

Annual Report of the Haffkine Institute

1990-91



**HAFFKINE INSTITUTE
PAREL, BOMBAY 400 012
INDIA**

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CHAPTER I

INTRODUCTION

In the year 1990 - 91 the public works department of the Government of Maharashtra started undertaking the maintenance and major repair work of the Institute and its laboratories. The recruitment of Scientific staff to fill the vacant posts and recruitment of fresh scientist are yet to be undertaken. The research activities continued as in the previous years. The following are the highlights of the various activities during the year under report.

Research :

The department of Bacteriology continued research on cloning of *V. Cholerae* surface antigen genes and studied R. plasmid mediated antibiotics resistance in *S. typhi*. They also studied the antibacterial activity of two medicinal plants viz. Aantomool and Pimpal.

The Biochemistry department continued studies on the biochemical characterisation of hypoglycemia causing factor purified from *Bordetella pertussis* and mechanism of action and the protective mechanism of *Bordetella Pertussis* extract against oxidative damage in animal. They also evaluated the protective effect of B. Pertussis against alloxan induced DNA strand breaks in mice pancreas.

In the department of Chemotherapy research in the field of drug against communicable diseases is being continued. Work is being pursued with biologically active heterocyclic compounds. Emphasis is being given on synthesis and testing of the new organic compounds for their antifungal, antibacterial, antimalarial, antitubercular and anthelmintic activities.

N- Substituted Acyl amino succinimides and succinimide were tested for its antifungal activity. Compounds in the 4- substituted benzoyl 2- chloroactamido thiazole series and 2- amino halogenated phenols condensed with adamantyl acid chloride (amido compound) were submitted for their anti T.B. and anti bacterial testing.

Amido derivatives of Quinoxaline were tested for anti malarial antibacterial and antifungal activities. The derivative showed anti malarial activity against *P. berghei* and antimicrobial activity against Salmonella and Staphylococcus organisms but were found inactive as antifungal agents.

Department of Immunology and Clinical pathology continued their investigation into the regulation of immune response to mycobacteria. Earlier it was found that help and suppression of immune response were mediated by distinct subsets of T. cells, the TH and TS. The investigation was further extended to study the mechanism of suppression. The possibility of suppressors competing for growth factors such as IL 2 was initiated. In another project, to study the regulation of T cell subset differentiation after ip and id immunization with *M. Vacecae*, investigation of triggered subsets recognising a single antigenic epitope was undertaken. The work is in progress.

The Phytochemistry unit continued screening of indigenous plants as vector control (mosquito larvicidal) agents. The Radiation biology and Human Pharmacology Unit was involved in the study of antimycotic and antibacterial activity of some herebal traditional remedies in superficial dermatomycosis and superficial dermal infections. The department of Toxicology are working on a *in vitro* system for screening and evaluating pesticide toxicity. The Virology department are studying the antihepatotoxic activity of *Phyllanthus niruri* plant extract and its active constituents in experimentally induced hepatitis B Virus infection in langur monkeys.

The Department of Zoonosis continued its screening programme of antimalarial, anthelmintic and insecticidal agents. Efforts are being made for developing *in vitro* techniques for screening authelminthic drugs. Research activities were also directed towards evaluation of DNA probe developed for diagnosing plasmodium vivax infection and assessment of sensitivity of *P. vivax* isolates to chloroquine.

Testing :

Various tests were conducted on products submitted by the Prohibition and Excise department of the Government of Maharashtra (Appendix VI) and on samples such as received from Commercial organisation and Institutes. In all 5922 samples of various types were tested during the year under report.

Patient oriented Services :

Clinical samples are accepted for investigations by the departments of biochemistry and clinical pathology. In all 4349 samples were investigated in the two departments. Various hospitals, charitable and private organisations from Bombay, suburbs and other places utilised their services. (Appendix II,111,111A).

Patients were immunised against Polio, Cholera, Typhoid, Diphtheria, Whooping cough and Rabies in the immunisation clinic. In all 713 patients attended the clinic and were immunised (Appendix VIII).

Training Programme :

The Institute offers various courses and training programmes for medical and paramedical students (Appendix X) A list of students registered for different degrees / courses during 1990- 91 is given in Appendix XI XII). Students who have successfully completed their thesis and obtained their degrees in given in Appendix XIII). Appendix XIV gives the list of recognised University teachers at the Institute.

Research Sponsoring Bodies.

The Maharashtra Council on Science and Technology, Education and Employment Department, Government of Maharashtra, Bombay, Department of Atomic Energy, Govt. of India; The Departments of Science and Technology, Government of India, The Indian Council of Medical Research financed some of the research projects. The Institute is thankful and gratefully acknowledges their financial support.

Administrative Bodies :

The Governing Council Presided over by the Hon'ble Ministry for public Health, Government of Maharashtra, met twice during the year under report. Apart from this the executive committee (Administration) and The Teachers Council met several times to provide guidelines for the smooth functioning of the Institute.

CHAPTER II

GENERAL DEPARTMENTAL ACTIVITIES

Bacteriology :

Two Students were registered for M.Sc. (Part II) in Microbiology by papers. One student secured first class and one secured second class. Five students were registered for M.Sc. (Part I) and all passed.

Testing activities were also carried out as part of the general activity by the department of Bacteriology. Bacteriological analysis of various products were undertaken. Water samples were analysed and water filters were tested. Lypholization of cultures were also undertaken (Appendix I).

Biochemistry :

The regular training courses in M.Sc. Biochemistry (by papers) part I and II, DMLT and short term courses in biochemistry were conducted in the departments of Biochemistry. The department also conducted lectures on special topics for M.Sc. (Part I) students of the Bombay University during the second term of the year.

Clinical investigation were carried out of samples referred by different city hospitals, private practitioners and medical officers of the institute. The total number of samples tested are shown in Appendix II.

Chemotherapy :

The Chemotherapy department in their routine activities sixteen compounds received from St. Xavier's college Bombay were tested for antitubercualr activity against H 37 Rv strain of M. Tuberculosis. None of them showed any antitubercula activity. One student was registered for Ph. D. under the head of the department Dr. Khadse. One student received the M. Pharm degree of the University of Bombay.

Clinical Pathology, Immunology :

The Clinical Pathology department received specimens from different hospitals and agencies (Appendix II) for investigation. Investigation were in the general are of cytology of the various body fluids, routine haematological examination, diagnostic serology, bacteriology, mycology and histopathological examination of biopsies (Appendix IIIA)

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

Human Pharmacology,, Bioavailability and Radiation Biology Unit

The Human Pharmacology and bioavailability Unit during the period under report undertook routine testing of all incoming samples for blood absorption studies in human volunteers. The list of Pharmaceuticals whose samples were accepted for the studies is given in Appendix IV.

Toxicology :

The department of Toxicology undertook toxicological evaluation of different types of chemicals and cosmetics . (Appendix-VII) . The samples were referred by various industries.

Virology :

General Activities in the department of virology included the immunisation services. In all 713 patients attended the clinic. Out of the total 295 patients received PCBC, 127 received ARV semple vaccine. The other vaccines administered included polio, triple, tetanus, dual typhoid MMR and cholera. The vaccines administered and the number of patients vaccinated are given in Appendix VIII. Herpes virus iselation studies *in Vitro* were carried out for eight patients.

The Department conducted the annual virology course for M.Sc., Ph.D. M.Phil and M.D. (Microbiology) students of the Uiversity of Bombay. In all twelve lectures and practicals were conducted. Two Ph.D., two M.Sc. and one M. Phil (Micrebiology) students are registered with the Head of the Department.

Zoonosis :

The Department of zoonosis continued their screening programme for screening antimalarial, anthelminatic and insecticidal agents. Maintenance of colonies of insects of public health and strains of parastites in experimental animals was continued. Venomous snakes and scerpions of the species *Buthus tamulus* and *Palannaes gravimanuus* (*Hoteremetrus indus*) were maintained at the venomous animal unit of the department. In all 75.42 g of venom was precoured and 194.36 g was supplied (Appendix IX).

The department received 492516 rats and shrews from different wards of Greater Bombay. Out of the redents received 72233 were examined for plague. None of the rats were found infected.

Academic Activities, Honours and Awards

Dr. B.G. Khadse head of the department of Chemotherapy along with his students attended the 42nd Indian Pharmaceutical congress held at Manipal from 27th to 30th December 1990 and presented two papers.

Dr. R.S. Kamat, Head of the department of clinical Pathology and Immunology participated as guest faculty in a continuation of Medical Education programme organised by Indian Immunology Society, at Lucknow in November, 1990. He also participated as guest faculty in the "Immunology Workshop" Organised by the Department of Pathology and Microbiology, Govt. Medical College, Nagpur, in Maarch 1991.

Dr. V.R. Rao, Head of the department of Toxicology attended as an expert the WHO meeting for revision of WHO guidelines for Drinking water Quality Inorganics I at Bilthoven, Netherland between 18-22 March, 1991. He delivered a lecture on Biological Evaluation of Plastic Medical Products at the UNDP/IAEA/RCA Regional Training course on Industrial Radiations Sterilization, Quality Control and Compatibility of Materials at B.A.R.C., Bombay from 17-28 September 1990. He also delivered a lecture on Preclinical Toxicity Requirements at the I.C.M.R. Training programme in Clinical pharmacology on 3rd December 1990.

Dr. R.A. Deshmukh, Medical officer in the department of Virology presented a paper titled "*In Vitro* testing of phyllanthus Niruri aqueous extract against viral DNA polymerase associated with hepatitis B antigen" at the XIV All India cell Biology Conference and cell Biology symposia ; held by Bhabha Atomic Research Centre, Bombay. She also attended the Fifth Annual review Meeting of programme officers of WHO/NICD viral Hepatitis Surveillance Centre at Madras, and presented the work done at the department.

Dr. D.M. Renapurkar, Head of the department of zoonosis visited the international centre of Insect Physiology and Ecology, Nairobi during 27-1-1991 to 16-3-1991. Dr. N.K. Sutar, Scientific Officer of the department of zoonosis participated in the VIII International Congress of Parasitology in paris and presented a paper on "Effect of tissue culture media on multiplication of plasmodium falciparum *in vitro*."

Mrs. M.V. Joshi, the Librarian, attended a course in MICRO CDS- ISIS at SHPT School of Library Science, SNDT, Bombay in June 1990.

CHAPTER III
RESEARCH PROGRESS
BACTERIOLOGY

The department of Bacteriology is currently pursuing the following research projects.

1) Cloning of *V. Cholerae* surface antigen genes in *S. typhi*. Ty 21a.

To obtain the desired antigenic clones and to study their immunogenicity, a small genomic library using pBR 329 has been constructed. Attempts are now being made to construct a genomic library using pHC 79 which will permit cloning of larger DNA fragments. Outer membrane protein (OMP) of *V. Cholerae* has been prepared and antibodies to OMP will be raised in rabbits. These antibodies will be used to identify desired antigenic clones.

2) Studies on R-plasmid mediated antibiotics resistance in *S. typhi*. To study the nature of the plasmid involved in multidrug resistant *S. typhi* strains from Bombay (including Dombivli strains) and some other cities in Maharashtra.

The plasmid coding for drug resistance has been isolated, visualized on agarose-gel. The stability of the plasmid in *S. typhi* has been studied. The transferability of this plasmid to *E. coli* is being studied. The plasmid will be characterized further through restriction mapping.

3) Studies on anti-bacterial activity of two medicinal plants viz. Anantmool (root) and pimpal (Bark).

In order to study it was decided to -

1) To prepare aqueous and organic extracts of these plant materials and test them for activity against gram positive and gram negative bacteria.

2) Isolate and identify the active principle.

The aqueous and organic extracts of these plant materials have been found to be active against *V Cholerae*, *S typhi*, *Str. faecalis*, *S. enteritidis* and *E. Coli* by agar diffusion technique.

On confirmation of these results, the active constituents will be isolated and characterized.

BIOCHEMISTRY

Biochemical characterization of the hypoglycaemia causing factor purified from *Bordetella pertussis* and to study its mechanism of action.

The objective of the study are :

ā) Biochemical Characterization of the hypoglycaemia causing factors and study of its mechanism of action with respect to :

I) Reversal of diabetic symptoms in alloxan diabetic rats.

II) To study its insulin secretory response in normal, alloxan diabetic and diabetic rats treated with *B. pertussis* factor.

b) Comparison of the hypoglycaemia causing effect of the *B. pertussis* hypoglycaemia causing factor with other clinically established oral hypoglycaemic agents such as sulfonyl ureas.

Cultivation of bulk quantities of *Bordetella pertussis* (strain 18323) about 38 litres at a cell count of 2.5×10^9 cells/ml. has been carried out.

Enzyme extraction of the crude protein from *B. pertussis* and testing it for its blood sugar lowering activity in mice was undertaken; following observation were recorded.

I) *B. pertussis* extract at a dose of 2mg/kg. b.w. given i.p. in rats showed max. blood sugar lowering at the end of 18 hours and the value of 64.16 mg% is 42.41% lower as compared to saline treated controls.

(II) Sub- cutaneous injection of *B. pertussis* crude extract at a dose of 3 mg/kg. b.w. given 37% blood sugar lowering at the end of 12 hours in rats.

III) Alloxan at a dose of 100 mg/kg. b.w. given s.c. in rats causes severe hyperglycaemia between 48 and 54 hours as compared to saline treated controls.

Work done on purification :

I) Sephacryl S - 200 gel filtration was carried out and two peaks recovered. Peak I has 37.61% blood sugar lowering activity, but peak II was not active. Both peaks are heterogeneous.

II) Density gradient ultracentrifugation using both sucrose (40%) and Cesium Chloride (CS CL) are being attempted. The former procedure showed improved purity, but recovery was less. The second procedure is in progress.

It is now proposed to undertake comparative studies on the hypoglycaemic factor from *B. pertussis* with other known oral hypoglycaemic agents such as sulfonyl ureas.

2. Study of protective mechanism of *B. pertussis* extract against oxidative damage in animal tissue.

Molecular oxygen at high concentrations, undergoes a series of 1-electron reduction to form highly reactive free radicals such as superoxide anion (O_2^-), hydroxyl anion (OH), besides hydrogen peroxide (H_2O_2). These accumulate in the cell leading to degenerative diseases.

Alloxan, by mediating stepwise redox reactions, leads to production of O_2^- and OH radicals. The hydroxyl radicals cause DNA strand breaks in the B cells of the pancreatic islets of Langerhans leading to the inhibition of proinsulin synthesis. This leads to diabetes mellitus.

An extract of *Bordetella pertussis* (strain 18323) has also been shown tentatively to have an antioxidant effect.

In view of these points, the objectives of this project have been defined as follows :

Detailed investigation of the mechanism of protective effect of *B. pertussis* derived factor against damage. This involves measurement of

- a) Levels of antioxidant enzymes such as
 - i) Superoxide dismutase (SOD)
 - ii) Catalase (CAT)
 - iii) Glutathione peroxidase (G Px)
- b) Level of an antioxidant metabolite, namely glutathione in its

reduced form (G.SH).

c) Extent of lipid peroxidation in the cell.

All the above parameters are assayed in mice pancreatic homogenates in Control, alloxan injected *B. pertussis* treated lots, and in groups injected with alloxan and groups treated with the extract.

3. Evaluation of protective effect of *B. pertussis* factor against alloxan induced DNA strand breaks in mice pancreas.

The extent of hyperglycemia induced by alloxan at various doses i.e. 30,40,50 and 75 mg/kg body wt (IV) was determined. The former two doses did not induce significant hyperglycemia, while a dose of 75 mg/kg was lethal to the mice. So alloxan at 50 mg/kg was selected as the sublethal diabetogenic dose.

Time rate response of alloxan was also studied. The hyperglycemia peaked at the end of 48 hours after i.v. alloxan administration. Hypoglycemic effect of freshly prepared B.P. extract (1mg/kg.b.w.) was also tested. There was 57.27% and 68.12% lowering in blood sugar as compared to control values (101.26 + 2.83 mg/dl) at the end of 6 and 12 hours respectively. *B. P. extract* was also found to significantly reverse the alloxan diabetes. The 48 hr. peak hyperglycemia (526.13 + 4.23 mg/dl) was lowered to normoglycemic levels by initial protection of the mice with *B. P. extract* 1hr. before injecting alloxan.

Similarly, the increase in lipid peroxidation values (index of peroxidative damage to the cell) caused by alloxan was preventive by *B. P. extract*.

The levels of catalase in mice pancreas were also found to be significantly suppressed by alloxan, but B.P. extract protected against this effect by elevating the catalase levels to normal levels.

CHEMOTHERAPY

Antifungal Agents

N- substituted aryl amino succinimides and succinimide

In continuation of synthetic work in this series with the reported high order of *in vitro* antifungal activity of succinimide with respect to miconazole, two compound have shown antifungal activity equal

to the standard drug miconazole under identical conditions of *in vitro* testing.

Antitubercular and Antibacterial Agents

4- Substituted benzoyl - 2 Chloro - 4 substituted benzoyl thiazoles

It is reported that 2 - Chloro - 4 substituted benzoyl thiazoles exhibited activity against INH resistance strain of *M. tuberculosis* bacilli, hence it was proposed to synthesise the above compounds and test against h 37 Rv strain of *M. tuberculosis*.

Twenty new compounds have been synthesised in the 4 substituted benzoyl 2 chloro acetamideo thiazole series and have been submitted for their anti T.B. and anti bacterial testing. Some of them showed good anti T.B. activity up to 0.2 ug/ml to 1 mg/ml.

The compounds namely 2- (4-morpholino -Glycylamino-) 4- chloro benzoyl thiazole showed antitubercular activity at 0.2 ug/ml and the other 2-(4'- ,Methyl - 1' piperazino) Glycylamino - 4 (p - chloro) - benzoyl/thiazole, 2 - (1 - piperzino) Glycylamino - 4 - (p-chloro) benzoyl thiazole, showed at 1.0 ug/ml

2-Amino halogenated phenols were prepared and condensed with adamantyl acid chloride to obtain the corresponding amido compounds for testing their antibacterial activity and also antitubercular activity.

Antimalarial Agents

Quinoxaline derivatives

6, 7 disubstituted 2-amino quinoxaline were condensed with hydroxy / methoxy benzoin acid chloride to obtain the corresponding amido derivative. They were tested by Rane's method for antimalarial activity. 6- acety 1 - 2 hydroxy quinoxaline and 2 - chloro 6 - propoxy quinoxaline of the same series showed anti-malarial activity against *P. berghei* in mice at 180 mg/kg.

Some compounds of this series were tested for antibacterial activity against *Salmonells typhi* and *Staphylococcus Aureus*. They were also subjected for antifungal screening. However, they were found inactive.

CLINICAL PATHOLOGY

IMMUNOLOGY

1. Study of regulation of immune response to mycobacteria

Earlier it was shown that help and suppression of immune response were mediated by distinct subsets of T. cells, the TH. and TS. The former recognised the antigen presented by IA whereas the latter recognised antigen presented by IE.

The investigation of T cell mediated suppression was extended to the study of mechanism of suppression. Investigation of the possibility of suppressors competing for growth factors such as IL 2 was initiated. Rat spleen cells were cultured with concanavlin A and IL 2- rich culture supernatant (Con A sup) was harvested. The IL - 2 content of the Con A sup was assayed by its ability to stimulate *in vitro* proliferation of a murine Lyt 2 + cytotoxic T cell line (CTLL).

The IL 2 induced proliferation of mouse spleen cells, non primed and primed with *M vaccae* by ip or id routes. Addition of antigen caused no effect on non-primed cells, minimum enhancement in proliferation of id primed cells and profound suppression of proliferation of ip primed cells. This was considered as prima facie evidence in support of the hypothesis that suppressors functioned by competing for growth factors.

This work was supported by a grant from Dept. of Science and Technology, Govt. of India.

2. Study of T cell subset differentiation after ip and id immunization with *M. vaccae*.

A new project was under taken to study the subsets of T helper cells triggered after ip and id immunisation with *M vaccae*. For this purpose, the investigation of triggered subsets recognising a single antigenic epitope, was considered essential.

Since 65 KD antigen was earlier shown in the laboratory to be an immunodominant T cell antigen. it was decided to employ the same in our studies.

Since 65 KD antigen is associated with the cell wall/ membrane of the mycobacteria. the following protocol was employed for purification.

Log phase culture of *M. vaccae*, grown on protein free medium, was killed with gamma radiation. It was then disintegrated by sonication. The sonicate was treated with lysozyme and EDTA to disrupt protein from cell wall / membrane. This was followed by a limited proteolytic digestion with papain. The final preparation was subjected to centrifugation at 50,000 g and the supernatant collected and protein content assayed. The protein was subjected to electrofocussing on agarose, employing 3.5 - 5.0 pH range ampholine. The precipitin band of pI 4.3 was identified with a surface electrode.

In order to raise antibody against 65 KD, the 4.3 pI band was cut, it was ground with the gel in 0.5 ml Freund's incomplete adjuvant and a rabbit was immunised. Antibody of 1:32 titre was obtained. This was then employed to standardise an ELISA for mouse anti 65 KD antibody assay.

For study of DTH response, the 4.3 pI band was cut, eluted with PBS and the eluate was employed in the mouse foot pad DTH tests.

For study of in vitro lymphoproliferation the 4.3 pI band was blotted on to nitrocellulose membrane (NCM) by electrophoresis. The (NCM) was solubilised with DMSO and reprecipitated to get NCM particles with adsorbed 4.3 pI antigen. The particles were suspended in RPMI 1640 and the optimum dilution of the suspension capable of supporting lymphoproliferation determined.

This work received a Jr. Research Fellowship grant of University Grants Commission and was also supported by a grant from Dept. of Science and Technology, Govt. of India.

PHYTOCHEMISTRY

Investigation of indigenous plant as vector control (mosquito larvicidal) agents.

Evaluation of mosquito larvicidal activity of extracts of *Trigonella foenum graecum* linn and *Ocimum basilicum* linn seeds.

a) *Trigonella Foenum graecum* linn - seeds (Marathi- Methi)

Five hundred grams powder of *Trigonella foenum graecum* seeds were extracted with benzene and methanol in soxhlet apparatus.

These extracts were concentrated and residue was tested for mosquito larvicidal (*Culex P fatigans*) activity as per WHO method. Twenty five mosquito larvae of 3rd 4th stage were taken in 500ml. beaker containing 250 ml. water. Benzene and methanol extracts were tested at the concentration of 0.016, 0.032 and 0.04% and mortality of mosquito larvae was observed at the end of 24 hours. Solvent and water control were also kept simultaneously. Among these two extracts of methi seeds, benzene extract showed promising mosquito larvicidal activity of 100% 76% and 40% at the concentration 0.04, 0.32 and 0.016 percent respectively. Methanol extract did not show mosquito larvicidal activity. There was no mortality in the control.

b) **Ocimum basilicum** : Linn seeds (Marath- Subja)

Ocimum basilicum seeds powder was extracted with benzene and methanol in the soxhlet apparatus. Extract were made free from solvent and tested for mosquito larvicidal activity. Benzene extract showed 100% mosquito larvicidal activity at the concentration of 0.032 and 0.04% respectively, and 80% with 0.01% concentration at the end of 24 hours. Methanolic extract of **Ocimum basilicum** seeds did not show larvicidal activity at 0.04, 0.032 and 0.016 percent concentration.

2. Investigation of India plants *Annona squamosa* and *Pongamia pinnata* as vector control (Mosquito larvicidal) Agents.

From the earlier research work, it has been seen that *Annona Squamosa* and *Pongamia pinnata* seeds hexane extracts (0.1) have shown hepatotoxicity. The biochemical parameters revealing these findings were investigated in albino rats for one month. Therefore it was necessary to extend this study for further three months to confirm the toxicity.

The seed oil of **Annona Squamosa** with experimental dose of 0.25 ml./ kg/day was orally given to albino rats for three months. Ten rats were taken for experimental purpose keeping simultaneously, ten rats as control group. At the end of every week weights were recorded of both the groups.

There was no significant difference in the weights of control and experimental group of rats. The rats of the experimental group were as active as control group. At the end of three months blood was drawn from the heart under anaesthesia. Alkaline phosphatase,

serum glutamate pyruvate transaminase (SGPT) , serum glutamate oxaloacetate transaminase (SGPT) and lactate dehydrogenase (LDH) from serum were estimated using standard methods. The experimental data showed that there was no significant difference in biochemical parameters in experimental and control group of rats.

Pongamia Pinnate Seeds (Marathi - Karanj)

The seed oil of *pongamia pinnate* was given orally in the dose of 1.5 ml/kg/ day for three months. Body weight and physical behaviour were recorded during the experimental period. The weights of experimental group of rats were low compared to control group. In the observation of behaviour it was noted that experimental rats were less active as compared to control group.

At the end of three months blood was drawn and biochemical parameters such as serum glutamate transminase (SGPT), serum glutamate, Oxaloacetate transaminase (SGPT), lactate dehydrogenase (LDH) and alkaline phosphatase were estimated from serum. There was no noticeable change in serum levels of SGPT, SGOT LDH and alkaline phosphatase serum levels in experimental and control group of rats.

RADIATION BIOLOGY, HUMAN PHARAMACOLOGY AND BIOAVAILABILITY UNIT.

Study of antimycotic and antibacterial activity of some herbal traditional remedies in superficial dermatomycosis and superficial dermal infections.

Objectives :

Phase I : 1.) Study of inhibitory activity of extract of traditional herbs against clinically isolated dermal pathogens

Phase II : Studies in suitable animal model for confirmation of efficacy **in vivo**.

Phase III : Concurrent study of dermal toxicity and further exploitation in human cases.

Plants with possible antifungal activities were selected from

citation index. Different extracts, such as petroleum ether, chloroform, methanol and aqueous, were prepared.

In vitro methods to assess antifungal activity were standardised employing known antifungal agents, Miconazole, Tolnaftate etc. For qualitative study of antifungal activity ' **ditch Plate** ' method was used and broth tube serial dilution method was employed for quantitative study of antifungal activities.

So far two plant extracts have been studied for antifungal activity. Antifungal activity of lime juice has been studied to assess whether it can be employed as vehicle to act in conjugation with other plant extracts such as **Butea monosperma** (Palas seeds) and **Luffa acutangula** (Dodaki Leaves). Other plants referred in Indian Materia Medica ' by Nadkarni are to be studied for antifungal activities *in vitro* they will be included in the phase II study.

TOXICOLOGY

In vitro system for screening and evaluating pesticide Toxicity

Red blood cells have been used for studying the haemolytic effect by different drugs and chemicals.

The present study is being undertaken to determine the effects of various pesticides (Cholinesterase inhibitors) on red cell and determine the order of lethality (lysing) with various concentration . This would reflect on the degree of toxicity and will be compared with existing acute animal toxicity (LD50). This method if proved sensitive and comparable to animal experimentation data with regard to LD50 will help in screening potentially toxic pesticides before elaborate animal experimentation are undertaken. The method could if found suitable also applied for residue analysis.

Preliminary work in screening the species variation and sensitivity was carried out with Malathion 50EC. Earlier it was reported that analysis could be done on plasma containing malathion. During the year under report analysis of spiked Mathion in blood and liver were undertaken and are in progress.

VIROLOGY

Antihepatotoxic activity of *Phyllanthus niruri* (PN) plant extracts and its active constituents in experimentally induced hepatitis B virus infection in the Langur monkey model (*presbytis entellus*).

The objects of the study is :

To verify the previous results obtained with this Langur model (*Presbytis entellus*) and HBV, and to assess the efficacy of extract ***phyllanthus niruri*** in experimentally induced Hepatitis B virus infection in Langur monkeys. Standardisation of the following assays was done.

I) SGOT

II) SGPT

III) LDH

IV) SOD

Estimation of viral polymerase.

VI) *In vitro* testing of crude aqueous ***phyllanthus niruri*** extract against viral polymerase and its kinetic study.

VII) *In vitro* testing of different ***phyllanthus niruri*** extracts, against viral DNA. Polymerase (viz. Petroleum ether, chloroform, ethyl acetate, methanol, water soluble in methanol, water insoluble in methanol).

VIII) HBs Ag positive serum samples were collected from 45 patients suffering from acute viral Hepatitis and were tested for 'e' antigen by ELISA method. Most of the 'e' antigen positive serum sample were found to contain replicative viral particles as judged by the presence of DNA polymerase activity associated with the isolated particles. The effect of aqueous extract of ***phyllanthus niruri*** was tested on the activity of virus associated DNA polymerase using viral DNA as the template primer and dNTPs as the substrate. Kinetic studies with various concentrates of the extract indicated that the mode of inhibition is non competitive with respect to the dNTP substrate.

Human HBc Ag and HBe Ag positive sera pool will be collected from acute viral hepatitis cases, and inoculated to each experimental monkey (1ml I.V.) Baseline studies for biochemical, histopathological and immunological parameters will be carried out. Presence of HBs

Ag and HBc Ag in the serum of infected monkeys will be tested by ELISA methods.

Presence of virus associated DNA polymerase will be estimated and action of *phyllanthus niruri* extracts (if any) on the out come of disease process will be studied.

ZOONOSIS

Malaria

Construction of A specific DNA probe for the diagnosis of Malaria

Construction of *P. Vivax* genomic library was undertaken by digesting the DNA with Sau 3A cloning into the Bam H. I. site of PBR 322 before transformation of *E coli* H. B. 101. Transformed colonies were identified by sensitivity to tetracycline and were then screened by hybridisation of duplicate nitrocellulose filters using radiolabelled *P. Vivax* DNA. Colonies that hybridised strongly with *P. Vivax* DNA were selected. Out of 8 repetitive clones obtained on the basis of sensitivity of hybridisation, one clone PHI 3 which could detect about 30 pg. of purified *P. vivax* DNA or approximately 300 parasites in a dot blot hybridisation assay and showed species specificity was selected. This clone, PH134 did not reveal hybridisation with either *P. falciparum* or *P. berghei* DNA in a southern blot assay and was thus found to be species specific. When the ability of this clone to detect parasites in the blood was assessed, it was observed to detect about 80 parasites per ul of blood using a 50 ul sample (ie a' total of 4000 parasites corresponding to a parasitaemia of 0.0016%). Therefore, the probe PH13 was used to detect *P. Vivax* malaria cases by random sampling assay and its sensitivity was compared to light microscopy. Evaluation of this probe is being continued.

Screening of antimalarial agents

In Vivo Screening of Antimalarial compounds and indigenous products was continued. *Plasmodium berghei* mouse model was employed for this study. Samples of drugs and indigenous products were obtained through the Department of chemical Technology and Indian Institute of Technology, pawai. It was possible to investigate over 40 products this year.

Influence of some growth promoting substances on multiplication of plasmodium falciparum in vitro.

A number of growth promoting substances used in plant tissue culture were investigated for their effect on cultures of malaria parasites. In all, seven such growth promoting substances were employed. These included 3 - Indoleacetic acid (IAA), 3 - indolophenoxy acetic acid (IBA), Gibberellic acid (GA), 2 - 4 dichloro phenoxy acetic acid, Naphtylacetic acid meso inositol and 4 - nitrophenyl phosphate. Out of these only 3 Substances, namely indoleacetic acid, indolebutyric acid and gibberellic acid improved *P. falciparum* growth in vitro.

Filariasis.

In addition to the study of using highly repetitive DNA sequence of *S. cervi* as a probe, studies were also carried out with radiolabelled whole DNA from *S. cervi*, adult worms. The adult worms irrespective of sex were washed thoroughly in PBS, pH, 7.2, 0.01M. The DNA was extracted. The probe was then prepared using radiolabelled *S. cervi* adult worms with ^{32}P (d ATP) by Nick translation to a specific activity of $2-3 \times 10^8$ cpm/ug of DNA. Hybridization assay was then carried out with 10ul samples of DNA of other filarial nematodes viz. *Brugia malayi*, *B. pahangi*, *A. canthocheilonema vittae*, *Litomosoides Carini*, *Setaria cervi* spotted previously on nitrocellulose sheets.

These sheets were then exposed to autoradiography and hybridisation signals were recorded. The DNA samples showing positive reaction ranged from 1 mg/ml to 0.1 ng/ml irrespective of the genus of the filarial nematode. No signals were obtained with leucocyte DNA.

Angiostrongyliasis

In vitro maintenance of Angiostrongylus cantonensis for chemotherapeutic studies.

After establishing the life cycle of the parasite *A. cantonensis* in the experimental albino rats, methods for cryopreservation were developed. 60 % viability of L_3 larvae of *A. cantonensis* was obtained by cryopreservation in DMSO. Viability of cryopreserved L_3 was confirmed by infecting albino rats with cryopreserved larvae.

Methods were standardised to purify L_1 and L_3 stage using

percoll gradient centrifugation. These purified larvae were utilised for *in vitro* maintenance of larval stages. L₁ and L₃ were maintained for 48 and 33 days respectively and their growth was studied. *A contonensis* adult females were maintained *in vitro* and development of eggs to L₁ stage larvae was studied.

Effect of mercury and cadmium on cell mediated immune response of mice in relation to an infections agent, *Hymenolepis nana*.

The objective of this study is to understand the mechanism by which mercury, an environmental pollutant (in subclinical and high dose) effects the immune capability of an experimental animal (mice), specially mediators and components of the cell mediated immune response in experimental *Hymenolepis nana* infection.

Experiments were designed to study the effect of mercury on *Hymenolepis nana* infectivity, retention of parasitic burden and cell mediated immune response.

All experimental recipients showed significantly greater worm burdan on all the observation days as compared to their counterpart controls. Worms persisted for a much longer period of upto 120 days as compared to control where they persisted only upto 60 days. Cell mediated immune response as assessed by migration inhibition test, rosette formation and foot pad swelling was significantly suppressed. Immune suppression of CMI was maximum on day 20, and was found to gradually taper off by day 120.

Results obtained from this study clearly indicate that mercury is a potential immunosuppressive agent, even a subclinical dose of mercury suppresses the immune response in the *H. nana* mouse model. It is also evident from the present study that mercury saverally affects the T- lymphocytes. Present study also shows that suppression of immune response is neither total nor permanent. Further work is in progress.

CHAPTER IV

GENERAL ACTIVITIES

Library :

The construction of the new Library Building was in progress. The Library has a collection of books on biomedical sciences and related fields. It collects and provides information to scientists and students in the field of Biochemistry, Communicable diseases, Immunology, Pharmacology, Zoonosis, Virology etc. of this Institute as well as other Institute in India.

Presently there are 8872 books in the Library. The number of books added during the year were 226. The Total number of journals subscribed were 141. The total number of volumes bound were 589 during the year.

Facilities :

1. The Library provides reprographic facilities to its staff and outside readers. The Library received 2335 requisitions for zerox.
2. **Inter Library loan :** The Library borrowed 221 journals from neighbouring Libraries for its staff and loaned 234 journals to other Libraries in the city.
3. **Visitors :** Around 4410 visitors utilised the Library facilities for their reference work.

APPENDIX - I
BACTERIOLOGY

Test	Number
Water samples	71
Disinfectants, Biocides	19
Cultures supplied	102
Filters	21
Resin Sample	1
Water purifying (ultraviolet unit)	2
Beverages	26
Air ioniser	1
Water storage tanks	2
Biological indicators (spore suspension or strips)	6
Polymeric rubber compounds for resistance to bacteria and fungi	2
Total	257

APPENDIX - II
BIOCHEMISTRY

Name of the body fluid	No. of Tests
Blood	375
Urine	74
Special Tests	90
Total	539

APPENDIX - III
CLINICAL PATHOLOGY

**Hospital and Agencies making use of the services of Clinical
Pathology Department**

Bombay Area

Came and Albless Hospital

St. Garage's Hospital

Jagivanram Hospital

Central Railway Hospital

B.Y.L. Nair Charitable Hospital

K.E.M. Hospital

M.G.M. Hospital

L.T.M.G. Hospital

E.S.I.S. Hospital

K.B. Bhatia Hospitals

Kasturba Hospital for Infectious Diseases

J.J. Group of Hospital

Police Hospitals

Fertilizer Corporation of India

B.J. Wadia Hospital for Children

Greater Bombay Municipal Dispensaries.

From Maharashtra

Civil Hospital, Thane

Civil Hospital, Alibaug, Raigad

General Hospital, Sawantwadi

ISP Hospital, Nasik

Public Health Centre, Buldhana

Civil Hospital, Akola

District Hospital for Women, Akola

Primary Health Centre, Jalgaon

General Hospital, Osmanabad

Civil Hospital, Dhulia

Civil Hospital, Nasik

APPENDIX - III - A
CLINICAL PATHOLOGY

Tests	Number
A. Histopathology	911
B. Haematology	979
C. Cytology for malignant cells	38
D. Clinical pathology	
Urine for routine examination	223
Stool for routine examination	184
Semen examination	4
C.S.F. routine cell count	-
Immunological pregnancy Test	43
Biological (Rat Test) pregnancy Tests	1
E. Bacteriology	
Direct smear for Tubercle Bacilli	-
Culture for Tubercle Bacilli	40
Animal inoculation for Tubercle Bacilli	4
Smear and culture for C. diphtheriae	-
Culture of Theatre swabs for Anaerobic	145
Urine Culture	315
Pus Culture	120
Sputum Culture	59
Stool Culture	69
Stool Culture (Cholera)	-
Blood Culture	43
Antibiotic Sensitivity Tests	319
Aspirated fluid Culture	17
C.S.F. Culture	20

APPENDIX - III - A (contd.)
CLINICAL PATHOLOGY

Tests	Number
Conjunctivities	2
Throat Swab Culture	17
F. Serology	
Widal Tests	98
Weil Felix Tests	73
Brucellosis Tests	54
V.D.R.L. Tests	7
Paul Bunnel Tests	25
Total Tests	3810

APPENDIX IV
HUMAN PHARMACOLOGY AND BIOAVAILABILITY
BIOAVAILABILITY STUDIES CARRIED OUT

Name of the company	Product
1. Concept Pharm	Tetracyclin caps
2. Kopran chemicals	Cloxicillin Ampicillin
3. Ajantha Pharm (HBPCL)	Amoxycillin
4. Armour Biotech	Ampicillin injection
5. U.S.U.P.	Erythromycin Tabs.
6. Vilco Laboratories	Ibuprofen
7. Lupin Laboratories	Cephalexin
8. Kopran chemicals	Ampicillin
9. Armour chemicals	Ampicillin
10. Uniphem Medico	Ampicillin
11. Lupin labs	Norfloxacin

APPENDIX V**LIBRARY**

Books Added	Journal Subscribed for	No. of journal bound
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226

141

589

Xerox requests received	No. of inter library loan request received	No. of inter library loan request made
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2335

234

221

No. of visitors

4410

APPENDIX VI**TESTING UNIT**

Product	Number
1) Samples of Drugs	49
2) I.M.F.L	2768
3) Beer	399
4) Toddy	1750
5) Ganja	18
6) Charas	1
7) Samples of Denaturants O.D.S. , S.D.S. french polish etc.	648
Total	5633

APPENDIX VII**TOXICOLOGY**

Classification of Sample	No. Tested	Toxicology parameters
Cosmetics	3	Acute Oral toxicity in mice.
	3	Mucous membrane irritation in rabbits.
Chemicals	2	Acute oral toxicity in mice
	1	Acute oral toxicity in rabbits.
	1	Acute dermal toxicity in rabbits.
	3	LC50 studies in fish
	4	Primary skin irritation in rabbits.
	1	Mucous membrane irritation in rabbits
	1	Toxicity test as per painted container.

APPENDIX VIIA

TOXICOLOGY

1. Acute Toxicity Study

Sr. No.	Sample	Species	Route	Results
1.	Aquaset	mice	Oral	LD50 > 15.300 mg/kg
2.	Cutex Kajal Blacks	mice	Oral	LD50 > 10.000 mg/kg
3.	Cutex Kajal Blue	mice	oral	LD50 > 10.000 mg/kg
4.	Cutex Kajal Brown	mice	oral	LD50 > 10.000 mg/kg
5.	Stella (Rin) concentrated	mice	oral	LD50 = 3.700 mg/kg Lower limit = 3274 mg/kg Higher limit = 4181 mg/kg
6.	Magnafloc 31	rats	oral	LD50 = 7192 mg/kg Lower = 5448 ml/kg Higher limit = 9493 ml/kg
7.	Vikram PAC - AC/100	Rabbits	oral	LD50 = 70 ml/kg. Lower limit = 5.84 ml/kg Higher limit = 8.39 ml/kg
8.	Vikram PAC-AC/100	Rabbit	dermal	LD50 > 10ml/kg
9.	Indfloc - 230	Fish		LC50 = 2.75 ppm
10.	Navian jade green	Fish		LC50 = 70 ppm
11.	4.5 Dinitrochrysazine	Fish		LC50 = 70 ppm

2. Primary Skin Irritation study in Rabbits :

Sr. No.	Sample	Results
1.	Navian jade Green	No skin irritation
2.	4.5 Dinitro Chrysarine	mild skin irritation
3.	Vikram PAC AC/100	mild skin irritation
4.	Steela (Rin) concentrated	mild skin irritation

APPENDIX VIIA (contd.)**TOXICOLOGY**

3. Mucous membrane irritation study in rabbits :

- | | |
|------------------------|-----------------|
| 1. Vikram PAC - AC/100 | No irritation |
| 2. Cutex Kajal Black | mild irritation |
| 3. Cutex Kajal Blue | mild irritation |
| 4. Cutex Kajal Brown | mild irritation |

4. Toxicity test as per painted containers :

1. Uniplas Plastics

Water was stored in these plastic containers for 7 days 14 days and 28 days and given ad lib to the mice. No Toxicity was observed

APPENDIX VIII**VIROLOGY**

Vaccines administered	Number of patients
PCEC	295
ARV Semple	127
Polio	118
Triple	83
Tetanus toxoid	60
Dual	5
Typhoid	10
Cholera	14
Total	713

APPENDIX IX
ZOONOSIS

Snake	No. procured	Venom produced (g)	Venum Supplied (g)
Cobra	121	40.87	136.16
R. viper	68	19.90	46.94
Krait	262	7.97	11.18
Echis	2217	6.68	0.08
Total	2668	75.42	194.36

Scorpions

1) Red	11270	0.37	0.37
2) Black	2	-	-
Total	11272	0.37	0.37

APPENDIX IX A**ZOONOSIS****NO OF VISITORS TO SNAKE FARM**

1) Students	2118
2) Staff of Scientific Institutions	1222
3) Distinguished visitors	-
4) Foreign Guests	10
5) Thursday visitors	113
6) Others	55
Total	3518

There were **16** enquiries regarding identification of snakes from Hospitals and individuals during the year **1990-91**.

APPENDIX X**POST GRADUATE COURSES OFFERED**

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

APPENDIX - XI
TRAINEES ADMITTED

M.Sc. by papers	10
Diploma in Medical Laboratory Technology (DMLT)	20

Research Fellowship and Scholarship

Post Doctoral Fellowship	3
Jr. Research Fellowship	11
Jr. Research Scholarship	15

APPENDIX - XII

LIST OF STUDENTS WHO REGISTERED DURING 1990-91

for M.Sc. Ph.D. M. Pharm, M.phill and M.D.

M.Sc.	Ph.D.
Smt. Brinda Krishnan	Smt. Viraga Haridas
Smt Reena Nanaiah	Shri. V. R. Lokhande
	Smt. Alpana Khirwadkar
	Smt. Smita Deshpande

M. Pharm	M. Phil
Smt. Vaidehi Deshpande	None

M.D.

Dr. R. Goyal

Dr. Tosha Usgaonkar

APPENDIX - XIII
DEGREE AWARDED DURING THE YEAR

Name of Candidate	Subject	Topic
Ph.D.		
1. Shri R.R. Hemkar	Appl. Biology	Studies on endo parasites of Commensal rats and evaluation of new rodent control agents.
 M.Sc.		
1 Smt. Jayashree Subramaniaum	Biochemistry	Biochemical Studies on Pongamia Pinnata Linn seeds, a potential mosquito larvicidal agents.
2. Smt. Manisha Pradhan	Appl. Biol.	Efficary of Polyvalent antivenin following injection of cobra venom with special reference to the histo pathological changes in different tissues of mice.
 M. Pharm		
1. Smt. Shraddha Nalavde	Pharm Chemistry	Synthesis and study of some thiazole derivatives as possible antitubercular agents.

APPENDIX - XIV
POST GRADUATE TEACHER

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

Name	Qualifications
Dr. S.R. Sengupta	M.D.
Dr. R.S. Kamat	M.D.
Dr. K.H. Dave	M.D., D.P.B.
Dr. D.M. Renapurkar	M.Sc., Ph.D., FMAS
DR. V.R. Rao	M.V.Sc., Ph.D.
Dr. P. Ramakrishnan	M.Sc., Ph.D.
Dr. S.V. Gadre	M.Sc., Ph.D.
Dr. B.G. Khadse	M.Sc., Ph.D.
Dr. R.P. Bhamaria	M.Sc., Ph.D.
Dr. S.R. Chavan	M.Sc., Ph.D.
Dr. S.T. Nikam	M.Sc., Ph.D.
Dr. B.D. Sathe	M.Sc., Ph.D.
Dr. (Mrs.) A.C. Desai	M.Sc., Ph.D.
Dr. (Mrs.) V.R. Kamath	M.Sc., Ph.D.
Dr. N.G. Chanderkar	M.B.B.S., B.Sc. (Med) path. & Bact., M.D., Ph.D.

APPENDIX - XV

PAPERS PRESENTED AT NATIONAL AND INTERNATIONAL COONFERENCES

1. The 42ND INDIAN PHARMACEUTICAL CONGRESS HELD AT MANIPAL 27th To 30th DECEMBER 1990

"Synthesis and study of 2 amino - 4- (Substituted) Benzoyl thiazole derivatives as possible chemotherapeutic agents". A.K. Manian, B.G. Khadse, P.A. Kirtikar and S.R. Sengupta.

"Synthesis and study biological activities of 6-chloro 5 (Biphenyloxy / quinoloxyl / naphtholoxyl - 2 - substituted (Benzoyl - thio hetero - acyl / thio) benzimidazoles". V. R. Lokhande, B. G. Khadse and S. R. Sengupta.

2. XIV ALL INDIA CELL BIOLOGY CONFERENCE AND CELL BIOLOGY SYMPOSIA AT BHABHA ATOMIC RESEARCH CENTRE, BOMBAY.

" *In vitro* testing of *phyllanthus Niruri* aqueous extract against viral DNA polymerase associated with hepatitis B antigen " S.R. Sengupta, R.A. Deshmukh, N.M. Betawar and S.A. Hassarajani.

3. VIII INTERNATIONAL CONGRESS OF PARASITOLOGY AT PARIS.

" Effect of tissue culture media on multiplication of *plasmodium falciparum in vitro*". N.K. Sutar, S.R. Sengupta and D.M. Renapurkar.

APPENDIX XVI

PAPERS PUBLISHED 1990-91

R. S. Kamat. Role of antigen presenting cells in the immunogenicity of mycobacteria. Trop. Med. and parasitology 41 : 225, 1990.

N.K. Sutar and D. M. Renapurkar. Influence of some growth promoting substances on multiplication of *Plasmodium falciparum in vitro* Ind. J. Malarior : 27 : 217 - 222, 1990

APPENDIX - XVI (contd.)
PAPERS PUBLISHED 1990-91

G.D. Nadkarni, V. R. Deshpande, V.R. Kamath and D.M. Renapurkar Plasma orosomucoid metabolism and susceptibility of malarial infection in rodents. Ind. J. Experimental Biol : 28 : 1078 - 1081 , 1990

V. R. Pradhan, N. K. Sutar and D. M. Renapurkar. Factors affecting the growth of *plasmodium vivax in vitro* : effect of animal serum. Rivista Ji parasitol 7 : 195 - 205, 1990

D.M. Renapurkar, T.G. Tare, N.K. Sutar and P.B. Deshmukh Observations on snake repellent activity of some plant extracts. Defence Science Journal 41 : 79-85 1991

A.J. Almeida, K.P. Deobhankar, M.K. Bhopale, V. Zaman and D. M. Renapurkar. Scanning electron microscopy of *Setaria cervi* (Rudolphi, 1967) adult male worms. Int. J. Parasitol 21 : 119 - 121, 1991

K.P. Deobhankar, A.J. Almeida, S.R. Sengupta and D.M. Renapurkar A specific DNA probe for diagnosis of *plasmodium vivax* malaria Med. Sci. Res. 18 : 689 - 690, 1990

A.J. Almeida, M.K. Bhopale and D.M. Renapurkar Evaluation of *Setaria cervi* antigen in the diagnosis of human filariasis. Folia Parasitologica 37 : 323-329.

N.K. Sutar and D. M. Renapurkar Effect of liver extract on growth of *plasmodium vivax in vitro* Ind. J. of Experimental Biology 29 286 - 287, 1991

APPENDIX - XVII**PAPERS SENT FOR PUBLICATION 1990-91**

K.E.Shroff, K.B. Sainis, S.R. Sengupta and R.S. Kamat, Major Histocompatibility Complex Restriction of T cell suppression of immune response to mycobacteria. *Int. J. Leprosy*.

T.G.Tare and D.M. Renapurkar, Studies on the yield of Echis Carinatus venom. *The Snake*.

T.G. Tare and D.M. Renapurkar Observation on cannibalism in cobra Naja naja in captivity. *The Snake*

T.G. Tare, N.E. Vad and D.M. Renapurkar, Circadian variation in venom yield by the Scorpion Heterometrus indus De Geer

Medical and Veterinary Entomology.

T.G. Tare, N.E. Vad and D.M. Renapurkar, Observation on ecology of the scorpion Heterometrus indus De Geer *Medical and Veterinary Entomology*

K.P. Deobhankar, A.J. Almeida, S.R. Sengupta and D.M. Renapurkar, A Specific DNA probe for diagnosis of plasmodium vivax *Malaria Med. Sci. Res.*
