two films on 'Automatic serology on brucellosis' and 'Barefoot doctors of China' were shown and created a great deal of interest to the scientists of the Institute.

CHAPTER III

RESEARCH PROGRESS

BACTERIOLOGY

Development of a polyvalent *Pseudomonas* vaccine

As ED_{50} values of all 8 LPS were similar in the range of 1-4 ng the polyvalent vaccine was prepared by mixing all 8 LPS in similar proportions. The vaccine was filtered through millipore filter and checked for sterility. Using this pooled vaccine active mouse protection tests were carried out. Challenges given were of heterologous strains to different groups. In these experiments, ED_{50} values obtained using different challenges were as follows :—

Challenge strains	ED_{50} values in ug/ml			
T ₁	...	...	...	0.02665
T ₂	...	...	...	0.03830
T ₃	...	...	...	0.0078125
T ₄	...	...	...	0.003401
T ₅	...	...	...	0.007707
T ₆	...	...	...	0.05112
T ₇	...	...	...	0.001371

These ED_{50} values suggest that after mixing different LPS, potency of pooled LPS remains in 'ng' quantity which is more or less equal to that of individual LPS. Apart from the efficacy studies, toxicity tests were also carried out with pooled LPS. These tests included—1. Subcutaneous local toxicity in rabbits ; 2. Toxicity in mice and guinea-pigs.

(1) *Subcutaneous local reaction*.—In this test, the local skin reaction to subcutaneous injections of pooled vaccine was recorded in rabbits. The area around site of injection was observed for 7 days, for signs of erythema, oedema, necrosis, induration and vaso-constriction. However, no such adverse reactions were observed upto the period of 7 days after inoculation of pooled LPS.

(2) *Toxicity tests*.—The toxicity studies in mice as well as in guinea-pigs were carried out by giving a dose of 1000 times more than ED_{50} values intra-peritoneally. The animals were observed for 7 days, weights were also recorded. Neither death nor decrease in weight was noted in the experimental animals.

Studies on Transferable drug resistance in Enterobacteriace : Fertility Inhibition typing.

The R-plasmids in the enteric isolates were classified into fertility inhibition types i.e. fi + and fi —. R-plasmids were transferred by conjugation to *E. coli* K 12 HfrC strain) which was then streaked on tryptone yeast extract calcium agar plate, and after drying, the surface culture was spotted with male specific phage U₂. On incubation, lysis of the K 12 HfrC culture indicated that the R-plasmind was fi — whereas no lysis indicated that the R-plasmind was fi +.

Seventy-four percent strains carrying M.D.R. transferable at 37°C and seven per cent with M.D.R. transferable at only 28°C were found to be fi +. The remaining strains were fi—.

Conjugal transferability and genetic stability

The standard R-plasmids were screened for their conjugal transferability and genetic stability in *Salmonella typhi* phage A, C5, E1, Vi degraded and Vi negative. The results indicate that 'phage type' of the *Salmonella typhi* strains does not influence the conjugal transferability and genetic stability of R-plasmids.

Microbiological transformation of steroids with special reference to alpha-hydroxylation of progesterone using *Aspergillus ochraceus* spores.

Pure crystalline 11 alpha-hydroxyprogesterone was obtained. The R_f of the product was exactly similar to the authentic sample and the melting point was 160-163°C, same as the authentic samples.

Different grains were tried to find out a cheaper substitute for barley Jawar and Bajara which are very cheap as compared to Barley seem to be the best. However, soyabean and cornsteap agar were also equally efficient.

Effect of various factors such as pH, concentration of conidia, aeration etc. are being studied. It has been observed that on increasing concentration of conidia and aeration, 11 alpha-hydroxyprogesterone formed is converted into 11 alpha 6 betadihydroxyprogesterone. Thus 11-alpha starts disappearing gradually. Along with 11 alpha and 6 beta-dihydroxyprogesterone appears a still more polar product (not yet identified) and two more less polar compounds (i.e. less polar than 11 alpha hydroxyprogesterone) also start appearing in traces.

Studies on Vaginal flora of Indian women of low socio-economic group

Eighty-one clinically asymptomatic females with normal pelvic examination were studied for cervical-vaginal flora. Out of 81, 50 females were with normal ovulatory cycle and 15 with anovulatory cycle. Rest were not followed up. Females with normal ovulatory cycle showed minimum aerobic flora on the 6th day whereas anaerobic flora was consistent throughout the cycle. The incidence of gram negative facultative anaerobes was less and usually present in the later half of the cycle. Strick anaerobes like bacteroides, clostridia and Veillonella were isolated from few cases :

1. The anaerobic growth was sparse.
2. Lactobacilli were predominant.
3. The aerobic flora isolated was not different from the previous study group of 81 cases.
4. The isolation rate of yeast was higher in this group.

BIOCHEMISTRY

Biochemical studies on *Bordetella pertussis* extracts

Purification studies on *B. pertussis* alkaline saline extract (SE) were continued. Studies on the hypoglycemia causing factor showed that DEAE cellulose column chromatography of the 0.45 M MgSO_4 precipitate from *B. pertussis* extracts yielded fractions with significantly enhanced hypoglycemic effect in mice. However there were no significant changes in the levels of liver glycogen and serum FFA (free fatty acids). Further studies are in progress.

Studies on cholinergic mechanism of brain in malnourished animals

Prenatal and postnatal undernutrition was found to affect the activities of acetylcholinesterase and choline acetylase in a differential manner. The deficits in the former were much more severe and also irreversible after the institution of dietary rehabilitation. Adult stage undernutrition and protein deficiency did not affect the activities of these two enzymes although acetylcholine levels were significantly reduced. Pre- and post-natal thiamine deficiency resulted into significant deficits in the brain regions, particularly the brain-stem where its weight and the activities of the enzyme were affected. Postweaning thiamine deficiency although resulted in reduced acetylcholine levels, the enzyme activities remained essentially the same. Acetylcholine turnover studies are in progress.

Isolation and purification of trypsin from bovine pancreas

The earlier attempts were restricted to standardization of a suitable and techno-economically feasible method to purify trypsin from pancreatic glands of sheep, pig and cattle. The batch size was of 0.5 to 1.0 kg. The present studies report the scaling up of batch size to 5-6 kg. with an activity of 4,000-5,000 BAEE units per mg. which is comparable to commercial grade II trypsin. A homogenous preparation of trypsin was purified from bovine pancreas using affinity chromatography technique.

At present the process is being scrutinised by NRDC for commercial exploitation.

Studies on Rickets—The effects of different dietary levels of Vitamin D and Calcium on lipid metabolism

Our earlier studies have shown that serum and liver lipids and liver lipogenic enzymes are affected differentially by different concentration of vitamin D in the diet, the effects depending upon the concentration of

dietary calcium. These peculiar changes suggest that vitamin D metabolites which are synthesised in kidney according to the calcium need of the body and not vitamin D *per se*, might be involved in altering lipid metabolism.

Keeping this in view, studies on lipid metabolism were extended to nephrectomised adult male albino rats. Serum lipoprotein pattern showed increase in pre-B-lipoprotein fraction along with increase in serum triglycerides and total cholesterol levels. Activities of lipogenic enzymes, viz. G.P.D., M.E., I.C.D. and C.C.E. were found to be elevated in the livers of the nephrectomised rats as compared to control or sham operated group. More of the ^{14}C of ^{14}C acetate, injected intraperitoneally, was incorporated in total lipids and non saponifiable fraction of the nephrectomised rat livers.

Further work with active metabolites of vitamin D is in progress.

Preparation of pancreatin

The project forms a part of the "Isolation of Insulin" project. Pancreatin powder was prepared with the batch size of 5/ kg. of cleaned pancreas. On an average the yield was 10-11 per cent of the raw material used. The enzyme activities of the preparation were tested and were found to comply with the specifications of National Formulary (NF) XIV, U.S.A. The preparation was also found to be free of Salmonella contamination.

National Research and Development Corporation (NRDC) have shown interest in obtaining the experimental know-how of the process for its possible commercial exploitation.

Isolation and Purification of lipase from pancreas

This project forms a part of the 'Isolation of Insulin' project. In the initial experiments, cattle and porcine pancreas, with the batch size of 0.5 kg. were processed. The specific activity of Lipase obtained was 2,500 units/mg. protein which was comparable to grade II lipase available due to low content of the enzyme in pancreas. Procedure is being suitably modified to increase the purity of the product and yield, if possible.

Preparation of Diagnostic Reagents Kits

Standardization of pregnancy test by immunological haemagglutination test using coated sheep erythrocytes has been completed. Purification of crude HCG (Human Chorionic Gonadotropin) obtained from pregnant women's urine is continued further by column chromatography. Columns used for purification are (a) DEAE cellulose with phosphate buffer 0.05 M pH 7.4 (b) Sephadex G-100. Biological activity of the purified HCG was found by rat prostate gland assay. It is planned to estimate the

following parameters from the serum of primary hepatoma patients (i)—Fetoproteins by enzyme immunoassay, (ii) LDH by electrophoresis (iii) Regans isozyme, (iv) oncofetal aldolase, (v) Gamma Glutamyl transpeptidase.

Reversed passive hemagglutination for the detection of Australia Antigen was formulated. Sheep antiserum specific for Australia Antigen in human serum, was processed to separate anti-HBS gamma globulin. Human group 'O' cells were sensitized by anti-HBs gamma globulin according to the standard procedure. Reversed passive haemagglutination test (RPHA) was performed using sensitized cells and microtitre plates with V bottomed wells.

A total of 71 human serum samples were tested by RPHA. The samples shown positive by RPHA were subjected to counterimmunoelectrophoresis and confirmatory neutralization test for confirmation. The false positive results amounted to 3 per cent. The stability of sensitized cell suspension was found to be six months.

CHEMOTHERAPY

ANTHELMINTIC AGENTS

Pyridothiazole derivatives

In continuation of the synthesis of pyridothiazole derivatives as anthelmintic agents, the new substitution of various benzophenones has been made at 2-position of pyridothiazole moiety. One of the pyridothiazole derivatives namely, 2-4-(p-nitrobenzoyl) phenylamino-pyrido (5, 4-b)-thiazole has shown very interesting anthelmintic activity against *H. nana* in mice.

Isothiocyanatobenzophenone derivatives

4'-substituted-4-isothiocyanatobenzophenones were prepared as possible anthelmintic agents. In this series, one of the compounds viz., 4'-nitro-4-isothiocyanatobenzophenone showed anthelmintic activity against *H. nana* in mice comparable to the standard drug Bitoscanate.

2-(substituted-Nitroaryl) imidazo (1,2-a) pyridines

The synthesis and study of a new line of compounds of 2-(substituted aryl) imidazo (1, 2-a) pyridine has been undertaken as possible anthelmintic agents. The required intermediates like substituted phenacyl bromides and substituted 2-amino-5 nitrophenylidines were prepared and these two type of intermediates are condensed to get the title compounds. Nine final compounds are ready for evaluation.

N-Substituted-N¹-(2-benzimidazoacetyl) piperazines

In continuation of synthesizing new N¹-substituted-N¹-(2-benzimidazoacetyl) piperazines, the substitution of groups like chloro, nitro, etc. at 5-position of benzimidazole moiety has been done. The screening results have shown two compounds to be active, which activity is more than 90 per cent of that shown by the standard compound.

Synthesis of Tetramizole type of compounds

These new compounds were prepared parallel to tetramisole with aryl substitution on thiazole part of tetramisole ring. These compounds were prepared by route other than the one used for tetramisole. The key intermediate 4, 5-dihydro-mercapto-5-phenyl-imidazole has been prepared from benzaldehyde. Eight new compounds have thus been prepared and submitted for anthelmintic screening.

ANTIMALARIAL AGENTS

2-(substituted-benzyl) thio-pyrido (5, 4-b) thiazoles

Some new pyridothiazoles have been synthesised as antimalarial agents. In this attempt, a few 2-substituted benzyl thiopyrido (5, 4-b) thiazoles were synthesised which are awaiting tests as antimalarial agents.

The pyrimidine derivatives

In continuation of synthesis of 2-anillinopyrimidines with noval diamine dimethylaminopropylamine, p-amino- α -di-di-ethylamino-o-cresol linkages at four position of pyrimidine nucleus, ten compounds have been prepared. In another series of 2-substituted-4-methyl 5, 6-dihydrofuro (2, 3-d) pyrimidine are prepared. Both these types of compounds are awaiting screening as possible antimalarial agents.

ANTITUBERCULOSIS AGENTS

Derivatives of 4-p-aminophenyl thiazole

In view of the high *in vitro* antituberculosis activity shown by certain thiazole derivatives, eight new derivatives have been prepared from 2-(3'-methoxy-4-propoxyphenyl)-4-(4'-aminophenyl)-5-methyl thiazole to get corresponding palmitamido butyramido, propionamido, acetamido,, etc. derivatives. These compounds are likely to favour water and lipid solubilities and as a result expected to give better antituberculosis activity. They have shown activity at concentrations from 10 ug/ml to 40 ug/ml. They will also be taken for *in vivo* screening.

Ethionamide and pyrazinamide type of compounds

In continuation of synthesising the ethionamide type of compounds a few additional 2-substituted-4-thio-isonicotinamides and 2-(2-aryloxy-pyridyl)-4-substituted phenyl thiazoles were prepared. One of these compounds inhibited the growth of *M. tuberculosis* *in vitro* at 5 ug/ml concentration.

Pyrazinamide is another well known drug in the Chemotherapy of tuberculosis. The synthesis of compounds parallel to pyrazinamide, and their intermediates has been undertaken recently to explore their biological activity.

2-Benzimidazolyl-benzylsulphides (and sulphones) and

2, 4-diamino-6-substituted-thiocarbanilidoquinazoline derivatives.

Ten compounds of benzimidazolylbenzyl sulphides have been prepared with various substituents on the benzyl and benzimidazolyl moieties with a view to get better antibacterial and particularly antituberculosis activity. Two compounds inhibited the growth of *M. tuberculosis* at 2 ug/ml and one compound at 1.56 ug/ml *in vitro* test system.

In another series, thiocarbanilides were prepared from 2, 4, 6 triamino-quinazoline and some substituted isothiocyanates to get the corresponding 2, 4-diamino-6-substituted thiocarbanilido-quinazolines. They have been submitted for antituberculosis activity.

2-Arylamino-benzthiazoles

A variety of benzothiazoles are reported in the literature to possess antibacterial and antituberculosis activities. However, the 2-substituted arylaminobenzthiazoles seem to be less explored. Therefore, 2-arylamino-benzthiazole having variety of substituents in the aromatic part are synthesised and are being evaluated for antibacterial as well as antituberculosis activity.

ANTIAMOEBIIC AGENTS

4-[p-(N-Methyl-N-dichloroacetyl-amino) phenyl]-thiazoles and semicarbazone and thiosemicarbazones of p-(N-dichloroacetyl-amino) acetophenone.

Diloxanide furoate is well known antiamoebic drug. Some 2-substituted thiazoles, having N-methyl-N-dichloro-acetamidophenyl grouping (same as in diloxanide) at 4-position are synthesized and submitted for antiamoebic activity screening. Similarly, preparation of 2-substituted aminothiazoles with same dichloroacetamido-substitution at 4-position is taken in hand.

P-(N-Methyl-N-dichloroacetyl-amino)-acetophenone was found to be more active than emetine-HCl *in vitro* test system. So further ketone derivatives such as semicarbazone, thiosemicarbazone, substituted-thiosemicarbazone, were synthesized. Attempts to cyclise the thiosemicarbazones are made and two cyclic compounds are obtained. In all, 15 compounds have been submitted for *in vitro* antiamoebic screening.

"R & D" ACTIVITIES

Process standardisation of 5, 5-diphenylhydantoin (Phenytoin)

The project for process standardisation of diphenylhydantoin has been undertaken recently in October, 1978. The process involves mainly four stages for the synthesis of diphenylhydantoin starting from benzaldehyde. Out of these the first stage of preparation of benzoin has been successfully completed on 1 kg. batch scale with high yield. Further work of this process is under way.

CLINICAL PATHOLOGY

Isolation and identification of *Candida* species from cases of leucorrhea and tests of their pathogenicity and sensitivity to various drugs.

This project has been concluded and the following are its salient features
 1) *Candida* could be isolated from 285 out of 700 cases, (2) The species identified were *C. albicans* 259, *C. tropicalis*-17, *C. krusi*-5, and *C. Pseudo-tropicalis*-4, (3) 150 isolates of *C. albicans* when tested for sensitivity to amycin, amphotericin, miconazole, mycostain and sporostasin, were found to be sensitive, (4) Hundred isolates were injected intramuscularly in thigh of mice and most of *C. albicans* (80%) produced localized abscess at the injected site in 7-10 days, (5) 150 soil samples from different parts of Bombay were collected and cultured but no *Candida* were isolated. These acts suggested that the *Candida* isolated were endogenous in origin and the I.M. pathogenicity test provided a suitable alternative to I.V. route test.

Preparation and utility of an antigen specific for mycelial phase of *C. albicans* in the diagnosis of systemic candidiasis.

In the previous year purified mycelial and yeast antigens were prepared and their immunogenecity was demonstrated by rabbit immunization. Sera of 100 normal individuals were tested against yeast and mycelial antigens by passive haemagglutination with sheep RBC coated with either of the antigens. A mean anti-yeast titre of 8 with a range of 0-64 could be demonstrated but none against the mycelial antigen. Sera of 49 cases of vulvovaginal candidiasis showed a mean titre of 40 and a range of 4-512. Forty-one of these cases did not show any titre against the mycelial antigen.

With a view to test the anti-mycelial reactivity of sera of known cases of chronic systemic candidiasis, the following types of clinical cases likely to have candida infection of deeper tissues, were collected, (1) drug resistant pulmonary T.B.-A.F.B. post & negt., (2) chronic bronchitis with or without bronchiectasis, (3) pulmanory abscesses and non-tuberculous granulomatosis. The rational for selecting these cases is that at least some of the drug resistant cases of pulmonary T.B. may have candida either as primary or secondary pathogens.

In all 252 cases were collected, their sputa cultured on Sabouraud's agar for number of candida/ml of each sputum sample. The results are shown in Appendix II B. Each serum sample was tested against anti-yeast and anti-mycelial antigens.

Studies on pathogenesis of glumerulonephritis

Antibody against BSA was raised in rabbits to a titre of 1/64. In vitro soluble antigen excess immune complexes were prepared and injected in 3 doses of 269 mcg at 8 hourly intervals into 6 mice. Two of the animals died due to anaphylaxis after the first dose and the surviving were sacrificed 12 hrs. after the last dose. The kidneys were taken out, half of each was frozen immediately for immunofluorescence studies and the remaining half for histology examination. The latter showed focal swelling and proliferation of few scattered glomeruli.

Future plans.—(1) Establish a dose response curve to antigen & antibody excess immuno-complexes, (2) To study the role of RE System in removal of immuno-complexes from circulation.

Studies of the immuno response against *V. cholera* antigens

The aims and objectives of this will be to establish if surface antigens of the organism can induce protective immunity and to compare the efficacy of oral systemic immunization using some of the useful antigens of the bacterium.

For the former, it was proposed to test the ability of antibodies against. (1) somatic antigens, (ii) any other known or unknown cell surface molecules, (iii) flagellar antigen, in providing protection against challenge with live *V. cholerae* by rabbit *ideal* loop technique. Anti LPS or anti-somatic antibodies were raised in rabbits by injecting boiled cells of *V. cholerae*. Antibodies against different surface molecules including LPS were raised by immunization with live organisms. The anti-LPS activity of serum was removed by absorbing the serum with boiled *V. cholerae* cells. The ability of anti-boiled cell serum, pre-and post-absorbed anti-live *V. cholerae* sera in providing protection against live challenge of *V. cholerae*, were tested by ileal loop technique.

Serum		Anti-LPS titre (passive haemagglutination)	Rabbit ileal loop Protection titre
1		2	3
Anti-EPS (antiboiled cell serum)		25,600	80
Anti-live		10,240	80
Anti-live cell serum absorbed with boiled cells	..	<20	>10

These results indicate that anti-LPS antibodies provide effective protection against challenge with live cholera vibrios. Cell surface antigens other than somatic antigens do not seem to, play significant role in bacterial adhesion to mucus membrane because when anti-live vibrio serum was absorbed with boiled cells, it lost almost all the protective effect.

This was followed by active oral immunization of rabbits with killed *V. cholerae* cells, culture supernatant and soluble LPS of *V. cholerae*. When the animals were challenged in animal loop they could withstand 100-300 times the standard EC 50 dose of live *V. cholerae*.

Future Plans.—(1) passive protection studies with flagellar antibodies, (2) oral immunization followed by testing the ability of the immunized animals to withstand challenge with live vibrios, (3) correlation of protective titre with specific antibody levels in loops.

Studies of the mechanism of immunity against salmonellosis.

The aim and objective is to assess the relative importance of cellular and humoral immunity in offering protection against salmonella infection. For this it was decided to carry out the investigation on mice employing *S. enteritidis* as the pathogen which causes in mice disease akin to human enteric fever.

In these animals LD_{50} values of the organism was established experimentally, also mean live counts of the organism per gram of liver, spleen and mesentric lymphnodes at various times after challenge with 10 and 100 LD_{50} doses of *S. enteritidis* were determined.

Future Plans.—(i) assessment of protection offered by immunisation with formalin killed and live *S. enteritidis* in sublethal doses. This will be done in two ways, namely estimation of LD_{50} values, and live bacterial counts per gram of various tissues at different times following challenge of immunised animals, (ii) assessment of ability of peritoneal macrophages of immunized animals to kill live *S. enteritidis*, (iii) assessment of antibody titres and delayed hypersensitivity against *S. enteritidis* sonicates in immunized animals and correlation with protection.

LPS (endotoxin) levels in clinical syndromes ascribed to the endotoxaemia of Gram-negative bacilli

The *aims and objectives* of this study will be to establish (i) a sensitive assay, namely ELISA for estimation of LPS of common Gram-negative bacilli in tissue fluids and serum, (ii) correlation of clinical symptomatology and endotoxin levels.

For this, a mutant *S. minnesota* "Re" was obtained from Max-Planck Institute. The LPC made by this mutant is composed of only Lipid A and KDO. The outermost polysaccharide chains are absent in the mutant LPS. The "RE" mutant was injected in rabbits to get antiserum against RE-LPS. This is expected to react against all LPS molecules containing KDO. Also LPS preparation have been made from *S. minnesota* and *E. coli*.

DIRECTOR'S RESEARCH CELL

Biochemical, Pharmacological and Immunological Studies of the venoms of snakes in India

Biochemistry

Venoms from cobra and Krait (family-elapidae) were fractionated using CM-cellulose chromatography and various fraction were obtained. From cobra venom we have obtained a purified homogenous neurotoxin and a cardiotoxin. The approximate molecular weight of both these toxins was similar (8000 Daltons) as confirmed by SDS gel electrophoresis.

Effects of Snake Venom on Blood Coagulation

The aim of this work of the Snake Venom Research Project was to study—(i) the effect of venom on factors of blood coagulation i.e. either activation or the inhibition of factors, (ii) isolation, purification and characterization of the active proteins from the venoms and (iii) again studying the detailed mechanism of action of the protein.

For the study of blood coagulation the parameters studied were :

- (i) Direct clotting time of plasma and fibrinogen.
- (ii) Plasma recalcification time.
- (iii) One stage prothrombin time.
- (iv) Thrombin clotting time of fibrinogen and plasma.
- (v) Thromboplastin generation test.

From the observation of the parameters studied showed that the venom of cobra (*Naja naja*) anticoagulates or inhibits the factor V, or destroys the brain thromboplastin when incubated for the same time. Factor VIII of the intrinsic blood coagulation is also inhibited.

In the case of Russel's Viper the venom is of coagulant type. It is also reported that it activates factor X of the blood coagulation by cleavage of high mol. wt. peptide of factor X. Our results also conclude that it has factor X activating activity.

In case of Krait venom both coagulate and anticoagulate activities, were found. The coagulate activity is worked by the anticoagulant activity. The venom inhibits for VIII of the intrinsic blood coagulation and activates factor X. This coagulant and factor X activating is seen only in TOLN generation test. This effect of the venom is not reported in the literature hence emphasis was given on the further study of the venom.

Pharmacology

After carrying out the initial toxicity studies the LD₅₀ values were determined for all the four venoms.

The LD₅₀ of the venoms of cobra and krait did not change significantly with age, storage and seasonal variations whereas the venoms of Russels viper and *Echis carinatus* very significantly depending upon the time lapse between milking and lyophilising. This may be cause partial destruction of the toxic proteins. It would, therefore, be difficult to establish a national standard for these viperine venoms.

In order to study the neurotoxic and cardiotoxic effects of crude venom and its fractions the following test systems were used : guinea-pig ileum, isolated rat fundal strip ; isolated rat phrenic nerve diaphragm ; isolated guinea-pig heart ; chick biventer cervicis and blood pressure in cats and rabbits.

The neurotoxic and cardiotoxic fractions isolated from cobra and krait venoms were studied using the above mentioned systems.

The toxoided venom of *Naja naja*, *Bungarus ceerulus*, *Vipera russeli* and *Echis carinatus* were studied for their pharmacological action on following biological systems, i.e. isolated guinea pig ileum rat fundal strip (to see whether their spasmogenic activities are masked or are unaffected), isolated hrenic nerve diaphragm of rat and on cat blood pressure and respiration *in vivo*.

The toxoids of all the venoms were devoid of any spasmogenic activity when compared with the equivalent dose of crude venom. However, 50 times does of toxoid showed spasmogenic activity. Similarly, the *Naja naja* and *B. caeruleus* toxoids did not show any neuromuscular blocking action with the equivalent dose of crude venoms, but 50 times does of krait toxoid, and 75 times does of cobra toxoid, the neuromuscular which was of reversible type. This was most surprising, because the crude venom produced a complete irreversible type of neuromuscular block.

In the experiments on cat blood pressure and respiration the toxoid was found to be non toxic as there was no change in the blood pressure and rate of respiration even at a dose of 100 mg/Kg.

In conclusion it can be said that the venom toxoid thus prepared for all the four poisonous venoms was free from any neurotoxic as well as cardio-toxic properties even when used in as high dose as 50 to 100 times that of crude venom.

Detailed pharmacology on the coagulant factor isolated from the venom of *B. caeruleus* was studied. This is the first report on the coagulant property of elapid venom. This factor was free from any neurotoxic or cardio-toxic property as we evident from our results on rat phrenic nerve diaphragm and isolated auricles of guinea-pig.

Immunology

Monovalent antiserum was prepared against all the four venoms using rabbit as an experimental model. The venom toxoid was prepared by using two methods :

- (1) Gamma-radiations (Co^{60} source),
- (2) Formalin.

The toxoid thus prepared was studied for its toxicity before using them for immunization. Initial results indicate that both the methods used for toxoidation can successfully form a toxoid. These toxoids were studied for their biochemical action and their immunological status using polyacrylamide, gel disc electrophoresis SDS gel electrophoresis and immunoelectrophoresis.

Formation of high molecular weight proteins by the treatment of Gamma radiation and formalin was confirmed by gel filtration of all the toxoids on sephadex G-200 column. These high molecular weight proteins were immunogenic proteins as shown by immunoelectrophoresis. In pilot experiments where rabbits and guinea pigs were immunised with formalised and irradiated toxoids respectively all the toxoids produced a high titre of neutralising antibodies in these animals. All the antisera could react with respective crude venom producing several precipitin lines. Encouraged with these results these toxoids were tried in monkeys. Normal monkeys sera showed precipitating type of antibodies against all the four venoms. However, no neutralization was possible. Initially, monkeys were immunised sub-cutaneously with formalised toxoids of cobra, krait, viper and echis venom without any adjuvant. After two weeks as no neutralising antibodies

could be detected, adsorption of these toxoids on to the aluminium phosphate gel was tried. Monkeys were immunised with 2 mg. of adsorbed toxoid proteins of cobra, krait, viper and *Echis carinatus* venoms. After 14 days the monkeys were bled and the sera was tested for their neutralising antibodies titre. One ml. of immunised monkeys serum could neutralise, 50 ug of cobra venom, 10 ug of krait venom and 80 ug each of *Russels Viper* and *Echis carinatus* venom. Further results with second does of immunization (1 mg. and 2 mg. of adsorbed toxoid protein) are awaited.

Pharmacological and Biochemical Studies on the venom of *Echis carinatus*

(i) *Acetylcholinesterase*.—Acetylcholinesterase activity was found to be present in the venom. As this fact was not reported in literature, the kinetics of the enzyme activity was studied. The Michaelis-Menton constant of the enzyme activity was $9.66 \times 10^{-5} \text{M}$. The optimum pH of the activity was 8.0. The activity was completely inhibited by 0.31 mM physostigmine salicylate. The enzyme was purified to about a 19-fold increase in activity by ion exchange chromatography on DEAE cellulose using a linear gradient of Tris HCL bufer 0.05 M (pH 8.4) to 2.0 M (pH 1.4). The enzyme activity was completely separated from the toxicity of the venom.

(ii) *The toxic protein of Echis carinatus venom*.—The toxic protein of the venom was been isolated by a three step procedure using ion-exchange chromatography on DEAE sephadex A-50, followed by gel filtration on sephadex G-75 and finally rechromatography on DEAE Sephadex A-50, and the protein was purified to electrophoretic homogeneity. The toxic protein of this venom had an intravenous MLD of 2.5 ug and an intracerebral MLD of 0.3 ug. It was completely free from the coagulant and haemorrhagic activities of the venom. Detailed characterization of the toxin molecule and the mechanism of its action is in progress.

Studies on artifertility activity of indigenous plant

It has already reported that the alcoholic extract of LF prevents pregnancies in rats and mice. Further, LF was studied for seasonal and regional variation in activities in different samples collected from different parts of Maharashtra State. The alcoholic extract of LF was also studied for its mechanism of action in rats and it was found to be antizygotic. This drug was also studied for its acute and chronic toxicity studied for its physical and chemical properties. NMR and MS analysis of this compound was conducted.

Effect of various indigenous studied for probable antisnake bite activity

In the year 1978-79, seven different samples from various parts of the country were received and tested for suggesting their antisnake bite properties. These preparations are in actual use by various people in case of snake bite treatment.

Unfortunately none of these seven different drugs showed any antisnake bite activity when used in crude forms or aqueous extract against laboratory mice. When petroleum ether, chloroform and alcoholic extracts of these

preparations were tried in mice, only one plant extract (Fangla) was found to possess significant activity against the venom of *Echis carinatus* (saw scale viper). Further detailed pharmacological studies on this plant are in progress.

Monovalent *Enhydrina schistosa* antivenin

Sea snakes were collected from local fishermen from the Bombay sea coast and kept alive in our laboratory and milked at weekly intervals by the standard method. The venom was lyophilised and stored at 20° c until use. Hyperimmunisation of horses with the venom was carried out for the preparation of commercial monovalent antivenin by the routine procedure. The serum from immunised horses was purified using ammonium sulphate precipitation and peptic digestion. The sterile purified plasma thus obtained was studied for the following tests. Potency, abnormal toxicity, residual ammonium sulphate; percentage solid contents and sterility as per the requirements for the biological products given in India pharmacopoeia.

One ml. of purified gamma globulin prepared by above mentioned method could neutralise 400 ug of *E. schistosa* venom which is approximately 307 MLDs in Kasauli mice. The antivenin prepared for commercial use was dispensed in a 10 ml. ampoule in lyophilised form. The concentration of purified antiserum is adjusted in such a way that 10 ml. of reconstituted serum will neutralise 450 ug. of *E. schistosa* venom i.e. 345 MLDs.

IMMUNOLOGY

Immunological studies in leprosy and other mycobacterial disorders

In the previous year PPD made from human tubercle bacilli ($H_{37}R_V$) was fractionated. A large mol. wt. protein containing fraction was isolated from PPD and it was shown to contain material capable of eliciting delayed type of hypersensitivity (DH) reactions in patients suffering from tuberculosis. This material was found to be a mixture of many proteins and further fractionation by gel filtration and ion exchange chromatography was not successful.

During the last year, sonicate of $H_{37}R_V$ were studied for protein antigens. Polyacrylamide disc electrophoresis with or without SDS showed approximate 15 bands in the sonicate. The latter did not separate into clear out sharp bands in spite of detergents. The sonicate was subjected to Gel filtration on sephadex G-100- & -200 with and without 8 M Urea. In both the chromatographic procedures one would obtain two fractions, one coming along with the end of void volume. This protein peak showed considerable training in G-100 and the 2nd fraction was eluted around 75,000 Daltons. In G-200 the 2nd fraction was eluted approximately at the same position. The fractions were studied for electrophoretic profiles by polyacrylamide gel electrophoresis (PAGE). They were also tested for their ability to elicit delayed type hypersensitivity (DH) in patients suffering from tuberculosis. Only the void volume eluates from both the gels could elicit DH. These did not constitute a single protein. PAGE profiles showed that the void

volume fractions contained a large mol. wt. material that could not enter the separating gel. In the separation gel at least 3 clear bands and many more faint bands could be seen. The clear bands were seen in the regions of 150,000 Daltons, and 20-30,000 Daltons. The 2nd fraction from both gels, could not elicit DH. These fractions on PAGE showed some of bands that were also seen in the void volume fractions but these did not show any protein band with mol. wt. of 150,000 or more. This permitted us to conclude that antigenic activity capable of eliciting DH resides in the proteins with mol. wt. 150,000 Daltons. Attempts at further separation by gel filtration and ion exchange chromatography was not possible even though the void volume fraction contained mixtures of proteins having as wide a difference in mol. wt. as 200,000 Daltons or more. This difficulty in separation was ascribed to high lipid content of the original sonicate as well as the fraction. Hence the void volume fraction was subjected to treatment with detergent Triton X-100 & KCL. The lipid and lipid-soluble material was then extracted with Butanol; the aqueous layer was concentrated, dialysed to remove Triton X-100 & Butanol. It was then subjected to PAGE and tested for ability to induce DH. The material could elicit DH in patients suffering from tuberculosis.

The PAGE profiles showed that the large mol. wt. band that could not enter the separation gel, was absent in the material subjected to Triton-X-KCL treatment. The other bands seen in the pretreatment fraction were sharper and clearer. Two fractions could be isolated one in the terminal portion of the void volume and immediately thereafter the other in the region of approximately 70,000 Daltons. PAGE profiles, of the fraction showed two bands in the region of 150,000-200,000 Daltons proteins. The first fraction could elicit DH whereas the second could not. This permits us to conclude that DH eliciting material has a mol. wt. of approx. 150,000-200,000 Daltons. Future Plan; (1) Further purification and characterisation of DH eliciting material, (2) Study of antigenic properties in terms of specificity and cross reactivity of DH eliciting materials and (3) Study of other antigens in the sonicate.

preparation of anti-human gamma, alpha, mu and light chain reagents.

Human serum was fractioned and IgG & IgM were prepared as follows :—
 (i) human serum was dialyzed against distilled water in cold, (ii) the ppt as collected and washed with distilled water in cold, (iii) the ppt was dissolved in 0.15 M NaCl and subjected to DEAE cellulose and G-200 gel chromatography, (iv) the fractions were tested against antihuman gamma lpha and mu chain reagents by micro-gel diffusion, (v) the mu positive fraction was concentrated. It also showed IgG and IgA impurities, (vi) the supernatant of the distilled water dialyzed serum was subjected to 37% $(\text{NH}_4)_2\text{SO}_4$ precipitation, (vii) precipitate was dissolved in 0.05 M PO_4 , (viii) IgG was precipitated by 0.05 M ZnSO_4 at pH 6.0, (ix) the precipitate as dissolved in 0.01 M PO_4 buffer and subjected to DEAE cellulose chromatography, (x) fractions were tested against anti-gamma, mu and lpha reagents. Fraction showing only IgG was pooled and concentrated.

For preparation of human IgA 500 ml. of human colostrum was collected.

The partly pure IgM and fairly pure IgG were mixed with Freund's complete adjuvant respectively and rabbits were immunized. The antisera raised had a titre of 1:8. Anti-gamma has very little activity against other heavy chains but reacts strongly against light chains. Anti-mu antiserum however reacts against IgG, IgA as well as light chains.

Future Plans : (i) Separation of IgA from colostrum, (ii) Preparation of light chains from IgG, (iii) Preparation of affinity columns of sepharose IgG, IgM, IgA and light chains for purification of antisera, (iv) Standardisation of Mancini technique for estimation of immunoglobulins in body fluids.

PARASITIC DISEASES AND ZONOSIS

Malaria

Host-parasite relationship between the rodent malaria *Plasmodium yoelii* and albino mice of varying age group and both the sexes are being studied. About 17 synthetic compounds have been screened out against *P. yoelii* but none of them was found active.

Amoebiasis

Seventeen strains of *E. histolytica* isolated from clinical cases of amoebiasis (acute amoebic dysentery), post-mortem amoebic hepatic abscess, chronic intestinal amoebiasis and from asymptomatic carriers were maintained *in vitro*. Hepatic amoebiasis was studied in Syrian golden hamsters and rabbits, and intestinal amoebiasis in the albino rat and guinea-pig. New models have been established, wherein the infant and newborn albino rabbit (*Oryctolagus cuniculus*, strain Hackine conventional non-inbred) inoculated intrahepatically with *E. histolytica* alone or following inoculation with HIBOMA virus, exhibited the most severe forms of hepatic amoebiasis. comparative serodiagnostid tests for hepatic and intestinal amoebiasis were evaluated by testing sera from appropriate patients and those with intestinal parasitic infections other than *E. histolytica* and from healthy controls.

The tests included complement-fixation, indirect hoemagglutination, amoeba-immobilization, fluorescent antibody test, double diffusion, immunoelectrophoresis and counter immunoelectrophoresis. Results showed positive reaction in : CF test (47.7-81.5 percent) FAT (67.8-96.3 percent) DD (73.3-83.3 percent), IEP (79.1-96.3 percent) and CIEP (82.6-91.7 per cent) after testing 246 human sera samples.

Schistosomiasis

The old focus of schistosomiasis (Gadgil and Shah, 1952) at Gimvi village in Ratnagiri District (Maharashtra State) was reinvestigated. Three types of snails were identified as *F. tunis*, *P. obesa* and *M. tuberculata* with the density population of snails ranges 10-12/sq. feet, 100/sq. feet and 100-120/sq. feet respectively. About 1151 snails were dissected and both the forked and unforked types of cerceria were observed. Skin test with

chistosome antigen (received from U. S. Public Health Department, Georgia) as employed to a total population of 598 persons in the Gimvi village and adjoining areas. Positive reaction in the endemic area (Gimvi) showed 0% and adjoining area 1.47-3.25% of the total population.

hookworm infections

A human *Necator americanus*-strain isolated from patients was passaged in infant rabbit model successfully upto the 6th generation without using any immunosuppressant. This rabbit strain (RSG-1-RSG-6) has been maintained from the year 1976 till now in our lab without any difficulty. Experiments in infant rabbits by buccal pouch route infection produced low mortality in animals, high worm yield and appreciable hookworm egg counts. This laboratory model appears to be an ideal model for various immunological and chemotherapeutic studies. The same rabbit strain failed to establish the infection in hamsters and mice with and without cortisone. Similar attempts to establish *Ancylostoma duodenale* in the infant rabbit was made successfully. The worm attained maturity earlier (30 days) as compared to *N. americanus* (50 days). Infective larvae were inoculated through buccal pouch route to 9 infant rabbits in the first generation and 5 infant rabbits in the second generation, the developing stages of worms were collected at different intervals and identified as *A. duodenale* only. Larvae could attain the adult stage to sexual maturity within 4 weeks, to the oviposition within 5 weeks and remained at that stage until seven weeks post exposure.

Cotton rat filariasis

Litomosodes caranii infection in cotton rats were maintained. Out of 39 infected rats 84 were microfilarial positive. In that pool, 66 rats were supplied to Pharmacology and Director's Research Cell for Chemotherapeutic and other research studies.

Besides the research work, of *N. dubius*, *H. nana*, *Ancylostoma ceylanicum* strains are being maintained for various drug screenings.

Hymenolopsis nana infection

Seventy-two male mice were infected each with a dose of 1000 *H. nana* eggs, three times at 5 days intervals. Out of these, 48 mice were used for MIF test and 24 were used for IHA test and worm recovery test. Equal number of uninfected mice were also kept as control. Antigen preparation and IHA were performed as per standard method whereas MIF test was done according to David and David (1971), Hudson and Hay (1976) and Takayanagi et al (1979). Cell-mediated immunity response during *H. nana* infection in mice was studied. MIF test was developed *in vitro* and results demonstrated significant level of inhibition (27.59%) on the day 10, maximum on the day 21 (51.70%) and remained constant on days 40 (40.89%) and 50 (50.69%). The simultaneous production of circulating antibody in serum gave very high titre (1:1024) initially on the day 10 but declined on day 21 (1:256) and remained at the same level up to day 50 when the

adult worms were being eliminated (21st day) or they were completely lost from the out (40-50 days).

Larvicidal activity of indigenous plants :

Twenty plant extracts were screened against 4th stage larvae of cosouito (*Culex fatigen*) in laboratory condition and 7 of the plant extracts were found to be effective.

ZOONOSIS

Plague

Rodents from different wards of Greater Bombay were tested against plague infection by the passive haemagglutination test using F_1 antigen of *P. pestis*. Out of 706 sera samples, only 100 samples gave low titre below 1:12.

Snakes.

Studies on output of Echis carinatus venom.

The study has been taken up with an object to enhance the output of venom and to standardise a suitable method for transporting and housing snakes in large numbers for venom collection. The modified methods yielded more venom production further study is in progress.

Rearing of young ones of R. viper.

The object of this study was to rear young ones of snakes in the captivity. Two hundred ninety-five young ones were fed by force with pieces of mutton of mice but the experiment failed. Further study in progress.

Snake repellent studies.

Previously in fabricated cages, extracts of *Acorus calamus* and Pine oil showed good repellent activities. These were tried in snake farm. Various concentrations of these two substances were put on filter papers and sprayed in snake-pits of the farm then Cobra, Viper and Krait were put in these areas. *A. calamus* at 75% concentration showed the best repellent activity. It was found non-toxic, produced repellent activity within 3-4 hours after spraying and retained the property upto 10-15 days.

PHARMACOLOGY

Leprosy

(a) *Drug in leprosy.*—The disadvantages of monotherapy in leprosy have been fully understood and the present trend is polytherapy. Unfortunately, the costs of Rifamycin and lamprene the alternative drugs are 600 and 500 times the cost of dapsone for each day treatment. It was therefore, necessary to find out the optimum dose and duration of therapy with this view a project was undertaken at Acworth Leprosy Hospital Wadala. The biopsy of the leprosy tissues was monitored at the department to assess the infectivity of the case and the persistent organism. The treatment schedules were kept coded.

(b) *Drug Resistance in Leprosy*.—The project under the financial assistance of ICMR was concluded last year. On analysis it showed that out of the 150 cases reported to be resistant cases on clinical impression, 14 were found to be total resistant i.e., to 100 mg. of the dapsone dose. This indicates that the incidence is not very high and frightening. But to have a clear picture it is necessary to screen the total leprosy population of a taluka or 3-5 years. This will give an idea about prevalence and incidence of the disease. A project has been submitted to ICMR.

(c) *A vaccine for leprosy*.—As *M. leprae* is poorly antigenic, there is a search for alternative *in vitro* cultured mycobacteria which can protect against *M. leprae* it has been shown in last year's report that ICRC bacillus of Cancer Research Institute is a good antigen. The work is extended further with the assistance of leprosy mission to study the mycobacterial antigens of Dr. B. R. Chatterjee of Jhalda (W. Bengal). They also show a promise. The work is under progress.

(2) Drug screening

Synthesised compounds of the Chemotherapy Department have been tested in the Pharmacology department for their biological activity as follows :—

(1) Antifilarial screening in cotton rats.

(2) Antiamoebic screening in hamsters and rats.

(3) Anthelmintic screening in mice using *N. dubius* and *H. nana* infestations.

A total of about 200 compounds (details of which can be seen in the report of Chemotherapy Department) were screened.

Indigenous drug screening

(1) *Study of neuromuscular blocking activity*.—There is an acute shortage of the popularly used N—M. blocking agent curare. The new **glucosteriod was isolated which produces reversible N—M. blocking activity** of curare in mice. Further work to characterise the full profile of N—M. activity and elucidation of its structure is in progress.

(2) *Hypoglycemic activity*.—(a) Hypoglycemic activity in the flavonoid fraction of plant coded was located. A dose of 500 mg/kg produces 38% activity at 5th hour in rats as compared to the 50 mg/kg of tolbutamide which produces 30 % activity at 5th hour. Further study is in progress.

(b) Hypoglycemic activity in the glycosidal fraction of plant coded PTN reported in the last year was pursued further and has found place in the report of Phytochemistry section.

(3) *Other studies*.—Plant products showing anti-amoebic and tuberculosis activities are also under study.

Studies on Tik 20 Antibodies

Tik 20 is used extensively for suicidal purpose. The treatment today rests on the use of atropine. We have developed antibodies to the active ingredient by combining it with albumin and raising antibodies in rabbits. Further work is under progress.

PHYTOCHEMISTRY

Larvicidal/Insecticidal activity of Indigenous plants collected from Ratnagiri District.

Plants were extracted with petroleum ether and methanol in soxhlet and the extracts were tested for larvicidal activity.

Among the collected plants following plants showed promising larvicidal activity—

- (1) *Durantaplumeri*
- (2) *Ocimumbasilicum*

Ocimum sanctum

Previously petroleum ether extracts showed promising larvicidal activity. Now the petroleum ether extract was subjected to column chromatography using neutral alumina column using Benzene and Benzene : Methanol as eluting solvents. Seventy fractions were collected and they were also subjected to T.L.C. on silica-gel using Hexane/acetone/chloroform solvent systems. Fractions 7, 26, 31 and 46, were given for testing the larvicidal activity and fractions 7 showed good activity. It was further purified by crystallization in Methanol and the same tested for larvicidal activity.

To carry research on Indian Medicinal plants to assess biological activity.

Pueraria tuberosa tubers were collected from Goa., and tested for hypoglycemic activity. Methanolic and watery extracts were passed through dowex ix column. Two fractions were separated out by this column. These fractions showed a single spot in TLC and paper chromatography and positive test for glycoside. Both fractions possessed blood sugar lowering property in normal rats. Hypoglycemic activity was compared with standard drug tolbutamide. It was observed that at the end of 7 hours blood sugar reduction was more than standard drug.

Isolation of glycoside by lead acetate method

Water extract was treated with basic lead acetate to remove inorganic salts and tannins. Excess of lead was removed by passing the H_2S gas. Filtrate was concentrated under reduced pressure. The residue was further purified by using silica-gel column and methanol-ethylacetate as eluent.

Two glycosides were separated which showed different R_f values on TLC and paper chromatography.

Collection and evaluation of Vinca rosea, Dioscoras, Solanum species and Insecticidal Plants.

Survey and collection was done from Panchgani, Ratnagiri and Thane districts. Following plants were collected and identified :—

- (1) *Terminalia tomentosa*
- (2) *Iphegenia stallata*
- (3) *Terminalia belerica*
- (4) *Duranta plumeri*
- (5) *Vitex negundo*
- (6) *Cymbopogon martini*
- (7) *Cuscuta reflexa*
- (8) *Ocimum basilicum*
- (9) *Vinca rosea*
- (10) *Dioscorea bulbifera*
- (11) *D. esculents*
- (12) *Hyptis suaveolens*
- (13) *Bambusa arundancea*
- (14) *Polygonum sps*
- (15) *Heliotropium indicum*
- (16) *Clerodendron inermis*

Vinca rosea collected from Institute campus, Thane and Ratnagiri Districts, was analysed for total alkaloidal content and following results were obtained.

Thane	Haffkine Institute Campus.	Ratnagiri District.
Roots. 1-20	1.6%	0.90%
Stem.	.43%	0.60%
Leaves 0.49%	0.56%	0.40%

Dioscorea bulbifera collected from Ratnagiri district was analysed for diosgenin content. No. diosgenin was found in the said species.

Dioscorea floribunda cultivated at Pimpri was analysed for diosgenin content. Total yield of diosgenin was 1.9%.

Solamun khasianum berries were collected from Pimpri; Solasodine was estimated by colorimetric method. Berries were found to content 1.8-2.0% of solasodine.

Project on grass Different grass were collected from Haffkine Institute campus and identified. The grass were extracted with petroleum ether and methanol. Presence of alkaloids and of steroids were detected. Following grass were tested :—

	Steroids	alkaloids
(1) <i>Setaria glauca</i>	+ ve	— ve
(2) <i>Cynodon dactylon</i> (Linn) Pers.	+ ve	+ ve
(3) <i>Setaria intermedia</i>	+ ve	— ve
(4) <i>Digitaria sanguinalis</i>	+ ve	— ve
(5) <i>Echinochloa colonum</i>	+ ve	— ve

RADIATION BIOLOGY UNIT

Studies on radiolabelled tetanus toxin

The project was continued during the current year. After completing the toxicity tests of the radiolabelled tetanus toxin in mice, further experiments were done in guinea pigs to study the tissue distribution. The labelled toxin was injected directly into the brain of the guinea pig and radio activity was measured in the circulating blood, liver and kidney after death of the animal within 4 hours. The observation of some radioactivity in these tissues was thought to be due to passage of the toxin through the brain-blood barrier. Tests were therefore undertaken to confirm whether the labelled toxin as such crossed the barrier or it was only the label i.e. ^{125}I that leaked out from the toxin and then passed through the barrier to appear in the blood and other tissues. This was done in cats, in both acute and chronic experiments. In acute experiments, the labelled toxin was injected intracerebroventricularly (i.c.v.) in anaesthetised cats and periodic samples of blood and urine were collected at fixed intervals upto 6 hours. These were counted for radioactivity. It was seen that less than 1% of the injected dose appeared in blood upto 6 hours. The urine samples and the thyroid gland also indicated some radioactivity. To test for the presence of toxin in the blood samples, the sera obtained from these blood samples were injected in mice to observe for the symptoms of tetanus toxicity. The animals did not present any symptoms, thereby indicating that the sera were devoid of toxin and contained only the tracer, ^{125}I . Further proof for leakage of the label alone from the brain was provided by the observation of radioactivity in the thyroid gland. It was thus confirmed that the toxin gets bound to the CNS structure. In chronic experiments the duration of this leakage of label was studied after i.c.v. injection of the labelled toxin and collection of the blood and urine samples upto 96 hours. In this case also, less than 1% of the injected radioactivity could be seen in blood during this period.

Experiments were undertaken to set up an assay system to study the antibody binding capacity of the labelled toxin as the antigen. The antigen was incubated with the standard antibody of known strength for a sufficient period to allow for the antigen-antibody reaction. The separation of antigen-antibody bound complex from the unreacted antigen and the excess antibody was affected by the use of dextran-charcoal suspension in different concentrations. This method did not work well since the non-specific binding was found to be much higher and it could not be reduced further. The second method employed for separation of the bound complex was the use of Polyethylene Glycol (PEG). This selectively precipitates the bound complex in the incubation mixture. However, this method also did not work since PEG could not differentiate between the globulins which it precipitated from the negative control (non-immune, normal serum) sample and the bound complex from the known antiserum.

positive control). In other words, PEG could not differentiate between the globulins and the labelled antigen of nearly equal molecular weights that of the globulins.

The use of a second antibody system thus became necessary. The second antibody directed against the globulins of the first antibody (which binds the labelled antigen) precipitates the bound complex selectively. Experiments are in progress to study this separation system.

Studies on radiolabelled antigens of M. tuberculosis.

This project was undertaken in collaboration with the Dept. of Immunology. Two antigenic fractions of *M. tuberculosis* were obtained after purification of crude soluble antigen by physico-chemical methods and these were labelled with radioactive iodine; ^{125}I by the standard chloramine-T method. Fraction I inducing delayed hypersensitivity was having a molecular weight 200,000 daltons while Fraction II which did not elicit delayed hypersensitivity indicated a molecular weight 200,000 daltons. However, both the fractions induced antibody production in rabbit. It was observed that incorporation of tracer into the protein molecule was comparatively poor for both the fractions. Similarly, the separation of labelled antigen from unreacted tracer iodine was also not very clear. This could be attributed to the aggregate formation in the antigens caused by the procedures used in our study. Further investigation by disc electrophoresis indicated the presence of 3 subfractions in Fraction I. Subfraction I and partially purified Fraction II which showed strong antibody binding reaction by immunodiffusion were subsequently used for radiolabelling. It was thought interesting to study whether antibodies in serum samples of infected cases can be detected by RIA, using these labelled antigens.

For the standardisation of the technique, known antiserum sample was used and the binding of the labelled antigen to the antibody was evaluated. Radioimmunoprecipitation test was initially employed, with the use of small filter paper discs to retain the antigen-antibody bound complex. The paper disc was washed well to remove the unreacted tracer antigen and the excess antibody added. A negative control was also run simultaneously. However, due to high non-specific binding of the tracer antigen on the discs. It was difficult to differentiate between the positive and negative control samples.

Subsequently, polyethylene glycol (PEG) was used to separate the antigen-antibody bound complex by its precipitation. It was seen that PEG could also not specifically differentiate between the bound complex and the globulins from the negative control sample, probably because of the high molecular weight of the antigen.

The use of second antibody system however seems to give better results with respect to the separation of the bound complex. Work is in progress to improve the methodology using this system.

TOXICOLOGY

Acute toxicity studies of commonly used pesticides.

Acute dermal toxicity of Dimethoate (Rogor), Fenitrothion, Ethion, Carboxin (Vitavax), Cythion 5% dust and Cythion 50% EC in rats were completed. Ethion was very toxic in comparison to the rest in rats (0.3 ml/kg produced 100% mortality in comparison to others). The least toxic being Cythion and Carboxin (no mortality even with 10 g/kg). Acute dermal toxicity of Azino-phosmethyl (tech., Devithion, Devisytox, Devilsulphan) were conducted. The most toxic being Devisystox (LD_{50} 72 mg/kg) in this species.

Studied acute oral toxicity in rats of Phenyl mercury acetate, Folithion 50EC, Devisystox, Devilsulphan, Dalpone, Acothion, Cycocel and Ethion (Tech.). Least toxic being Dalapone (LD_{50} 8 g/kg) in this species. The acute oral toxicity in mice of Dimethoate and Ethepon were also studied. Dimethoate was found to be more toxic (LD_{50} 130 mg/kg).

Appendix VI, VII show the results of the tests.

Protein deficiency & drug action

The project was under taken to study the effects of using therapeutic drugs in mal nourished (protein deficient) population. The studies are conducted in rats fed with 3% protein diet (malnourished) & compared the data obtained in from experiment on rats fed with 15% protein diet (control group). The drug selected for this study was Aspirin.

(i) *LD Studies* : Earlier work on LD_{50} studies of Aspirin by oral route indicated LD_{50} value for rats fed with 3% protein level was about $\frac{1}{2}$ the value obtained in the animals that were fed 15% protein diet (control). These were confirmed.

(ii) *ED₅₀ Studies* : Normal and malnourished rats were subjected for ED_{50} determination of Aspirin (anti-inflammatory activity) by rat foot pad edema method. The values are :

Normal rats—520 mg/kg.

Malnourished rats—150 mg/kg.

(iii) *Absorption, metabolism, distribution and excretion of Aspirin in normal and malnourished rats* : Blood absorption studies of Aspirin were determined in normal rats earlier. At present their pattern are being studied in malnourished animals.

(iv) *Standardisation of the methods* : The method for studying the metabolism, distribution and excretion pattern of Aspirin were also standardised during this period.

VIROLOGY

The In Vivo Development of Vaccines Against Viral Hepatitis Type B Using the Newborn Oryctolagus cuniculus Model System.

Newborn and Foetal Rabbit Model Systems.

Further intrahepatic passages of HIBOMA virus and reisolation and passage of human HBV were effected in newborn (intrahepatic) and foetal (transplacental) *O. cuniculus*, strain Haffkine, conventional, non-inbred. This work was carried out in spite of the continued presence of severe intercurrent infections, such as coccidiosis and salmonellosis in the animal house breeding colonies of the Haffkine Bio-Pharmaceutical Corporation Limited (HBPCL), from which the rabbits were being supplied to this Department for the HIBOMA experimental work. For this reason, amongst others, it became necessary to curtail, or entirely suspend, for long periods of time, development of the rabbit model. In addition, it was realized that the earlier obtained high mortality figures in rabbits intrahepatically inoculated at the newborn stage with HIBOMA virus, were due, in no small measure, to the fact that parturient mothers of the newborn rabbit litters supplied to our Department from the breeding colony were either too old to adequately feed their young, or that certain young females, who did possess ample milk reserves, refused to feed their young, possibly as a result of the sudden change of environment from the breeding colony nursery to the experimental animal house. Both these factors (which could only be detected when large numbers of comparatively healthy, coccidiosis and salmonella-controlled rabbits were made available to the Department by the HBPCL breeding colony, especially for the purpose of long-term, chronic infection studies on HIBOMA infection, which studies are reported below) contributed very significantly to the delay in development of the *Oryctolagus* model.

Nevertheless, the results accruing from studies involving very substantial numbers of newborn and foetal experimental and control groups of rabbits, unequivocally confirmed our earlier findings concerning highly significant weight loss in animals inoculated intrahepatically at birth, or intravenously (pregnant female inoculation) with HIBOMA (the rabbit-adapted human HBV), or HIBOMA-HSP (human HBV), a series of 5 experiments being performed, 3 in the newborn rabbit and 2 in the pregnant rabbit. The purpose of the first series of three similar experiments was to test whether growth inhibition could be accomplished following intrahepatic inoculation with HIBOMA-I(5), the 5th passage of the original lapinized human HBV; by HIBOMA-HSP (human HBV from an HBsAg-positive serum pool from 24 patients of hepatitis B) and by HIBOMA-HSP(RP1), the first intrahepatic rabbit passage of HIBOMA-HSP into the newborn rabbit. The purpose of the next two individual experiments, in which a different inoculation route was used (intravenous in the pregnant animal in the 4th gestational week), but two of the viruses utilized were the same as those in the first set of 3 experiments

(HIBOMA-HSP and HIBOMA-HSP(RP1), was to test whether these viruses could, also, show the same growth-inhibition *in vivo*, following delivery. As already indicated, the results of all five experiments unequivocally demonstrated that HIBOMA-I(5), HIBOMA-HSP and HIBOMA-HSP(RP1) could all produce marked growth-inhibition and stunting, in comparison with uninoculated, or ^{60}Co -irradiated virus controls.

Work continued briskly on the project entitled "Biochemical Studies in Viral Hepatitis with Particular Reference to HIBOMA Virus". In order to obtain *definitive data* on the glutamic pyruvic transaminase (SGPT) levels of 100 normal rabbits belonging to different age-groups (weight range 50g-3.5 kg), such estimations were made soon after receipt of the animals in the laboratory, prior to subjection to virus inoculation, or any other such experimental procedure. The assay method was that of Reitman and Frankel (1957). The 3-sigma SGPT limits were computed and shown to lie between 1.36 IU for rabbits (of all ages). This revises our previous figures of 1.52 IU, as stated in the 1977-78 Annual Report of the Institute, as the latter range was determined from a smaller number of normal animals.

For study of the elevation of the SGPT level (as a marker of acute hepatitis), following intrahepatic inoculation at birth with HIBOMA-I(5) virus, a total of 96 experimental and 38 control animals was utilised. The SGPT levels at days 1-3, 7-10, 20 and 40 post-virus inoculation were found to be significantly higher (p : 0.01 to 0.001) in the experimental animals, in comparison with uninoculated controls.

It had been earlier observed (Annual Report of the Haffkine Institute, 1975-76) that following intrahepatic HIBOMA-I(5) inoculation into newborn rabbits, a considerable proportion of the animals failed to gain weight normally, remaining stunted. A number of such experimental and control (uninoculated) animals were not sacrificed, but observed for long periods of time (100-700 days). Here, again, SGPT elevation was considered a marker of chronic hepatitis. The data from 71 experimental and 55 control animals showed, unequivocally, that, at days 100-140, 150-175, 180-200 and 250-700, the levels in experimental animals were very significantly elevated, in comparison with controls ($P < 0.001$).

Although the clinical parameter of weight loss and the biochemical marker of significant elevation of the SGPT beyond the 3-sigma level have provided unequivocal evidence concerning the presence of acute and chronic hepatitis following HIBOMA-I(5) and HIBOMA-HSP inoculation, the final demonstration of infection will depend upon virological techniques—the presence of HBsAg and HBV will have to be demonstrated in the hepatocytes of infected animals. In addition, it would be interesting to attempt to obtain further histopathological evidence of the presence of viral hepatitis lesions in the livers of such animals.

on-human Primate Model Development-The Langur System

As stated in the Annual Report of the Haffkine Institute (1977-78), the angur (*Presbytis entellus*) studies initiating Phase II (Non-human Primate experiments) of the SERC-Sponsored Project commenced in February 1977. A single adult langur had then been inoculated intravenously twice (6 days apart) with HIBOMA-I(5) and, 8 weeks later, appeared to have lost weight. Twenty-one weeks and four days following the first rabbit virus injection by which time the animal was already emaciated, the langur received a single intravenous inoculum of HIBOMA-HSP, the human virus. Thirty-four weeks following the first virus inoculation, the animal was necropsied and the liver showed a well-marked portal triaditis, of the type seen in human chronic persistent hepatitis.

During 1978-1979, a liver homogenate, stored at necropsy from the above sick animal, was inoculated into 2 fresh langur. One of these animals developed the clinical syndrome, as described for the first langur, along with biochemical (significantly elevated SGPT levels beyond the 3-sigma limit) and histopathological evidence of liver damage. The second langur, though clinically apparently healthy, did show histopathological evidence of hepatitis. Thus, it appeared that HIBOMA-I(5), or HIBOMA-HSP (or, possibly, a mixture of both viruses together), could be passaged in the langur, thus satisfying one of the most important criteria of demonstrable virus aetiology.

A second experiment of similar design was next performed. In the Annual Report for 1977-78 it had been reported that a group of 7 langur had been intravenously inoculated with HIBOMA-HSP and that the same clinical syndrome of anorexia and emaciation had been observed in 5/7 animals. In addition, ascites had been seen in 3/7 animals and that, in all, 5/7 animals had shown histopathological evidence of acute or chronic hepatitis. During 1978-79, a longitudinal (prospective) study was carried out in four fresh langur. A pooled liver homogenate, from those langur of the previous experiment which had evidence of hepatitis, was inoculated intravenously. Two out of the four animals showed the typical clinical syndrome and presented biochemical (elevated SGPT levels) and histopathological evidence of hepatitis. In one of the seriously affected langur there was a very close correlation between the clinical, virological, histo-pathological and biochemical criteria of hepatitis. Thus, once again, it was demonstrated that HIBOMA-HSP could also be passaged in the langur system.

Another interesting experiment carried out during 1978-79 was a longitudinal study, involving a total of 9 langur. In this experiment, re-inoculation biopsies showed normal liver histology in 6/9 animals. One, out of the remaining 3, had fatty change and the other 2 questionable (minimal), lobular infiltration. Following intravenous HIBOMA-HSP inoculation, 3 langur developed the *typical clinical syndrome, which progressed until the animals*

became moribund, just prior to necropsy. Progressively, too, histopathological change suggestive of hepatitis appeared, until at necropsy, 25-28 weeks following virus inoculation, 8/9 animals showed one, or the other changes (lobular or portal) infiltration with mononuclear cells, several of the animals showing higher grades of such changes at necropsy as compared with the intervening liver biopsies, which were carried out routinely at 6-week intervals. In the sera of 4/9 langur, HBsAg was detectable by RIA for 14 days following virus inoculation. The FITC labelled anti-HBs, prepared as described in the Annual Report (1977-78), was utilized in this experiment for the demonstration of HBsAg-positive direct immunofluorescence. It was found that, whereas, in the pre-inoculation biopsies, none of the nine langur livers showed immunofluorescence, the 3/9 animals, which had exhibited the clinical syndrome described above, did show such fluorescence between 6-26 weeks (the usual incubation period of hepatitis B in man) post-HIBOMA-HSP inoculation. The fluorescence was specific and intracytoplasmic.

The foregoing data, accruing from experiments conducted on a total of 27 langur suggest that this non-human primate is susceptible to infection with HIBOMA-HSP and, probably, HIBOMA-I (5) viruses. It is believed, further, that this is the first time that any non-human primate has been reported to exhibit a clinically recognizable disease syndrome following inoculation of the lapinized, and human hepatitis B viruses. Again, ascites and progressive emaciation leading to death have not, heretofore, been reported in any animal, non-human primate, or otherwise.

The stunting syndrome exhibited by newborn-and *in utero*-inoculated rabbits [with HIBOMA-I(5), HIBOMA-HSP and HIBOMA-HSP (RPI) viruses], is again the first such demonstration in a non-human mammal. It is of great interest that the single, almost universally present sign of clinically apparent viral hepatitis infection in man, that of anorexia, should be consistently also noted in the rabbit and the langur.

RABIES RESEARCH

Development of a safe and effective vaccine against rabies prepared in tissue culture.

Primary Hamster Kidney Cultures.—Study of rabies viral dynamics in cell cultures of primary hamster kidney (pHKTC) were continued confirming the earlier results that virus adsorption was better from uncentrifuged seed suspension at 37°C for 1 hour and in presence of 100 ug/ml of DEAE-dextran. Though the viral presence was demonstrated in the Spent culture medium as tested from the 4th to the 36th post-infection day, the yield was quite low (only 1 : 10 dilution showing deaths on inoculation in mice).

As the infection of fresh cultures required some minimum amount of infectious virus in the previous passage, it became evident that sub-passages of virus could not be carried out without recourse to concentration at each passage level. This work will be continued further as soon facilities for concentration and purification of virus (gel-filtration and ultrafiltration equipment) become available.

Mouse Embryo cultures—Growing rabies virus in primary cultures of mouse embryo, another substrate being attempted. Standardization of condition for satisfactory growth and maintenance of METC was therefore undertaken. Different growth media (Hank's medium with lactalbumin hydrolysate and MEM) and sera (sheep and goat sera at different concentrations) were tried. Excellent growth of cells occurred when MEM was supplemented with 10 per cent. normal goat serum. Work on viral dynamics in these cultures is being started.

Human Diploid cell cultures—In keeping with the current trend of utilizing homologous cells for growth of viruses for human vaccine development, a strain of human diploid cell cultures (HDCC) has been obtained for pilot experiments for testing its suitability for vaccine development. The screening tests include: chromosome analysis (for genetic stability and diploidy), bacterial sterility, freedom from fungus, mycoplasma, latent viruses, extraneous agents and carcinogenic activity. The tests are in progress. If necessary a certified cell line strain will be obtained for adaptation of rabies virus at appropriate time.

CHAPTER IV

TESTING

Testing Unit carried out its usual routine activity in the year. It received samples from Prohibition and Excise Inspectors from all over the State. Various pharmaceutical manufactures also availed facility of testing fine chemicals, raw materials and finished products for specialised pharmacopoeial tests for which sophisticated equipments and expertise are required. During the year, 2584 samples received from prohibition and exercise inspectors were reported (*see* appendix XV). The unit undertook testing of microbiological/biological tests and various chemical tests were also carried out by the unit during the year (*see* appendix XVI, XVII).

The following drugs were assayed for Blood-Absorption studies by the Bioavailability Unit:—

<i>Erythromycin</i>	...	Ipca Laboratories.
<i>Tetracycline (oral)</i>	...	Kemp & Co. Bios Pharmaceuticals. DWD Pharmaceuticals. Biddle & Sweyer Co. Pvt. Ltd. Mercury Pharmaceutical Industries. Martin & Harris Pvt. Ltd.
<i>Oxytetracycline (oral).</i>		Kerala State Drugs & Pharmaceuticals Ltd. Indian Drugs & Pharmaceuticals Ltd.
<i>Oxytetracycline</i>	...	IDPL.
Injection.		
<i>Chloramphenicol</i>	...	IDPL. Mercury Pharmaceuticals. Biddle & Sweyer Co. Pvt. Ltd. Kerala State Drugs & Pharmaceuticals Ltd.
<i>Chloramphenicol and Streptomycin Combination.</i>		Indian Drugs & Pharmaceuticals Ltd.
<i>Ampicillin</i>	...	DWD Pharmaceuticals. Kerala State Drugs & Pharmaceuticals Ltd. Kopran Chemicals. Biddle & Sweyer Co. Pvt. Ltd. IPCA Laboratories. IDPL. Martin & Harris.
Capsules.		
<i>Ampicillin</i>	...	IDPL.
Injection.		
<i>Althrocin Tablets</i>	...	Alembic Pharmaceuticals.

<i>Gentamycin</i>	...	Unique Pharmaceuticals. DWD Pharmaceuticals.
<i>Prestet D</i>	...	(Tetracycline & Doxycycline Combination) by Citadel Fine Pharmaceuticals Pvt. Ltd.
<i>Doxicillin</i>	...	Apex Pharmaceuticals. Super Pharmaceuticals. IDPL.
<i>Amoxicillin</i>	...	DWD Pharmaceuticals.
<i>Methyl Dopa</i>	...	IDPL. Ciffis Pharmaceuticals.
<i>Diphenyl-Hydantoin</i>		IDPL.
<i>Kinex</i>	...	Unique Pharmaceuticals.

Dose Schedule be as suggested by the respective pharmaceuticals covering about a week's repeated dose regimen were also carried out to assess the ojourrn of Prestet D and Althrocin.

The results were interesting to note, however, are treated as confidential s suggested by the pharmaceuticals themselves.

otoxicity Studies

The toxic unit of the units tested toxicity effects of various substances Appendix XX) experimentally in animal models.

CHAPTER V

GENERAL ACTIVITIES

Biostatistics

A biostatistician attached to the institute provides expert advice regarding the subject to post-graduate students, research project workers and takes part in teaching programme of the institute.

Instrumental cell

This Unit has been in existence for last two years. It looks after maintenance and proper functioning of near about hundred electronic instruments. It also provides expert advice regarding purchase of new equipments and takes interest in development of electronic instruments.

Bulletin of Haffkine Institute

The Bulletin provides information regarding the various research activities of the institute. It is published thrice a year and abstracts important bio-medical articles published in leading journals of the world.

Library

It is one of the oldest and finest libraries in India. It works from 9 a.m. to 7 p.m. on weekdays and upto 5 p.m. on Saturdays. This year a photocopier machine manufactured by OCE has been purchased by the library to share the workload of xerox machine. The grant for purchasing the machine has been provided by UGC.

During this year, library committee met five times to discuss the library matters. The library continued to participate in International exchange programme sponsored by WHO. Ciba Research Center and WHO presented 40 books to the library.

Welfare activities

Haffkine Sports Club arranged Annual Tournaments in February. Staff of the institute participated in great enthusiasm in various sports like badminton, table tennis, carrom, kabaddi, volley-ball, cricket, chess, etc. The institute participated in various tournaments in and outside Bombay, and won trophies in kabaddi, table tennis and cricket.

Administration

The Institute continued in the grip of financial crisis. The critical financial condition coupled with labour unrest not only prevented future programme being undertaken, but also hampered the normal working of the Institute to a great extent. Amidst these difficulties, Dr. B. B. Gaitonde relinquished the charge of the Director to take up a new assignment in W.H.O. Nevertheless during the year, action was initiated to find a solution to the labour problems. Government was also approached to augment the financial resources of the Institute.

APPENDIX I

Department of Biochemistry

Routine and special Tests.

Name of the Test					No. of the Test
<i>Blood Serum.</i>					
Acid phosphatase	...	...	...	...	22
Alk. phosphatase	...	...	...	...	70
Bilirubin	...	...	...	...	227
Calcium	...	...	...	...	27
Chlorides	...	...	...	...	63
Creatinine	...	...	...	...	23
Cholesterol	...	...	...	...	115
Icteric Index	...	...	...	...	148
Amylase	...	...	...	...	5
Phosphorus (Inorganic)	...	...	...	...	21
Proteins-Total	...	...	...	...	127
Albumin	...	...	...	...	31
Globulins	...	...	...	...	31
Sugar	...	...	...	...	225
Thymol Turbidity	...	...	...	...	141
Urea Nitrogen	...	...	...	...	46
Uric Acid	...	...	...	...	12
Total				...	1334

Urine and Stool.

Albumin	...	...	...	...	1
Bile salts and pigments	...	...	...	...	216
Calcium	...	...	...	...	2
Porphyryns	...	...	...	...	2
Sodium	...	...	...	...	1
Sugar	...	...	...	...	34
Creatinine	...	...	...	...	1
Urobilinogen	...	...	...	...	1
Total				...	258

C.S.F. and other body fluids.

C.S.F. Chlorides	...	...	...	...	2
C.S.F. Proteins	...	...	...	...	3
C.S.F. Sugar	...	...	...	...	3
S.M. Test	...	...	...	...	2
Total				...	10

Name of the Test					No. of the Test
<i>Special Tests.</i>					
Catecholamines (Urine)	...	...	...	...	1
Fat in stool	...	...	...	...	1
Gastric Analysis	...	...	...	...	4
Glucose Tolerance Test	...	...	...	...	3
17-Ketosteroids (urine)	...	...	...	...	11
Lange's Colloidal Gold Curve (C.S.F.)	...	...	...	...	197
Potassium (serum)	...	...	...	...	45
Protein Bound Iodine (serum)	...	...	...	...	44
Serum proteins by paper Electrophoresis	...	...	...	...	37
Sodium (serum)	...	...	...	...	81
S.G.O.T.	...	...	...	...	166
S.G.P.T.	...	...	...	...	171
Triglycerides (serum)	...	...	...	...	3
Urinary calculi	...	...	...	...	15
Vinyl Mandelic Acid (urine)	...	...	...	...	8
				Total	787
<i>Summary</i>					
1. Blood/serum	...	...	...	...	1334
2. Urine and stool	...	...	...	...	258
3. C.S.F. and their body fluids	...	...	...	...	10
4. Special Tests	...	...	...	...	787
				Grand Total	2389

APPENDIX II A

DEPARTMENT OF CLINICAL PATHOLOGY

Hospitals and Agencies making use of the services

Bombay Area

From Maharashtra

Cama and Albless Hospital	...	Civil Hospital, Thana.
S. George's Hospital	...	Civil Hospital, Alibag, Kolaba.
Jagjivanram Hospital	...	General Hospital, Sawantwadi.
Central Railway Hospital	...	I. S. P. Hospital, Nashik.
B. Y. L. Nair Charitable Hospital	...	Public Health Centres, Buldhana.
K. E. M. Hospital	...	Civil Surgeon, Sangli.
M. G. M. Hospital	...	District Hospital for Women, Akola.
L. T. M. G. Hospital	...	Civil Hospital, Akola.
E. S. I. S. Hospitals	...	Primary Health Centre, Jalgaon.
K. B. Bhabha Hospitals	...	General Hospital, Osmanabad.
Kasturba Hospital	...	Civil Hospital, Dhulia.
G. T. Hospital	...	Civil Hospital, Nashik.
J. J. Group of Hospitals	...	Hindustan Aeronautics Ltd., Nashik.
Police Hospitals	...	
M. A. Podar Hospital	...	
Greater Bombay Municipal Dispensaries.		

The routine investigations carried out during the year.

Name of the test	No. of samples
A. Histopathology	1115
B. Hematology	768
C. Cytology for malignant cells	17
D. Clinical Pathology	
Urine for routine examination	238
Stool for routine examination	147
Semen examination	8
Cerebrospinal fluid for cytological examination	1
Immunological Pregnancy Tests	1312
Biological (Rat Test) Pregnancy Tests	297
E. Bacteriology	
Culture for Tubercle Bacilli	90
Animal Inoculation for Tubercle Bacilli	31
Smear and Culture for <i>C. diphtheriae</i>	68
Urine Culture	334
C.S.F. Culture	3
Stool Culture	37
Blood Culture	466
Sputum Culture	11
Miscellaneous Body Fluids and Exudates Cultured	138
Cultures of Theatre Swabs for Anaerobes	201
Antibiotic Sensitivity Tests	333
Special Bacteriological Examinations	12
F. Serology	
Widal Test	430
Weil Felix Test	430
Brucellosis Test	430
V.D.R.L. Test	151
Kahn Test	10
Wasserman Test	3
Paul Bunnell Test	258
Casoni's Test	13
R.A. Test	40
Cold Agglutinins	2
G. Mycology	
Isolation and identification of Pathogenic Fungi from Clinical Material.	8
Isolation and identification of Fungi from commercial samples	2
Total	7404

APPENDIX II B

Isolation of *Candida albicans* from Sputum

Class	Total	Count Not done.	Orgs./ml. 500	Orgs./ml. 501 5,000	Orgs./ml. 5,001 20,000	Orgs./ml. 20,001 50,000	Orgs./ml. 50,001 1,00,000	Orgs./ml. 105
I Chronic bronchitis clinically and radiologically diagnosed.	83	2	66	10	4	1	..	..
II Drug Resistant Pulmo T.B. (Clinical and Radiological) AFB—ve.	107	29	51		17			..
III Drug Resistant tuberculosis AFB+ve.	115	19	65		13			
IV Miscellaneous non tuberculosis/ pyogenic and granulomatous disorders.	40		27		5		..	
V Pulmo. T.B. (?) AFB—ve, Nodrugs given.		..				..	..	..

APPENDIX III

Rabies Research Unit

Materials (brains)	Nos.	Positive by Negri body	Positive Biological test	*Inconclusive by biological test
Human	.. 12	Nil	5	3
Dog	.. 3	Nil	Nil	1
Cattle	.. 4	Nil	2	1

*Highly putrified hence unsuitable for inspection or materials which showed nonspecific toxicity killing all the inoculated animals within a few hours.

APPENDIX IV.

Radiation Biology Unit.

The details of the routinely done diagnostic test for evaluation of the Thyroid Function are as follows :—

(i) Total patients diagnosed	...	...	...	...	145
(ii) Detected hyperthyroid	...	...	...	...	32
(iii) Detected hypothyroid	...	...	...	...	16
(iv) Detected euthyroid	...	...	...	...	79
(v) Thyroxine suppression test advised	...	...	...	...	13
(vi) Cases indicating positive suppression	...	...	...	...	5

APPENDIX V

Samples Tested for Toxicity

Serial No.	Classification of samples	No. of tested	Toxicity parameter
1	Drugs ..	5 6 63	(i) Acute oral Toxicity. (ii) Undue Toxicity. (iii) Histamine like substances.
2	Cosmetics ..	1 1	(i) Acute oral Toxicity. (ii) Primary skin irritation.
3	Pesticides ..	11 10 1	(i) Acute oral Toxicity. (ii) Acute Dermal Toxicity. (LD ₅₀) (iii) Primary skin irritation.
4	Chemicals ..	3	(i) Acute oral Toxicity.
5	Plastic ..	2	Toxicity of plastic containers.
6	Painted containers ..	3	Toxicity of water stored in painted containers
7	Metallised polyester film ..		Toxicity of water stored in Metallised polyester film.
8	Aluminium foil/paper laminate coated pieces.		Toxicity of water in which Aluminium foil/paper Laminate coated pieces were kept dipped.

APPENDIX VI

Acute oral toxicity

Serial No.	Name of the compound	Species	LD 50	Fiducial limits	Symptoms.
1	Dimethoate (Rogor)	.. Mice	.. 130 mg./kg.	.. 93-182 mg./kg.	.. Motor inco-ordination, loss of righting reflex, gasping and death.
2	Phenyl Mercury Acetate..	Rats	.. 33 mg./kg.	.. 275-39.6 mg./kg.	.. Initially sluggish then excitation, loss of righting reflex, respiratory disturbances.
3	Folithion 50 EC	.. Rats	.. 445 mg./kg.	.. 356-556 mg./kg.	.. Excitation, respiratory disturbances, convulsion and death.
4	Dalapone ..	.. Rats	.. 8 gm./kg.	.. 7.142-8.969 gm./kg.	Sluggish, respiratory disturbances, loss of righting reflex, death.
5	Devisystox 25 percent EC	Rats	.. 165 mg./kg.	.. 103-268 mg./kg.	.. Depression, tremors, respiratory disturbances, excitation, loss of righting reflex, gasping and death.
6	Devisulphan 35 percent EC	Rats	.. 16 mg./kg.	.. 9.4-28 mg./kg.	.. Excitation, convulsions and death.
7	Ethephon ..	.. Mice	.. 1900 mg./kg.	.. 1520-2375 mg./kg.	Excitation, respiratory distress and death.
8	Accothion 50 per cent EC	Rats	.. 550 mg./kg.	.. 363-824 mg./kg.	.. Tremors, sluggish and death.
9	Cycocel ..	.. Rats	.. 1305 mg./kg.	.. 1088-1566 mg./kg.	Excitation, respiratory distress, convulsions and death.
10	Ethion (Technical)	.. Rats	.. 33.8 mg./kg.	.. 27.3-44.2 mg./kg.	Excitation, respiratory distress and death.
11	Phenyl Mercury Acetate ..	Rats	.. 36 mg./kg.	.. 27.7-46.8 mg./kg.	Excitation, respiratory disturbances and death.

APPENDIX VII
Acute Dermal Toxicity

Serial No.	Name of the compound	Species	L.D. 50 value	Fiducial limits	Symptoms
1	Dimethoate (Rogor)	.. Rats	.. 961 mg./kg.	.. 620—1,490 mg./kg.	Eyes protruding, irregular respiration, loss of righting reflex and death.
2	Fenitrothion	.. Rats	.. 1,588 mg./kg.	.. 1,204—2,250 mg./kg.	Eyes protruding, irregular respiration, loss of righting reflex, emaciation and death.
3	Ethio	.. Rats	.. Dose 336 mg./kg. (0.3 ml./kg.). Produces 100 per cent mortality.		Eyes protruding, irregular respiration, loss of righting reflex, emaciation, and death.
4	Carboxin (Vitavax)	.. Rats	.. L.D. 50 could not be determined.		Animals appeared normal.
5	Azinphos Methyl (Tech.)	.. Rabbits	.. 500 mg./kg.	.. 357—700 mg./kg.	.. Excitation, tremors unsteady gait and death.
6	Devithion 50 per cent EC.	.. Rabbits	.. 271 mg./kg.	.. 150—486 mg./kg.	.. Excitation, irregular respiration and death.
7	Devisystox 25 per cent EC.	.. Rabbits	.. 72 mg./kg.	.. 43—123—mg./kg.	.. Excitation, irregular respiration, salivation, convulsions and death.
8	Devisulphan 35 percent EC	.. Rabbits	.. 165 mg./kg.	.. 89.1—306 mg./kg.	.. Excitation, irregular respiration, salivation convulsions and death.
9	Cythion 5 per cent dust	.. Rats	.. LD 50 could not be determined.		Animals were normal.
10	Cythion 50 per cent EC	.. Rats	.. 9,142 mg./kg.	.. 7,749—10,789 mg./kg.	Bulging of eyes, motor incoordination respiratory disturbances and death.

APPENDIX VIII

Snake and Scorpion Venom Processing

Type of venom				Venom in gms. Processed	Supplied
Cobra	...	...	...	104.50	138.00
Krait	...	...	...	17.00	43.00
Viper	...	...	...	123.00	115.00
Echis	...	...	...	85.50	33.3
Red scorpion	...	...	...	0.23	5.20
Black scorpion	...	...	...	1.4	2.8

No. of snakes and scorpions purchased

species				number
Cobra	...	...	...	172
Krait	...	...	...	264
Viper	...	...	...	507
Echis	...	...	...	9017
Red scorpion	...	...	...	484
Black scorpion	...	...	...	2184

APPENDIX IX

*Post-graduate Courses**Degrees.**By Research.*

M.Sc.	...	...	Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology.
M.Pharm	...	...	Pharmacology and Pharmaceutical Chemistry.
Ph.D.	...	...	Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology, Pharmacology.

By Paper.

M.Sc.	...	...	Microbiology, Biochemistry.
M.D.	...	...	Pathology, Microbiology, Pharmacology.

Diploma.

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

Certificates.

Drug Chemical	...	...	
Drug Bio-assay	...	...	
Short Term Courses	...	...	Clinical Pathology, Biochemistry, Parasitology and Entomology.

Post-graduate

APPENDIX X

Post-graduate Teachers

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

Name	Qualifications.
Dr. M. B. Bhide	... M.D.
Dr. R. S. Kamat	... M.D.
Dr. F. L. Saldanha	... M.Sc., Ph.D., FIC (Cal.).
Dr. A. J. Baxi	... M.Sc., Ph.D.
Dr. D. M. Renapurkar	... M.Sc., Ph.D.
Dr. M. H. Shah	... M.Sc., Ph.D.
Dr. K. H. Dave	... M.D., DPB.
Dr. M. V. N. Shirodkar	... M.B.B.S., AB., SCD., PBK.
Dr. S. Mukerji	... M.B.B.S., M.Sc., Ph.D.
Dr. P. Ramkrishnan	... M.Sc., Ph.D.
Dr. S. V. Gadre	... M.Sc., Ph.D.

APPENDIX XI

Statistics Relating to Post-graduate Students

Name of Degree			Subjects			No of students registered
M.D.	..	..	Pharmacology	..	..	1
			Microbiology	..	..	2
			Pathology	..	..	2
Ph.D.	..	..	Applied Biology	..	..	13
			Biochemistry	..	..	10
			Microbiology	..	..	6
			Organic Chemistry	..	..	8
			Pharmacology	..	..	1
M.Sc.	..	..	Applied Biology	..	..	4
			Biochemistry	..	..	3
			Microbiology	..	..	9
			Organic Chemistry	..	..	9
			Zoology	..	..	2
M.Pharm	..	..	Pharmacology	..	..	2

APPENDIX XII

Degree Awarded and Research Topics

Name of students	Degree awarded		Topics
Shri S. C. Talwalkar ..	..	Ph.D. (Biochemistry)	Studies on protein malnutrition <i>vis-a-vis</i> Adrenal gland function in albino rats.
Shri A. V. Gosavi ..	..	M.Sc. (Zoology) ..	Studies on nematode parasites on cockroach <i>P. americana</i> L.
Shri S. N. Joshi ..	..	M.Sc. (Biochemistry)	Biochemical aspects of <i>E. carinatus</i> venom.
Shri P. S. Narse ..	..	M.Sc. (Org. Chemistry)	Synthesis of organic compounds of possible therapeutic value (Thienyl sulphonamide de and Piperazine Derivatives).
Kum. S. R. Salvekar ..	..	M.Sc.(Microbiology)	Study of Anaerobic Cultures.
Smt. Alka Gupta ..	..	M.Sc.(Microbiology)	Study of bacterial growth on a selective medium with special reference to <i>V. cholerae</i> .

RESEARCH PROJECTS OF RESEARCH FELLOWS

Purification of diphtheria toxin and biochemical studies with the toxin : Shri A. S. GHATAGE.

Diagnostic Reagent Kits : Kum. R. S. HIRWE.

Natural immunity against tetanus among the Adivasis of Virar : Kum. A. K. DAVE.

Synthesis of and development of anthelmintic compounds : Shri P. P. DALVI.

Synthesis of anthelmintic compounds : Shri S. R. LOKHANDE.

Synthesis of organic compounds of possible therapeutic value : Shri D. S. SANGHVI.

Synthesis of anti-amaebic agents : Shri V. J. KHAIRNAR.

Salmonella and Shigella Oral Vaccine : Shri V. M. RANGNEKAR.

Dynamics of cervical vaginal microflora with respect to changes in estrogen and progesterone levels in asymptomatic human females : Shri V. M. RANGNEKAR.

Immunological studies of leprosy : Kum. S. W. AWACHAT.

Studies of an antifertility activity of an indigenous drugs and mechanism of action of IUCD : Shri R. T. MAHAJAN.

APPENDIX XIII

Short Term Training Courses

Seventy-nine students attended short-term training courses during the year. Details of subject offered and the number of students attending these courses are recorded here.

Clinical Pathology	...	...	...	...	14
Blood Banking	...	...	...	...	7
Biochemistry	...	...	...	...	15
Preparation of Vaccines and parenteral solution (Bacteriology and Media).	...	...	...	...	1
Entomology and Parasitology	...	...	...	...	1
Testing Unit	...	...	...	...	1
Grand Total	...	...	...	...	39

APPENDIX XIV

Trainees and Research Fellowships and Scholarships

Trainees admitted :

Diploma in Medical Laboratory Technology (D.M.L.T.)	...	...	14
Course in Drug Analysis (Chemical)	...	...	8
Course in Drug Analysis (Biochemical and Microbiological Methods) ... Methods).	...	...	3
Short Term Training Course	...	...	39
Number of research Fellowship and Scholarship :			
Senior Research Fellowships (Haffkine Institute)	...	...	4
Junior Research Fellowships (Haffkine Institute)	...	...	2
Senior Research Scholarships (Haffkine Institute)	...	...	1
Junior Research Scholarships (Haffkine Institute)	...	...	5
Senior Research Fellowships (Platinum Jubilee)	...	...	2

APPENDIX XV

Prohibition and Excise Samples

Samples						No. of Samples
Indian Made Foreign Liquors	...	...	...	...	...	1352
Beer	...	...	...	...	...	795
Toddy	...	...	...	...	...	357
Rectified spirit	...	...	...	...	...	10
Bhang	...	...	...	...	...	20
Ganja	...	...	...	...	...	42
Opium	...	...	...	...	...	3
Charas	...	...	...	...	...	1
Mahua	...	...	...	...	...	4
Total						2584

APPENDIX XVI

Biological and Microbiological Tests

Tests					No. of tests carried out
Pyrogen	...	...	...	...	276
Microbiological assays	...	...	...	...	89
Bioassay for adrenaline content	...	...	...	...	94
Toxoids (complete testing)	...	...	...	...	22
R.W.C., S.A. (germicidal value)	...	...	...	...	103
Toxicity	...	...	...	...	20
Depressor substances	...	...	...	...	77
Sterility	...	...	...	...	61
Insulin assay	...	...	...	...	26
Other tests	...	...	...	...	31
Total				...	799

APPENDIX XVII

Chemical Tests

Tests					Test numbers
Complete analysis	...	...	...	...	75
Polymorph Content	...	...	...	...	166
Infra-red Scanning	...	...	...	...	54
Gas liquid chromatography	...	...	...	...	14
Assay of active ingredients	...	...	...	...	6
Ultra-violet scanning	...	...	...	...	3
Total				...	318

APPENDIX XVIII

Critics Club Lectures

Speaker	Department	Subject
<i>Institute Scientists' Lectures</i>		
r. P. N. Akolkar	.. Biochemistry ..	Studies on Indian fermented foods : Bio-chemical, Nutritional and Microbiological changes.
hri J. N. Deshpande	.. Virology ..	Studies on the incidence of Hepatitis in Bombay.
r. M. B. Bhide	.. Pharmacology	Effect of vaccine from ICRC bacilli against <i>M. leprae</i> infection in mouse foot pad.
um. Sunita V. Utgi	.. Bacteriology ..	Development of polyvalent <i>Pseudomonas</i> vaccine.
r. N. G. Chanderkar	.. Radiation Biology	Studies in Thyroid function.
r. V. Dabholkar	.. Toxicology	A study of Autonomic Functions in Leprosy.
hri S. Y. Guirges	.. Zoonosis ..	Infant Rabbit as a New model for hepatic amoebiasis with other studies on <i>E. histolytica</i> pathogenicity.
hri P. P. Dalvi	.. Chemotherapy	Synthesis and study of some New Anthelmintic agents.
hri J. P. Mehta	.. Clinical Pathology	Preparation of an Antigen specific to Mycelial form of <i>C. albicans</i>
um. Anita K. Dave	.. Biochemistry ..	Natural Immunity against Tetanus among Adivasis of Virar.
r. (Kum) R. M. Baride	.. Director's Laboratory	Toxidation of Venoms of poisonous Indian snakes.
hri P. Y. Naik	.. Pharmacology	Phytochemical and Pharmacological Investigations of a plant <i>Imperata cylindrica</i>
r. H. J. Mansharamani	.. Rabies Research Unit.	Study of Rabies virus in Tissue culture
um. S. R. Kolhekar	.. Bactriology	Microbiological transformation of steroids with special reference to 11 Hydroxylation of Progesterone using Fungal spores.
Miss Sushila Menon	.. Zoonosis ..	Complete Development of Human Hookworm, <i>Necator americanus</i> in Infant Rabbits.
Dr. M. H. Shah	.. Chemotherapy	Plastic containers for transfusion liquids
Miss Theresa Joseph	.. Clinical Pathology	Bacteriology of Diarrhoea in infants and children.
Shri S. P. Gawade	.. Director's Research Cell	Sea snake antivenin

Guest Lectures

Prof. A. Philippu
Head, Neuropharmacology Section
Dept. of Pharmacology and Toxicology
University of Waerzburg
West Germany

Hypothalamic Regulation of Arterial Blood Pressure.

Dr. Parvinkumar B. Sehgal
Assistant Professor
Rockefeller University
New York, U.S.A.

Super-induction of Human Fibroblast Interferon production.

Prof. George Wald
American Biologist and
Nobel Prize Winner in Physiology
and Medicine, 1967

Vision and more Important Matters.

Dr. Dawn Owen
M.R.C. Laboratory
Animal Centre (LAC)
Surrey, U.K.

Animal Health, Breeding and Maintenance.

Do.

Parasites of Animals

Films

Automatic serological testing for Brucellosis
(Courtesy of British-Council).

Barefoot doctors in China
(Courtesy of Dr. C. G. Pandit).

APPENDIX XIX

Working of the Library

The following figures indicate the working of the library during the year :—

Number of outside readers visiting the library	Number of books and periodicals issued to our staff	Number of periodicals issued under i inter library loan service	Books nad Periodicals obtained for our staff from other libraries
1	2	3	4
7,040	3,023	261	46

Additions to Library

Number of books Number of books added during the year	Number of Number of Vol. Bound	Number of Number of Current Periodicals	Number of Current Periodicals received free of charge	Number of Number of reports received from Scientific Institutions
348	797	183	159	43

APPENDIX XX

Papers sent for publication

BARIDE (Kum.), R. M. and GAITONDE, B. B. : Biochemical changes in experimental Rabies-I.

Indian Journal of Medical Research.

BARIDE (Kum.), R. M. and GAITONDE, B. B. : Biochemical Changes in experimental Rabies-II : Brain monoamines and Free amino Acids.

Virology.

BARIDE (Kum.), R. M. and GAITONDE, B. B. : The affect of prior immunisation and subsequent challenge with Rabies virus on biochemical paremeters in Mice.

Indian Journal of Medical Research.

BARIDE (Kum.), R. M. GAITONDE, B. B. and JAIN, S. D. : Biochemical studies on the toxoids of venoms of poisonous Indian Snakes.

Toxicon.

BARIDE (Kum.), R. M., JAIN S. D. and GAITONDE, B. B. : Immunological and Biochemical Studies on the formalised toxoid of red scorpion venom.

Toxicon.

BHATTACHARYA, S. and GAITONDE, B. B. : Detection and partial purification of chotinesterase from the venom of the saw scaled viper (*Echis carinatus*) .

Toxicon.

BHOPALE, M. K. and KAMATH (Mrs.), V. R. ; Immunological Role of Intestinal mucus and tissue extract of mouse during *Hymenolepis nana* infection.

Bulletin of Haffkine Institute.

BHOPALE, M. K. and MENON (Kum.), SUSHILA : Complete developoment of Human Hookworm, *Ancylostoma duodenale* (Dubini, 1843) in infant rabbits.

Experientia.

BHOPALE, M. K. MENON (Kum.), SUSHILA and KULKARNI (Kum.), LAXMI : Adaptation of *Necator americanus* to infant rabbits, humoral antibody, leukocyte response and serum protein changes during infection.

Ir. of Helminthology.

BHOPALE, M. K., SAVITRI (Kum.), C. R. and KAMATH (Mrs.), V. R. : Cell-mediated immunity responses during *Hymenolepis nana* infection in mice.

Bulletin of Haffkine Institute.

CHAVAN, S. R., DESHMUKH, P. B. and RENAPURKAR, D. M. : Investigation of Indigenous plants for mosquito larvicidal activity.

Bulletin of Haffkine Institute.

GAITONDE, B. B. and BARIDE (Kum.) R. M. : Toxoidation of venoms of poisonous Indian Snakes.

Indian Journal of Medical Research.

GAITONDE, B. B., RENAPURKAR, D. M. and APTE, V. H. : Comparative efficacy of three anthelmintic drugs in treatment of guineaworm infection.

Bulletin of Haffkine Institute.

GAWADE, S. P., BUDUK, D. P. and GAITONDE, B. B. : Preparation of Monovalent *Enhydrina Schistosa* (Common Sea Snake) Antivenin.

Indian Journal of Medical Research.

GOSAVI, A. V., KULKARNI, A. B. and RENAPURKAR, D. M. : Some Biochemical studies on *Hammerschmidtella diesing*, Leidy, 1850.

Bulletin of Haffkine Institute.

GOSAVI, A. V. and RENAPURKAR, D. M. : Infection Patterns in Multiple Species of Nematodes Parasitic in *Periplaneta americana* L.

International Journal of Parasitology.

GUPTA, R. M. and NIMBKAR, Y. S. : Good Manufacturing Practices in Parental.

IDMA Bulletin.

PAVRI, (Kum.), ROSHAN, BAXI, A. J. and NAIK, S. N. : Red cell ATP, 2, 3 Diphosphoglycerate and haemoglobin types in Deccani Sheep from Maharashtra.

Bulletin of Haffkine Institute.

PRABHU, S. R., PATEL, (Mrs.), M. R. and SHAH, M. H. : Synthesis and study of substituted terephthalamides and bis-hydrazones of terephthalic acid as possible antituberculosis agents.

Bulletin of Haffkine Institute.

RAMKRISHNAN, P. : Studies on Hypoglycemic effect of fractions from $MgSO_4$ precipitate of *Bordetella pertussis* extracts.

Bulletin of Haffkin Institute.

SHETTY, K. T. and GAITONDE, B. B. : Effect of contraceptive steroids on Gama Aminobutyric acid Metabolism and Pyridoxal Kinase activity in Rat Brain.

Biochemical Pharmacology.

SHETTY, T. K. and GAITONDE, B. B. : Effect of the chronic administration of Ethinyl estradiol and Norgestral on biogenic amine (S) level and monoamine oxidase enzyme activity in Rat Brain.

Biochemical Pharmacology.

SULE, D. P., SHAH, M. H., GHOOI (Mrs.), S. R. and BHIDE, M. B. : 2-[5'-Substituted-1', 3', 4'-oxadiazol-2'-YL) thiomethyl]-5 (or 6)-substituted-benzimiduzoles as anthelmintic agents.

Bulletin of Haffkine Institute.

APPENDIX XXI

List of Papers Published.

BHIDE, M. B., NAIK, P. Y. and JOSHI, R. S. : Studies on the Neuromuscular blocking activity of seeds of *Solanum khasianum*, Clarke.

Bulletin of Haffkine Institute, 1978, 6., 31-35.

BHIDE, M. B., PRADHAN, K. S. and BAPAT, C. V. : A vaccine from ICRC bacilli against *M. Lepræ* infection in mouse foot-pad.

Leprosy in India, 1978, 50, 332-344.

BHOPALE, M. K., MENON (Kum.), S. and RENAPURKAR, D. M. : Development of *Ancylostoma ceylanicum* in Laboratory Hosts.

Bulletin of Haffkine Institute, 1978, 6, 68-72.

DHABUWALA, J. B., VALAVALKAR (Mrs.), K. D., MUKERJI, S. and DAVE (Mrs.), J. K. : Cultural and serological diagnosis of Gonorrhoea.

Bulletin of Haffkine Institute, 1978, 6, 57-61.

GAWADE, S. P. and BHIDE, M. B. : Chromatographic separation of the venom of *Enhydryna schistosa* (Common Sea Snake) and characterization of principal neurotoxic component from the Western Coast of India.

Indian Journal of Medical Research, 1978, 67, 854-861.

KHOT, V. S., PATEL (Mrs.), M. R. and SHAH, M. H. : Synthesis of N⁴-(5-Nitro-2-Furoyl)-N-Substituted piperazines as possible antimicrobial agents.

Bulletin of Haffkine Institute, 1978, 6, 40-42.

NERULKAR, C. B., CHAUDHARI, D. T., PATEL (Mrs.), M. R. and SABNIS, S. S. : Synthesis and study of 2-(4-Aminophenyl) thiazoles for *In Vitro* antituberculosis activity and as useful chemotherapeutic intermediates.

Bulletin of Haffkine Institute, 1978, 6, 65-67.

PATIL, A. M., CHAUDHARI, D. T., PATEL (Mrs.), M. R. and SABNIS, S. S. : Synthesis and study of 2-substituted-4-[4 $\frac{1}{2}$ -Methoxy-3-(2-Hydroxy-5-halo-or 5-nitrobenzylideneamino) phenyl] thiazoles hydrochlorides for *In Vitro* antituberculosis activity-II.

Bulletin of Haffkine Institute, 1978, 6, 46-39.

RAO, (Kum.), S. M. and SHIRODKAR, M. V. N. : Rous sarcoma virus blockade *In Vivo* and *In Ovo* by Rabies-induced Plasma Factor.

Indian Journal of Medical Research, 1978, 68, 717-723.

SALVEKAR (Kum.), S., MUKERJI, S. and DAVE (Mrs.), J. K. : Anaerobic Bacteriology of Septic Wounds.

Bulletin of Haffkine Institute, 1978, 6, 19-24.

SONI, H. K., BAXI, A. J. and VAIDYA, A. B. : Effect of Drugs on Red Cell Metabolism *in vitro* studies.

Bulletin of Haffkine Institute, 1978, 6, 12-18.

SONURLIKAR (Mrs.), U. A. and BHIDE, M. B. : Antifilarial activity of some anthelmintic drugs used in clinical practise.

Bulletin of Haffkine Institute, 1978, 6, 25-30.

SULE, D. P., SHAH, M. H., GHOOI (Mrs.), S. and BHIDE, M. B. : Synthesis and anthelmintic activity of 2-(N⁴ substituted-N¹ piperazinyl) Methyl-5 (or 6)-substituted benzimidazoles.

Bulletin of Haffkine Institute, 1978, 6, 62-64.

