

**Annual
Report of the
Haffkine Institute
1987-88**



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RESEARCH AND TESTING**

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CHEMOTHERAPY

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TESTING UNIT

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Medical Officer :

RANJANA A. DESHMUKH, M.B.B.S., M.D.

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B.D. SATHE, M.Sc., Ph.D.

V.R. KAMAT, M.Sc., Ph.D.

N.K. SUTAR, M.Sc.

CHAPTER I

INTRODUCTION

The year 1987-88 continued to be plagued with financial crisis and lack of a full time Director of the Institute. Efforts to solve the financial problems of Haffkine Institute and to bring the Institute back to its original glory has been one of the priorities of the Government. Firstly a full time Director, Dr. S.R. Sengupta was appointed by the Government of Maharashtra to steer the Institute in its crisis. Then in order to solve the problems of the Institute high level committees were instituted by Central and State Governments. Under the Chairmanships of Dr. M.G. Dev and Dr. V.K. Iya, committees were constituted by the State Government, while a Committee was constituted under the Chairmanship of Mr. Lov Raj by the Central Government. The Committees will be looking into the working problems and suggest remedial measures. Both the committees will be submitting their reports along with their recommendations to the Government of Maharashtra.

In spite of unsolved financial crisis, the research activities continued mainly due to minor financial relief in the form of research grants from funding agencies such as DST, ICMR and commercial organisations. The following are the high lights of the various activities during the year under report.

Research :

The department of Bacteriology continued their research on transformation in **S. typhi** and on industrially important bacterial cultures and their phages.

The Biochemistry department were involved in studying the role of **B. pertussis** extract on levels of superoxide dismutase in pancreas of normal and diabetic animals. The effect of **B. pertussis** extract on the levels of glucose - 6- phosphate dehydrogenase in liver was also studied. Studies have shown that **B. pertussis** extract has a unique mechanism of action in that it not only increases glucose utilisation in the normal as well as the diabetic animal, but also appears to exert a protective effect in pancreas against free-radical induced oxidative damage by alloxan.

The Chemotherapy department continued their synthetic research programme of drugs against communicable diseases like tuberculosis, fungal diseases, malaria and helminthiasis. A number of benzimidazole

and substituted thiazole derivatives, sulfonamide derivatives, aromatic and heterocyclic diethyl - carbamoylamide derivatives were synthesized and tested for their activities. The department also undertook projects sponsored by pharmaceutical and chemical industries.

The Clinical pathology and Immunology departments continued their research on DTH eliciting antigens of **S. typhi** and regulation of immune response to mycobacteria. In their research on the study of DTH eliciting antigens of **S. typhi**, in their attempt to obtain in pure form, the DTH eliciting antigen, it was observed that the protein focussing at pl 7.1 - 7.2, could elicit DTH response in mice immunised with **S. typhi**. In their second study on regulation of immune response to mycobacteria, it was concluded that the non-responder state of mice to **M. vaccae** by ip route was due to poor presentation by peritoneal cells.

The Human pharmacology and Bioavailability Unit is subjecting their bioavailability data obtained over the years to analysis for obtaining baseline data of bioavailability pattern in normal healthy Indian population.

The phytochemistry Unit in their continued studies on indigenous plants found that the one hexane extract of the leaves of **Ixora coccinia** showed considerable mosquito larvicidal activity at 0.04 and 0.08% concentration; but extracts of chloroform or methanol does not show promising activity. The hexane extract of **Ixora coccinia** leaves using column chromatography showed that fraction II exhibited 100% mortality at 0.03% concentration where as fraction III at the concentration of 0.04% resulted in 100% mortality. The oil from the seeds of *A. Azadirachta Indica* Juss, was studied for mosquito larvicidal activity. Various concentrations of oil were studied and it was noted that 0.04% exhibited 100% mortality.

The department of Virology conducted preliminary studies to see the efficacy and immunogenicity of PCEC rabies vaccine in post exposure cases. Protective antibody response was observed in every patient and no side effects. The activities of the WHO/NICD surveillance of viral hepatitis continued with survey confined to selected areas of reported outbreaks as well as hospitalised cases and in blood donors.

The Zoonosis department continued their research activities on malaria, filariasis, schistosomiasis and angiostrongyliasis. Efforts were made for developing better medium for long term cultivation of

P. vivax and *P. falciparum*. A number of antibiotics were evaluated *in vitro* for their antiplasmodial activity. Synthetic compounds were also screened for antimalarial activity *in vivo*. Screening for anthelmintic activity was continued against *A. cantonensis* of synthetic compounds developed at the chemotherapy department.

Testing :

One of the important functions of the Institute is testing of various products submitted by the Prohibition and Excise department of the Government of Maharashtra and samples such as drugs, chemicals, biologicals, pesticides, biocides filters, water samples, plastics etc. In all 4509 samples of various types were tested during the year under report.

Patient oriented services :

The departments of clinical Pathology and Biochemistry accept clinical samples from patients for investigations. In all 3574 samples were investigated in the two departments. A number of hospitals, charitable and private organisations from Bombay and other places utilised their services (Appendix III).

In the immunisation clinic patients of different ages are immunised against Cholera, Typhoid, Diphtheria, Whooping cough, Rabies, Measles Tetanus against nominal charges. In all 1905 patients attended the Clinic and were immunised (Appendix X).

Training Programme :

The Institute offers various courses and training programmes for medical and paramedical personnel (Appendix XII). A list of students registered during 1987-88 for the different degrees / courses and students who have successfully completed their thesis and obtained their degrees are given in Appendix XIV, and Appendix XV. Appendix XVI gives the list of recognised University teachers at the Institute.

Sponsoring Research Bodies :

The department of Science and Technology, Indian Council of Medical Research, Central Council of Research in Ayurveda and Siddha System and Maharashtra Council on Science and Technology, Education and Employment Department, Government of Maharashtra, 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 Minister for Public Health, Government of Maharashtra met twice during the year under report. Apart from this the Executive committee (Administration) and the Teachers Council met several times to provide guidelines for the smooth functioning of the Institute.

CHAPTER II

GENERAL DEPARTMENTAL ACTIVITIES

Bacteriology :

Out of five students registered for M.Sc. (by papers) in Microbiology, four passed in first class and one in Second Class. One Research student, Mrs. Suchitra N. Dani (nee Ms. Suchitra Tatke) submitted her thesis on "Transformation in *S. typhi* Ty 21a" and was awarded M.Sc. degree in Microbiology by the University of Bombay.

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

Biochemistry :

Training courses in M.Sc. Biochemistry (by papers) Part I and II and D.M.L.T. were completed during the year under report. Lectures on special topics in advanced biochemistry were held in the Haffkine Institute during the year. The department carried out Clinical investigations of samples referred by various city hospitals private practitioners and Medical officers of the Institute. The total number of samples tested were 571 (Appendix II).

Chemotherapy :

Two students were awarded degrees from the University of Bombay under guidance of Dr. B.G. Khadse. Miss. Zarine Khetty obtained her M. Pharm. (Medicinal chemistry) and Mr. Shekher B. Bhirud was awarded Ph.D. in organic chemistry.

The department also assisted outside researchers from various universities and research organisations for *invitro* testing in the field of Tuberculosis, Antifungal and Antibacterial activities on payment basis. Around 100 compounds have been tested, fifty compounds belonging to outside research workers were also analysed for Carbon, Hydrogen and Nitrogen.

Clinical Pathology and Immunology :

The department of Clinical Pathology received specimens from different hospitals and agencies (Appendix III) for investigation. The services rendered were in general areas of cytology of the various body fluids, routine haematological examination, diagnostic serology, bacteriology, mycology and histopathological examination of biopsies (Appendix III-A).

The DMLT 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 Bio-availability Unit tested formulations referred by various pharmaceutical companies. The list of Pharmaceuticals whose samples were accepted for the studies are given in Appendix - IV.

Toxicology :

The department of Toxicology was actively engaged during the period under report in toxicological evaluation of different types of pesticides, drugs, chemicals, growth regulators, plastics (Appendix VII, VIII). These samples were referred by various industries. The department was also involved in teaching programmes of post graduate students.

Virology :

The routine activity of the Virology department also include the Immunisation Clinic as a public service. In all 1905 patients attended the clinic during the period for vaccination. The vaccines administered and the number of patients vaccinated are given in Appendix - X.

The Clinical Virology section performed the following tests by Elisa method. Rubella Igg-10, Rubella Igm-3, Cytomegalovirus antibody - 6 samples. Four urine samples were tested for Cytomegalovirus inclusion body cell.

Rabies virus in mice were isolated from post mortem tissues of four patients. Anti rabies antibody titration was done for 2 patients who received the semple type of antirabic vaccine and developed neurparalysis.

Zoonosis :

Screening of synthetic compounds for antimalarial activity *in vivo* was undertaken for evaluating synthetic products developed at the Chemotherapy department. Screening of anthelmintic preparations against *A. cantonensis* was continued. Facilities for cryopreservation of strains of parasites were established. Maintenance of colonies of insects of public health importance and strains of parasites were continued. The following strains of insects and parasites were maintained by the department : **Culex fatigans**, **Musca domestica**

nebulo, Periplaneta americana. Among the parasite strains that are available in the department are **P. berghei A. cantonensis. A ceylanicum** and **S. cervi**.

A large number of scorpions and snakes were purchased and maintained in the venomous animals unit for their venoms and research. The total quantity of venom produced and supplied to various organisations is shown in Appendix XI.

As a part of plague surveillance programme 619829 rodents collected by the Municipal Corporation of Greater Bombay were identified. Out of these rodents 83866 were examined for plague.

Large number of students from medical colleges, schools and other Institutions attended lecture cum demonstration programmes conducted by the department for educating the people about snakes and first aid in snake bite cases (XI-A). The department also continued to extend expert assistance to various Institutions, hospitals in matters relating to the identification of venomous animals.

Academic Activities, Honours and Awards :

Dr. B.G. Khadse, Head of the department of Chemotherapy attended, the Indian Pharmaceutical congress held at Calcutta from 26th to 28th December 1988. He also attended the 8th National Congress of parasitology sponsored by Organon Research Centre at Calcutta from 26th to 28th February 1988.

Dr. V.R. Rao, Head of the department of Toxicology delivered an invited lecture on Good Laboratory practices at the Work Shop on Inhalation toxicology, organised by Indian Institute of toxicology in Pune from 16th to 18th March 1987.

Dr. M.K. Bhopale, Sr. Scientific Officer, Class-II of the department of Zoonosis was awarded fellowship for training in advanced parasitology in the field of Chemotherapy of Parasitology at Hammamatsu University, Japan from July 1987 to Sept. 1987. Dr. B.D. Sathe and Dr. N.K. Sutar of the same department participated in the Second Symposium on vectors and vectorborne diseases in February 1988 at Trivendrum.

CHAPTER III

RESEARCH PROGRESS

BACTERIOLOGY

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

Various techniques used in the isolation and purification of plasmid DNA were standardised and the purified plasmid DNA was used to transform *S. typhi* Ty 21a.

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

Relationship between the phage 39 and the host *C. mediolanum* was studied with respect to phase resistance. Also some of the phage characteristics such as burst size were studied.

BIOCHEMISTRY

Biochemical studies on the mechanism of action of the hypoglycaemic factor from *Bordetella pertussis*.

Studies have shown that *B. pertussis* extract has an unique mechanism of action in that it not only increases glucose utilization in the normal as well as the diabetic animal, but also appears to exert a hitherto unknown protective effect in pancreas against free-radical induced oxidative damage by alloxan. The purified extracts were found to significantly elevate the levels of the primary free-radical scavenging enzyme superoxide dismutase (SOD) in pancreas of the normal animal, and also significantly restore the severely depleted SOD levels of the alloxan diabetic animal. Such effect has not been shown by any of the drugs and alcohols which are also reported to exhibit hypoglycaemic effect.

The effect of *B. pertussis* extract on the levels of glucose-6-phosphate dehydrogenase, the rate limiting enzyme of the pentose cycle was studied in liver. This is because this enzyme levels are influenced by a diversity of agents including diet and hormones.

In response to *B. pertussis* treatment, the levels of glucose-6-phosphate dehydrogenase showed a rise at the end of 24 h, with the maximum increase observed at the end of 72 h. This effect persisted for a period of 10 days after which the levels of the enzyme returned to the normal values. The fall in the blood glucose levels

corresponded with the increase in glucose-6-phosphate dehydrogenase levels. Further, the enzyme levels which were severely depleted in the alloxan diabetic, were found to be significantly restored if the animals are pretreated with **B. pertussis** preparation. The mechanism involved in this effect is not completely understood.

Hence further studies are being undertaken to throw light on the specific role of **B. pertussis** extract on enzyme synthesis in the liver.

CHEMOTHERAPY

The synthetic research programme mainly involved in the field of benzimidazole, substituted thiazole, succinamide derivatives and N, N-diethyl carbamoyl derivatives of aromatic as well as heterocyclic amines.

1) Tuberculosis : Benzimidazole and substituted Thiazole derivatives :

In continuation of synthetic programme as well as testing programme, about hundred new compounds have been synthesized and tested for their *in vitro* anti-tubercular activity. It was suggested that previously synthesized compounds which showed better potency should be exploited further.

2) Antifungal : Succinimide derivatives :

Some succinamide derivatives were observed to be much active than the marketed drugs like Miconazole, Clotrimazole and Tolnaftate against fungal infection. They were taken for *in vivo* study against **T. rubrum**, **T. mentagrophyte** and **Candida albicans**.

The testing is under progress.

3) Antimalarial Activity : Substituted Aromatic and Heterocyclic diethyl-carbamoylamido derivatives :

In this year about twenty-five new compounds have been synthesized from thiazole series bearing N, N-diethyl carbamoyl grouping for evaluating them as antimalarial agents against **P. berghei** in mice. These compounds have been characterized for C, H, N analysis and I.R. spectroscopic data. They will be taken up for antimalarial studies.

N, N-Diethyl carbamoyl amido derivatives of substituted amines which were found to be potent similar to Chloroquin Phosphate, and the activity of these compounds have been also confirmed through

W.H.O. report by Prof. W. Peter at London School of Tropical Medicine. These compounds are underway for further animal studies. This antimalarial programme is proposed to be continued in collaboration with M/s. Alkylamine Chemicals.

4) Anthelmintic Activity : Benzimidazole derivatives

Synthetic programme to develop anthelmintic drug, in collaboration with M/s. Organon Research Centre, Calcutta, is continued, and about 20 new compounds have been synthesized and sent to them for screening.

CLINICAL PATHOLOGY AND IMMUNOLOGY

1. Study of DTH eliciting antigens of *S. typhi*

Earlier it was shown that the DTH eliciting antigen in the sonicate of *S. typhi* focussed within the pl range of 6-8. For improvement in the focussing quality it was essential to subject the sonicate to papain digestion.

Papain digested sonicate of *S. typhi* was run on Sephadex G 200 column and the eluates were tested for their ability to generate DTH response in immunised mice. It was observed that the fractions eluting at the end of the void volume gave a positive DTH response. These were pooled and subjected to preparative electrofocussing on IEF grade Sephadex employing ampholine of pl range 6-8. The protein focussing at pl 7.1-7.2 could elicit DTH response in mice immunised with *S. typhi*.

2. Study of regulation of immune response to *Mycobacteria*

Earlier it was shown that *M. tuberculosis* was immunogenic to mice by both ip and id routes whereas *M. vaccae* was immunogenic by id route only. Further the non-immunogenicity by ip route could be corrected by treatment of mice with poly I : poly C, 3 h prior to immunisation. Based on this it was proposed that the non-immunogenicity of *M. vaccae* and the other rapid growers by ip route could be due to inability of peritoneal cells to present rapid grower antigens.

Employing *in vitro* lymphocyte transformation response, ability of peritoneal cells and irradiated spleen cells to present *M. vaccae* and *M. tuberculosis* sonicates were compared. It was observed that both peritoneal and spleen cells could present *M. tuberculosis* sonicate efficiently. In contrast, only spleen cells could present *M. vaccae* sonicate efficiently; *M. vaccae* induced proliferation of

primed T cells in presence of peritoneal cells as antigen presenters was very poor.

Thus based on these data it was concluded that the non-responder state of *M. vaccae* by ip route was due to poor presentation by peritoneal cells.

HUMAN PHARMACOLOGY AND BIOAVAILABILITY

Data for the last ten years involving 13 antibiotics in over 130 formulations, infections, capsules, tablets or syrup is being subjected computerized analysis. This would give the base line data of bioavailability pattern in normal healthy Indian population.

PHYTOCHEMISTRY

General Activities :

(1) Survey and collection of Indian plants from different parts of Maharashtra State and investigate them for mosquito larvicidal/insecticidal activity.

(2) Phytochemical and toxicological studies of active extracts/constituents.

Following plants were taken for mosquito larvicidal/insecticidal activity.

- (1) **Pithecellobium dulce**, Roxb Benth leaves (Chinch)
- (2) **Ixora Coccinia**, leaves (Banduk)
- (3) **Azadirachta Indica**, A juss. Seeds (Neem)

Ixora coccinia :

Leaves were powdered to uniform mesh and extracted in soxhlet apparatus with hexane, chloroform and methanol serially. All these extracts were dried on water bath and evaluated for mosquito larvicidal activity. Hexane extract showed 72% and 100% mortality at 0.04 and 0.08% concentration respectively, where as chloroform and methanol extracts at the above concentration did not show any promising activity. Therefore hexane extract was subjected to column chromatographic fractionation to locate the active principle.

Column Chromatography :

The Column was packed by using silica gel in benzene. The hexane extract of **Ixora coccinia**, leaves was allowed to adsorb on the top of the column in a narrow band. The elution was continued

with following solvent systems (1) benzene (2) benzene methanol (95:5) (3) benzene; methanol (90:10) (4) benzene; methanol (80:20) (5) benzene methanol (50:50) and (6) methanol, till there was no residue obtained at the end of each elution. Six fractions were collected and made free of solvent on water bath. They were then subjected to mosquito larvicidal activity following W.H.O. specification. It was observed that fraction II showed 100% mortality at 0.03% concentration, where as fraction III at the concentration of 0.04% resulted in 100% mortality. Other fractions did not show promising activity.

Azadorachta Indica, A Juss, (seeds)

The oil from the seeds was extracted using petroleum ether and kept at room temperature after complete removal of petroleum ether. Precipitate was settled at the bottom of flask and it was filtered out. The total oil was subjected to larvicidal activity. Various concentrations of oil were used and it was noted that the minimum concentration i.e. 0.04% exhibited 100% mortality. Precipitate was dried and tested for mosquito larvicidal activity. Different concentrations were tested and out of these 0.01% and 0.03% concentration showed 84% and 100% mortality of mosquito larvae respectively.

Further work of purification of active principle will be carried out.

TOXICOLOGY

Teratogenic studies on Indigenous pesticide formulations.

In continuation of last year's project, teratogenic potential of Dimethoate 30 EC, a selective insecticide was undertaken. Initially an acute toxicity was undertaken. The result obtained indicate that the acute toxicity of Dimethoate 30 EC is less than its pure technical grade material. Dimethoate 30 EC at 38.8 mg/kg dose was teratogenic. A high percentage of resorption and skelital anomalies were observed with 38.8 mg/kg dose.

In vitro system for screening and evaluating pesticide toxicity.

Many types of pesticides are being used all over the world. The demand for less toxic but potential pesticide has resulted in extensive research and development. Toxicity testing of newer developed products will involve animal experimentation which are elaborate, expensive and many times objected by anti - vivisectionists. Animal experimentation can not be enitely substituted if one desires to

extrapolate to human situations. However, one can look for tests that do not involve live animals specially, for preliminary screening of potential toxic pesticides. With this in view *in vitro* studies are in progress with red cells to determine the effects of various pesticides on them.

VIROLOGY

1) Efficacy of PCEC rabies vaccine and HIRG in post exposure protection against rabies.

The Semple type of vaccine which has many serious side effects and poor antigenicity, is being used in our country since last 50 years. The safe and more immunogenic vaccine like PCEC and Human diploid cell antirabies vaccine are being administered in our Immunization clinic since last 2 years. We wanted to do preliminary studies to see the efficacy and immunogenicity of PCEC vaccine in post exposure cases.

One ml of the purified chick embryo cell culture (PCEC) rabies vaccine was given intramuscularly in the deltoid muscle on day 0,3,7,14,30 and 90 as post exposure treatment to ten patients. Simultaneously with the vaccine, human rabies immune globulin was administered on day zero in the amount 20 IU/kg of body weight. The antibody titre for each patient was carried out on day 0,3,7,14,30,90 and 100 by suckling mouse brain inoculation test. The protective antibody response was observed in every patient without any side effects. The patients are in good health after several months.

Activities of WHO/NICD Viral Hepatitis Surveillance Centre :

During the current year, the study of Viral hepatitis was confined to selected areas of reported out breaks as well as to hospitalized cases from Kolhapur and Sangli. The routine survey of voluntary blood donors from Haffkine Bio-Pharmaceutical blood bank using RPHA and ELISA kits was also carried out.

The out breaks investigated were reported from Kolhapur, Sangli and Deolwadi-District Sindhudurg, in the months of Feb. 88 and Dec. 88 respectively.

The Deolwadi out break was found to be due to contaminated spring water causing infection by the feco/oral route. The causative agent appears to be ETNANB. The hospitalized cases from Kolhapur and Sangli out breaks were showing high prevalance (56% and 90% respectively) of HBsAg positively. However unless all the markers like

anti HBc-Igm, Anti HBc total and Anti HBs are studied it is very difficult to rule out an out break or high prevalence of blood borne transmitted Non A Non B hepatitis. In Kolhapur sporadic cases were also studied for the period of 8 months starting from February 88 to December 88.

An overall incidence of Viral hepatitis situation from all over Maharashtra for the period of 6 years i.e. starting from 1983 to 1988 is presented in table No. 1 (Appendix IX). The attack rate and death rate has been markedly reduced due to early detection of out-breaks and decontamination of water supply.

The viral hepatitis situation for rural and Urban areas for 1988 is shown in Table No. II (Appendix IX-A).

The figures mentioned here are compiled from the reports of the Directorate of Health Services of Maharashtra, for all the forty districts and Corporations in Maharashtra. Table No. III (Appendix IX-B) gives the summary of markers studied at the three locales mentioned earlier.

Table No. IV (Appendix IX-C) gives the figures of blood banks and their distribution in the State.

Table No. V (Appendix IX-D) gives the age and Sex distribution of hepatitis cases in the three out breaks.

Table No. VI (Appendix IX-E) shows the out breaks in Maharashtra for 1987.

Kolhapur and Sangli

The representative samples were collected from acute viral hepatitis hospitalized cases, in both these urban areas. As many as 57% & 90% of samples showed presence of HBsAg by ELISA. Only 17.7% and 4.5% of the samples showed presence of anti HAVIgm by ELISA. In Bombay's Kasturba Hospital it has been shown by one of our laboratories (Dr. R.S. Kamat's personal communication) that parenterally transmitted non A non B hepatitis is the major cause of ordinary and fulminant variety of acute Viral hepatitis and it is endemic in the urban areas.

It may be suggested that unless we study the previously mentioned markers it is very difficult to come to a definite diagnosis. The situation was brought under control by giving the directives to the general practitioners and medical staff in the Government and

municipal hospital to use disposable syringes and needles when possible and properly autoclaving the glass syringes and needles.

Deolwadi (District-Sindhudurg)

This is small 'basti' situated on the hill top. There are 60 Kachha houses. The total population is 377. Out of which 102 cases of jaundice were detected from November '88 to December '88.

The water supply to the village comes from a natural spring, which flows for nearly eight months in a year. There is a small nullha which runs across the village and is dry for most of the year except in the monsoon. This is also used as a dumping ground for night soil and garbages. The sex and age distribution of these cases is shown in table No.V (Appendix IX-D).

There were four pregnant women. The mortality in this out break was nil. The water supply was found to be highly contaminated. Measures for decontamination of water supply were undertaken and the out break was brought under control.

It seems that the situation of non A non B hepatitis in Rural and Urban Maharashtra is different. In rural areas it is due to mostly the enterically transmitted agent while in an Urban area its mode of transmission appears to be different from feco-oral route, may be parenterally or Sexually transmitted.

ZOONOSIS

Research on malaria, filariasis, schistosomiasis and angiostrongyliasis was continued.

Malaria :

Screening of synthetic derivatives and indigenous drugs for antimalarial activity in in-vitro and in-vivo systems.

Strains of *P. vivax*, *P. falciparum* and *P. berghei* have been successfully established by *in vitro* cultivation. A method for evaluating the drug sensitivity of plasmodia to chloroquine has been standardised and sensitivity of 30 isolates has been determined with this technique. Methods have also been developed to screen antimalarial drugs against *P. vivax* *in vitro*. So far the effect of antibiotics like erythromycin, tetracycline, ampicillin, streptomycin on *P. vivax* has been studied. The evaluation of antimalarial agents *in vivo* system is being pursued for the past 6 years. Over 45 compounds have been investigated during this year.

Filariasis :

Studies are in progress to maintain various stage of filarial nematodes of the species **S. cervi** and stages of human filarial nematodes under **in vitro** conditions for obtaining ES and other antigens. Methods are also being developed for using the **in vitro** maintenance technology for **in vitro** drug evaluation.

Ancylostomiasis :

Evaluation of Immunological responses in hamsters during **A. ceylanicum** infection was carried out by humoral and CMI parameters during 20 and 60 days post infection. **A ceylanicum** worm antigens (Ac ww Ag-som. and Ac ww Ag-Es.) were analysed and characterised. They were observed to show 9 bands and 2 bands, ranging around 205 Kd and 66 Kd mol. wt. These antigens were proteins in nature. They were heat stable and capable of inducing immunity, particularly Ac. WW Ag-ES antigen and Ac WW Ag-Som fraction 2. **A ceylanicum** UV-irradiated larval vaccine was prepared and 90% and 100% protectivity obtained when 15 minutes and 20 minutes UV-exposed larvae were used. The role of CMI was confirmed. Gamma-irradiated **A. ceylanicum** larvae (50 Kds) also conferred 97.79 protection. Hyper-immune serum raised in hamsters by using UV-irradiated larvae adult worm hemogenate with F C A adjuvant in rabbit gave protection ranging from 33.3% and 93.9%. Ig G fraction collected after ammonium sulphate treatment and D E A E ion-exchange chromatography conferred 97.6% protection. Chemotherapy and immunological response studies after oral administration of 6 standard drugs suggested that serumantibody demonstrated a sudden increase to high titre on the 10th post treatment day. The antibody titre then dropped attaining the level of untreated infected controls. Similarly PCA titre, ITH and DTH measurements were also observed to decline.

Chemotherapeutic study revealed that albendazole (50mg/kg) and ivermectin (0.0005 mg/kg) are very effective (100% efficacy) anthelmintic drugs against all the developing (1,3,14,15) and adult stages of the parasite.

Angiostrongyliasis :

Various intermediate hosts of **A. cantonensis** viz. slugs, snails, prawns, crabs and vertebrate host like rodents were surveyed. **Laevicaulus altae** (slug), **Macrochlamys indica** (snail), **Rattus norvegicus** (rat) were found infected with **A. cantonensis**. The

efficacy of the drugs flubendazole, levamisole and their combination against **A. cantonensis** in mice on the 15 post infection day was evaluated. Flubendazole was effective at 25 mg/kg, 50mg/kg, 100mg/kg 200mg/kg when given each day for 5 days. Levamisole at a dose rate of 200 mg/kg was completely effective in a single treatment. The combined therapy of levamisole (20mg/mg) and flubendazole (15mg/kg) also proved 100% effective. Both drugs given prior to experimental infection however showed no prophylactic activity.

CHAPTER VI

GENERAL ACTIVITIES

Library :

The library added about 118 books during the year 1987-88 out of which 40 books were received as gift and on exchange. The total collection of books in the library as on 31st March 1988 was 8596. The library subscribed to 141 journals during the year and 436 volumes were bound in the year.

The library remained open on all working days except Saturdays, Sunday and Public holidays.

Facilities :

(1) The library provided reprographic facilities to its staff and outside readers. The library received xerox requisition for approximately 16089 pages during the year.

(2) Inter library loan : The library borrowed 41 journals from other libraries in the city for its own staff and loaned 232 library bound journals/items to other libraries in the city.

(3) Visitors : About 5200 outsiders visited the library for reference, during the year.

APPENDIX - I

BACTERIOLOGY

Tests	Number
1. Biocides including disinfectants and antiseptics	37
2. Filter candles	41
3. Water samples	82
4. Supply and lyophilization of bacterial cultures	56
5. Polyethylene tanks	2
Total : 218	

APPENDIX - II

BIOCHEMISTRY

Name of the body fluid	No. of tests
1. Blood	330
2. Urine	85
3. Special tests	156
Total : 571	

APPENDIX - III

CLINICAL PATHOLOGY

Bombay Area	From Maharashtra
Cama and Albless Hospital	Civil Hospital, Thana.
St. George's Hospital	Civil Hospital, Alibag, Raigad
Jagjivanram Hospital	General Hospital, Sawantwadi
Central Railway Hospital	I.S.P. Hospital, Nasik
B.Y. L. Nair Charitable Hosp.	Public Health Centre, Buldhana
K.E.M. Hospital	Civil Hospital, Akola
M.G.M. Hospital	District Hospital for women, Akola
L.T.M.G. Hospital	Primary Health Centre, Jalgaon
E.S.I.S. Hospital	General Hospital, Osmanabad
K.B. Bhabha Hospital	Civil Hospital, Dhulia

APPENDIX III (Continued)

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

APPENDIX - III A
CLINICAL PATHOLOGY

Tests	Number
A. Histopathology	382
B. Haematology	1145
C. Cytology for malignant cells	18
D. Clinical Pathology	
Urine for routine examination	242
Stool for routine examination	190
Semen examination	6
C.S.F. routine cell count	-
Immuological (Rat Test) Pregnancy Tests	117
E. Bacteriology	
Direct smear for Tubercle Bacilli	-
Culture for Tubercle Bacilli	35
Animal inoculation for Tubercle Bacilli	25
Smear & Culture for C. diphtheria	5
Culture of Theatre swabs for Anaerobes	154
Urine culture	141
Pus culture	47
Sputum culture	19
Stool culture	16
Stool culture (cholera)	1
Blood culture	5
Antibiotic Sensitivity Tests	203
Aspirated fluid culture	13

APPENDIX - III A (Continued)

Tests	Number
C.S.F. culture	2
Conjunctivitis swab	5
Throat swab culture	2
F. Serology	
Widal tests	146
Weil Felix Tests	38
Brucellosis Tests	32
V.D.R.L. Tests	9
Paul Bunnei Tests	5
Total Tests	3003

APPENDIX - IV

HUMAN PHARMACOLOGY AND BIOAVAILABILITY

Name of the Company	Product
1) M/s Tata Pharm	Cap. Ampicillin - cross over
2) M/s Cadilla Lab.	Syr. Ampicillin - cross over
3) M/s Concept Pharma	Syr. Rifampicin - cross over
4) M/s SOL - Pharmaceuticals	Cap. Ampicillin - cross over
	Tab. Ampicillin - cross over
	Cap. Cephalexin - cross over
	Tab. Cephalexin - cross over
5) M/s Ranbaxy Laboratories	Cap. Doxycyclin - cross over

APPENDIX V

LIBRARY

The following figure indicate the working of the library during the year 1987-88

Number of outside readers visiting the library	Number of periodicals issued under inter library loan service	Books and periodicals obtained for our staff from other Libraries	Number of Books added during the year
5200	232	41	118
No. of volumes bound	Number of Current periodicals subscribed	Current periodicals received free of charge	
436	141	40	

APPENDIX - VI

TESTING

Indian Made Foreign Liquors	2397
Beer	499
Toddy	908
Ganja	68
Bhang	17
Charas	04
Country Liquors	95
Denaturants	65
French Polish	01
Caramel	03
Drugs	200

APPENDIX - VII

TOXICOLOGY

Sr. No.	Classification of sample	Number tested	Toxicity parameter
1.	Surgical Suture	1	Abnormal toxicity
2.	Chemicals	6	Acute Oral toxicity in mice
		2	Acute Oral toxicity in rats
		3	Acute dermal toxicity in rabbits.
		7	Primary Skin irritation test in rabbits.
		3	Mucous membrane irritation test in rabbits.
3.	Plastic tanks	1	Test as per painted containers
4.	Polyethylene drapes	1	Biological tests as per U.S.P.

APPENDIX VIII

TOXICOLOGY

Sr. No.	Sample	Results	
		LD 50 value mg/kg	Fiducial limits/Remarks
A.	Acute oral toxicity in Mice:		
1.	Ureflow	> 16,800	-
2.	Sugaret	6298.5	5120.7 - 7747
3.	Fatty acid - A	> 13,500	-
4.	Fatty acid - B	> 13,500	-
5.	Polycryl - 7249	17 ml/kg	13.93 - 20.74 ml
6.	Amino acetic acid (glycine)	6400	5952 - 6912
B.	Acute oral toxicity in Rats :		
1.	Odomos	> 10,000	-
2.	Plant growth regulator	> 20,000	-
C.	Acute Dermal toxicity in Rabbits :		
1.	Fatty acid A	> 13,500	-
2.	Fatty acid B	> 13,500	-
3.	Odomas	> 15,000	-
D.	Primary Skin irritation study in Rabbits :		
1.	PEG - 400	-	mild irritation
2.	Aquafloc - 1001	-	mild irritation
3.	Sugaret 18	-	mild irritation
4.	Fatty acid - A	-	mild irritation
5.	Fatty acid - B	-	mild irritation
6.	Odomos	-	mild irritation
7.	Plant growth regulator	-	No irritation
E.	Mucous Membrane Irritation in Rabbits:		
1.	PEG 400	-	mild irritation
2.	Odomos	-	mild irritation
3.	Plant growth regulator	-	mild irritation
1.	Test for Plastic container		
	Polyethylene drapes	-	complies as per U.S.P.
2.	Test for Painted container - Fusion Tanks	-	Except odour other paraneters were comparable with the controls.
3.	Abonormal Toxicity Absorbable surgical sutures.	-	No undue toxicity was observed.

APPENDIX IX VIROLOGY

Viral Hepatitis Status in State of Maharashtra - 1983 - 1988

Year	Attack rate	Deathe rate
1983	24121	838
1984	25437	663
1985	23679	624
1986	18080	513
1987	18160	473
1988	5376	292

Data collected from 40 Districts and Corporations of State of Maharashtra.

APPENDIX - IX-A VIROLOGY

1988 Infective Hepatitis Outbreaks having more than 10 cases.

Sr. No.	District	Taluka	Village	Month of outbreak	Attack	Death
1.	Raigad	Mangaon	Amoosi	Jan-88	11	0
2.	Sindhudurg	Deogad	Manache Deuhwadi	Dec-88	93	0
3.	Dhule	Nandurbar	Nandurbar Town	Oct-88	12	3
4.	Jalgaon	Pachora	Bhoj	Feb-88	18	0
5.	Nasik	Igatpuri	Igatpuri Town	Aug-88	79	1
6.	Kolhapur	Karvir	Kithali	Sept-88	60	0
7.	Sangali	Shirala	Vakurde Bk	Aug-88 & Sept-88	194	1
8.	Satara	Jawali	Kudal	June-88	108	5
9.	Satara	Paitan	Malharpeth	Dec-88 to Feb-89	124	0*
10.	Buldhana	Khamgaon	Shapur	Oct-88	13	0

- 1) Cases are reported from Bombay and Kolhapur, Corporation throughout year (high incidence).
- 2) Sporadic cases are reported throughout the year by Pune, Nasik, Solapur and Aurangabad Corporations. (* up to 6-2-89).

APPENDIX - IX-B

VIROLOGY

MARKERS OF VIRAL HEPATITIS IN SELECTED SERA

(Out breaks in Maharashtra 1987-88)

Place	Number Sera tested	HBsAg (ELISA)	HAV-Igm (ELISA)
Kolhapur** (Feb. '88 - Nov. '88)	107	(60) 56.7%	(19) 17.75%
Sangli** (Aug. '88 - Sept. '88)	44	(42) 95.5%	(2) 4.5%
Deolwadi* Dist.-Sindhudurg (Nov. '88 - Dec. '88)	44	(2) 4.5%	-
Bombay (Voluntary Donors)	138	(10) 7.2%	(3) 2.1%

* Out patient department cases.

** Hospitalized cases.

APPENDIX - IX-C

VIROLOGY

Information Regarding the Blood Banks in the State :

a) Agency wise :	
1) Government	39
2) Corporation	5
3) Red Cross	10
4) Private / Commercial	31
5) Voluntary	23
Total	108
b) Location-wise :	
In Corporations	63
At District Head-quarters	30
At periphery	25
Total	118

APPENDIX - IX-D

VIROLOGY

Age and Sex Distribution of Hepatitis Patients

Place	Age in years					Sex		Total samples Collected
	1-10	11-20	21-30	31-40	40	Male	Female	
Kolhapur	82	57	123	53	27	237	95	342
Sangali	11	17	10	5	1	22	22	44
Deolwadi District : Sindhudurg	1	5	15	15	8	21	23	44

APPENDIX - IX-E

VIROLOGY

Infective Hepatitis 1987, Outbreak, having more than 10 cases.

Sr. No.	District	Taluka	Village	Month of outbreaks	Attack	Deaths
1.	Raigad	Roha	Roha Town	March-87 to April-87	111	0
	Raigad	Roha	Surrounding villages	March-87 to April-87	56	0
		Kolhapur	Khopoli Town	April 87	52	0
2.	Jalgaon	Pachore	Varsale	August 87	54	0
3.	Nasik	Molegaon	Sajwal	Sept. 87	10	0
4.	Beed	Beed	Town	Sporadic cases throughout the year		
5.	Jalna	Jalna	Bharakheda	Dec. 87	10	0
6.	Parbhani	Hijoli	Chikahagar	August-87	13	0

- 1) High incidence is observed throughout the year in corporation areas of Bombay, Kolhapur and Pune.
- 2) Sporadic cases are reported throughout the year by Corporations of Aurangabad, Solapur and Nasik.

APPENDIX - X

VIROLOGY

Vaccine administered	Number of Patients
Antirabic	1224
Cholera	23
Typhoid	17
Measles	2
Triple	202
Dual	6
Oral Polio	277
Tetanus	48
Plague	2
Hepatitis	48
MMR	56
Total	1905

APPENDIX - XI

ZOOONOSIS

Snakes	No. Procured	Venom Produced (g)	Venom Supplied (g)
1. Cobra	68	163.28	77.20
2. R. Viper	58	25.71	67.99
3. Krait	14	11.28	12.54
4. Echis	3217	19.52	24.57
Total	3357	219.79	182.30
Scorpions :			
1. Red scorpions	8393	3.07	4.90
2. Black Scorpions	338	0.06	-
Total	8731	3.13	4.90

APPENDIX - XI-A ZONOSIS

Statistics of visitors to snake farm :

1.	Students	3869
2.	Staff of Scientific Institutes	820
3.	Distinguished visitors	65
4.	Foreign Guests	14
5.	Thursday Visitors	175
6.	Others	70
Total		5013

There were 37 enquiries regarding identification of snakes from hospitals and individuals.

APPENDIX - XII 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.
M. Pharm.	- Pharmacology and Pharmaceutical Chemistry.
M. Phil.	- Microbiology
M.D.	- Pathology, Pharmacology and Microbiology.
D.M.L.T.	- Diploma in Medical Laboratory Technology.
D.A.	- Drug Analysis

APPENDIX - XIII Trainees Admitted

M.Sc. by papers (Part I and II)	20
Diploma in Medical Laboratory Technology (DMLT)	20
Drug Analysis	15
Short Term Training Courses	20

Research Fellowship and Scholarships Awarded :

Sr. Research Fellowships (Platinum Jubilee)	2
Jr. Research Scholarships (Platinum Jubilee)	3
Sr. Research Fellowships (Chinchwad Land)	2
Jr. Research Scholarships (Chinchwad Land)	2

APPENDIX - XIV

List of students who registered during the year 1987-88
for M.Sc. (by research) Ph.D., M. Pharm, M.Phil and M.D.

M.Sc. (by research)	Ph.D.
Shri S.B. Tayade	Dr. N.G. Chanderkar Mrs. A.A. Gandhi Dr. (Mrs.) C.H. Pandit Mr. K.P. Deobhankar Ms. S. Sengupta Ms. S. Nagvekar
M. Pharm	M. Phil
Ms. S.V. Nalavde	Ms. P.A. Joshi
M.D. -	

APPENDIX - XV

Degree awarded during the year 1987-88

Name of Candidate	Subject	Topic
Ph.D.		
1. Mr. S.V. Joshi	Org. Chem.	Synthesis of heterocyclic compounds as possible antitubercular agents.
2. Mr. V.G. Shirke	Org. Chem.	Synthesis and study of organic compounds as possible antitubercular agents.

Appendix - XVI
List of Post-Graduate Teachers

Name	Qualifications
Dr. M.V.N. Shirodkar	M.B.B.S., A.B., Sc.D. (Johns Hopkins) P.B.K. (U.S.A.)
Dr. R.S. Kamat	M.D.
Dr. K.H. Dave	M.D., D.P.B.
Dr. D.M. Renapurkar	M.Sc., Ph.D., 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. M.K. Bhopale	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. D.P. Buduk	M.V.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 - XVII

Papers presented at National and International Conferences

Indian Pharmaceutical congress held at Madras 1 - 3 January, 1988.

V.G. Shirke, V.R. Lokhande, A.S. Bobade and B.G. Khadse *in vitro* screening of synthesised 2 - substituted aryl - 5, 6 - Disubstituted aryl, 5, 6 - Disubstituted imidazole (2, 1b) Benzothiazoles and corresponding benzothiazoles.

V.G. Shirke, A.S. Bobade, V.R. Lokhande and B.G. Khadse. Synthesis and study of 2, 5 - Dichloro - 4 - nitro benzamido and 3, 5 - Dinitro - 0 - Tolumide as a possible antitubercular agents.

V.G. Shirke, S.M. Patil and B.G. Khadse. Synthesis and study of antitubercular activity of 2 - alkyl - 5 - Nitro - 6 - substituted Benzothiazoles - 2 - yl - carbamates.

APPENDIX - XVIII
PAPERS PUBLISHED 1987-88

S.R. Naik and R.S. Kamat. Mechanism of liver necrosis in hepatitis B Viral infection. J. Trop. Gastroenterology 8, 59, 1987.

S. Rath, V. Bal, B.J. Mohite, S.R. Naik, A.J. Zuckerman and R.S. Kamat. Anti-idiotypic humoral and cellular responses to antibody to hepatitis B virus infections. In "Viral Hepatitis and Liver Diseases"; proceedings of international symposium on viral hepatitis and liver Diseases, 1987 in London. A.J. Zuckerman ed. Alan. R. Liss Inc. Publ.

S.R. Chavan and S.T. Nikam. Investigation of alkanes from *Azadirachta indica*, A Juss (neem leaves) and their mosquito larvicidal activity. Pesticides 22(7), 32-33, 1988.

V.R. Kamath, S. Menon, D.M. Renapurkar. Larvicidal effect of perbendazole on experimental infection of *A. cantonensis* in mice. Chemotherapy 33:45-447.

L.S. Limaye, V.R. Pradhan, M.K. Bhopale, D.M. Renapurkar and K.D. Sharma. *Angiostrongylus cantonensis*. Study of intermediate, paratenic and definitive hosts in greater Bombay. Helminthologia 25:31-39, 1988.

Vidya R. Pradhan and D.M. Renapurkar. Succptibility of different hosts to *P. berghei* infection. Ind. J. Microbiol 28:123-124, 1988.

V.R. Kamath and D.M. Renapurkar. Evaluation of insecticidal activity of propetamphos against common house hold insect pests. Pesticides 21(12) : 27-28, 1987.

D.M. Renapurkar. Observation on ecology and distribution of *xenopsylla brasiliensis*. Pesticides 22(5) 22-24, 1988.

V.R. Kamath and D.M. Renapurkar. Protection against *P. berghei* in rats by immunization with adult worm homogenate of *S. cervi*. Current Science. 56 : 499-500, 1987.

M.L. Ramaiya, V.R. Kamath and D.M. Renapurkar - Long-term in vitro cultivation of *P. berghei*. International Journal for parasitology 17:1329-31, 1987.

A.J. Almedia, M.K. Bhopale, V.R. Kamath, S. Menon and D.M. Renapurkar. Immuological response due to *S. cervi* in albino rats. Helminthologia 24:43-51, 1987.

A.J. Almedia, M.K. Bhopale, D.M. Renapurkar. Evaluation of *S. cervi* antigen in the immuno diagnosis of human filariasis. Bratisl lek-Listy, 87:641-648, 1987.

APPENDIX XIX

PAPERS SENT FOR PUBLICATION

S. Rath, V.Bal. B.J. Mohite, V. Haridas, S.R. Naik, S.A. Kamat, A.J. Zuckerman and R.S. Kamath. Anti-idiotypic humoral and cellular responses to antibody to hepatitis B surface antigen in hepatitis B viral infections. Clinical and Experimental Immunology.

V.G. Shirke, B.G. Khadse, A.S. Bobade, R.P. Bhamaria and Sengupta S.R. Synthesis and Antitubercular activity of some new 2 - substituted aryl amino-5/6 disubstituted / 6 substituted benzothiazoles. Indian Journal of Pharmaceutical Sciences.

V.G. Shirke, S.V. Nalavade, B.G. Khadse and S.R. Sengupta. Synthesis and study o 3, 5 Dinitro - 0 - Toluamide derivatives as Antituberculosis Agents. Indian Drugs.

Zarine Khetty, Vijayalaxmi R. Kamath, D.M. Renapurkar and B.G. Khadse. Synthesis and Evaluation of 4, 6 Diamino - 1, 2 - Dihydro - 2' - Dialkyl or Aryl - 1 - (substituted) - S - Triazines as antimalarial agents. Indian Journal of Malariology.

Saroj Bapana, D.M. Renapurkar S.R. Chavan, Cyclopicidal activity of three indigenous plants. Insect Science and its Application, Nairobi, Kenya.

D.M. Renapurkar, **Bandicota bengalensis gray**. Observation on ecology, distribution, status to plagus in Bombay. J. Hyg. Epidemiology, Micro and Immunol.

M.L. Ramaiya and D.M. Renapurkar. Use of various animal erythrocytes for *in vitro* cultivation of **P. berghei**. International Jr. Parasitology.

D.M. Renapurkar, T.G. Tare, N.K. Sutar and P.B. Deshmukh. Observation on snake repellent activity of some plant extracts. Defence Sci. Journal.

M.L. Ramaiya, N.K. Sutar and D.M. Renapurkar. Use of human plasma for the *in vitro* cultivation of **P. berghei**. Ann Trop Med. Parasitology.

D. M. Renapurkar, V.R. Kamath, N.K. Sutar, R. Deshmukh, C.H. Pandit and S.N. Marathe. Micro test for assaying sensitivity of **P. vivax in vitro**. Chemotherapy.

