TUBERCULOSIS RESEARCH CENTRE

CHETPUT CHENNAI-600 031

REPORT ON RESEARCH ACTIVITIES DURING 1996 - 1997

INDIAN COUNCIL OF MEDICAL RESEARCH
NEW DELHI

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PREFACE

Tuberculosis continues to be a major problem in our country. The increase in HIV infection and, multidrug resistant M.tuberculosis is adding to the existing burden of the problem. The presently available tools if utilised properly would help a great deal in controlling the disease. That has not happened for more than one reason. The nation is now at a stage where it is desperately trying to apply the present existing tools with a revised strategy in the most cost effective manner. The failure of controlling this dreadful disease in the past has highlighted the present necessity for more basic, applied and operational research to develop better tools and better strategies for disease control.

The multi disciplinary approach of the Centre has been the basis of its significant contributions in the field of chemotherapy of tuberculosis. The outcome of the clinical studies undertaken by the Centre will be helpful in formulating new policies for the treatment of drug resistant organisms of pulmonary tuberculosis in adults and also contribute significantly towards the treatment of childhood and extra-pulmonary forms of the disease. In order to fully rehabilitate patients who have been treated for pulmonary tuberculosis, they are being assessed for their cardio-pulmonary status. In a major shift in the emphasis of research over the last few years, this Centre has been undertaking operational studies that would help transfer the benefits of research to the control program of the Govt of India. This annual report highlights the results of the recently completed studies. Among the important findings are (a) the economic impact of tuberculosis on patients and family was considerable (b) the first point of contact was a private provider for more than half of the chest symptomatics who took action. Their economic status , literacy, age and gender did not influence action taking and the type of facility used (c) private practitioners rely more on x-rays for diagnosis and are aware of the RNTCP. Other important operational studies are examining the following issues a) women and tuberculosis b) patients attitudes to providers of TB services. The Centre's systematic studies on surveillance of HIV infection in tuberculosis patients and surveillance of individuals infected with HIV for the development of tuberculosis are continuing and will provide authentic information of the dual infection.

During this year special efforts were also made at our Centre to link various governmental and non-governmental agencies that are actively involved in TB control. In order to achieve this the Centre organised five major training programmes and national workshops jointly with Lepra India, DANIDA, NACO,

and Central TB Division(DGHS, Ministry of Health and Family Welfare, Govt of India). These activities were aimed at (i) Training of medical officers involved in RNTCP, (ii) Integrating TB control with HIV management, (iii) Reviewing the current status of drug resistance in the country, to evolve uniform sampling techniques, to record the accurate history of patient's treatment and to evolve mechanisms of quality assurance and to develop reporting and feedback systems. In addition the Centre also held a joint review meeting with TINP and Dept of Adidravidar and Tribal Welfare, Govt. of Tamil Nadu on the collaborative study "Feasibility of utilising literate youth volunteers to improve the nutritional status of antenatal and postnatal women and children under five years in Jawadhu hills tribal area".

On the laboratory side, the emphasis of the Centre on quality control in mycobacteriology since its inception received recognition from the Nation and WHO which designated the Centre as a supranational laboratory for mycobacteriology. The Centre's commitment for quality assurance of anti-tuberculosis drugs was similarly acknowledged by WHO which included the Centre as a member of the global network for quality control of anti TB drugs. In the area of frontier biology the Centre has continued to its commitment to the development of diagnostic and monitoring tools based on DNA technologies. It is hoped these will ultimately be beneficial for the control of tuberculosis. Histopathological studies of tuberculous lymphnode and skin highlighted the possible involvement of humoral immune factors in necrotic inflammation. The development of new statistical methodologies by the Centre for the analysis of biomedical data is continuing.

The environment conducive for research provided by the Centre is being utilized by the scientists of the Centre to turn out high quality research. The fact that a large number of young and bright scholars are attracted every year by the Centre to pursue doctoral research programmes bears testimony to the quality of guidance. Often these programmes form part of the research activities of the Centre.

The Institute has talented, enthusiastic and well-trained research and technical staff to face greater challenge that is ahead of it. I am confident that with additional resources primarily for infrastructure building this Centre of excellence can climb further heights in making significant contributions not only in development of more tools for TB control but also provide the lead in frontier areas of modern biology.

P.R.Narayanan
Director

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AS ON 31.12.97

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OPERATIONAL RESEARCH STUDIES - COMPLETED

Utilisation of sub-centres for drug delivery and its impact on case-holding

(Completed study, 1993-96)

Patients' poor compliance for drug intake under programme conditions is attributed to many reasons and one important factor identified is the inability to attend PHC due to long distance from patients' residence and consequent loss of wages. In this study, drug delivery was organised through the sub-centres situated closer to the patient's residence as a measure to improve compliance of patients. Twelve PHCs in West Godawari district of Andhra Pradesh were randomly selected for the study and were further randomly allocated to the intervention(6) and non-intervention(6) areas. These centres were distributed in tribal, upland and delta areas. As a preliminary measure, training on all aspects of tuberculosis and the programme was given to the PHCs staff including pharmacists and MPWs incharge of sub-centres.

In the study centres when a treatment card was opened, for all newly diagnosed cases, the option of collection of drugs was given to the patient; he was asked to collect either from the main PHCs or sub-centres. The MPW was asked to issue the drugs to the patients on a weekly basis and in case of default, MPW will do the defaulter chasing by sending a message or by personal visit. In control centres, the drug supply was through main PHCs only.

The study population consists of 85 patients in study centres and 36 in control centres; 58% in study centre opted for treatment at PHC and 42% at sub-centres. In the study area, only 47% had collected more than 80% of the prescribed treatment at the PHC as compared to 92% at the sub-centre. In the control centre the treatment completion rate was 47%. Fifty-one percent of the patients were lost in the study centre who opted for treatment at PHC in contrast to 3% among sub-centre patients; 50% were lost in the control centre.

It was observed that it was feasible to supply drugs at sub-centres and the completion rate at the sub-centre was high (92%). The proportion of lost cases was also negligible (3%). This observation has relevance in the present context of DOTS under programme conditions.

Evaluation of the reasons and level of delay in diagnosis of sputum positive pulmonary tuberculosis under programme conditions in South India

(Completed study, 1996-97)

Majority of the TB patients are aware of their chest symptoms but several studies have shown that only half of them attend general health institutions. They have varying preferences for health care, ranging from government institutions to practitioners of indigenous medicines. There are several reasons for failure to attend the health centres. Studies done in Korea have shown the existence of a delay which can be classified as patients' delay and the doctors' delay. The extent of these delays in India has not been estimated.

The objectives are to find out (a) is there a delay in the diagnosis (b) If 'Yes', the reasons for the delay - reasons attributable to patients and/or health services and (c) to identify the level of delay; patient's delay or doctor's delay in the programme.

The sampling frame was the city of Chennai and the district of Vellore. In the city of Chennai, two TB Centres and one other randomly selected General Hospital were included as participating centres. In the district, the study covered the District Tuberculosis Centre (DTC), two x-ray centres, 4 microscopy centres and respective referral centres including a few representative of private hospitals. The total number of patients aimed at is 300 rural and 300 urban.

All patients diagnosed to have sputum positive tuberculosis were administered a questionnaire by a trained social worker within a month of diagnosis of tuberculosis. The questionnaire included details regarding

general information on health seeking behaviour of the patient and awareness and action taken by family members.

A total of 531 patients were interviewed. It was observed that the average delay in diagnosis was 48 days on the part of the patients and 60 days on the part of the doctors and the total delay in the diagnosis of a sputum positive pulmonary tuberculosis patient under programme conditions was considerable(108 days). There was no difference in the overall delay among male/female patients. However, the location of the patient's residence played a role in the delay - the average delay for rural patients was more(125 days) as compared to the delay observed among urban patients(93 days).

Behaviour pattern of "chest symptomatics" in urban and rural populations

(Completed study, 1997)

The Tuberculosis Control Programme is felt-need oriented and depends on symptomatics taking action and reporting to health facilities. The health seeking behaviour pattern of "chest symptomatics" is therefore the main determinant in the success of the TB control programme. Information regarding their action taking could be crucial in the planning of future strategies to improve both the case-finding and case-holding components of the programme.

A community based cross-sectional study in 2 urban (Chennai and Madurai) and 2 rural (Poonamallee Panchayat and Mellur Block) areas of Tamil Nadu was undertaken to find out the behaviour pattern of chest symptomatics. The study area was selected based on PPS linear sampling method and the sample by random sampling of the street and the house. An initial qualitative exercise was carried out prior to designing of the interview schedule. The interview schedule was semi-structured and pre-coded and prepared based on information gathered from focus group discussions held in all the four areas and were pretested. The questionnaire sought information regarding health-

seeking behaviour (for major and minor ailments) and for chest symptoms in detail. The responses were then analysed using SPSS/PC+ + statistical software. From each area it was aimed to obtain information from atleast 150 symptomatics.

A total of 4624 respondents were interviewed and 689 symptomatics were identified. Of these, 649 (91 %) were interviewed, 310 in the urban and 339 in the rural area. This sample included 161 symptomatics who had been treated for tuberculosis.

Around 70% of chest symptomatics had taken action by approaching a health facility. Ninety percent of them did so within 4 weeks, with 70% taking action within 1 week.

The first point of contact was a private provider for more than half of those who took action. Their economic status, literacy, age and gender did not influence action-taking and the type of facility utilised.

Even among the economically underprivileged family (income <Rs.500/month), the preference was to go to the nearest private provider, despite the cost involved.

The awareness of the symptoms of tuberculosis was low, even among patients who were earlier treated for TB. This clearly indicates a deep lacuna in the level of awareness in the community.

Thus, there is a need to create and awareness in the community on the symptoms of tuberculosis and on the availability of diagnostic and treatment services, free of cost at Govt. Hospitals. A methodology to involve private practioners in the programme has to be evolved.

Economic impact of TB on patients and families

(Completed study, 1997)

Majority of the TB patients are in the economically productive age group and hence a study was planned to quantify the economic impact

of TB on patients and families from the costs incurred by patients attending different health providers, in rural and urban areas.

Information regarding patients' socio-economic, demographic characteristics employment particulars, income, expenditure incurred towards illness and effects of illness on children was collected using an interview schedule prepared from analysis of 17 focus group discussions. Direct and indirect costs included money spent for diagnosis, drugs, investigations, travel and the loss of wages and total costs were projected for the entire period of treatment (6 months).

A total of 304 (Govt. 202, Non-Govt. 77, Private 25) newly detected sputum positive pulmonary tuberculosis patients including 120 females on 2 to 6 months of treatment were studied. The average direct, indirect and total costs were Rs.1,443/-, Rs.2,206/- and Rs.3,469/-, respectively, and the costs were related to region, sex and type of health providers. The average work days lost was 83 days and average debts were Rs.2,079/-. Fifteen percent of rural and 11 % of urban patients faced rejection by their family. Among school going children 11% discontinued studies and 8% took up employment to support the family. More than half of the women in both areas were not able to do household activities.

The total costs and in particular, indirect cost due to TB were significant for all patients. There was a significant decrease in caring activities of women while a fifth of school going children of TB patients discontinued schooling.

Thus, it was observed that the economic impact of tuberculosis on patients and family was considerable.

Impact of tuberculosis on private for-profit providers

(Completed study, 1997)

The Health services offered by the private for-profit health care system in India parallel the public health services. The "private for-profit

providers (Pps)" also have a role in the management of tuberculosis (TB). Very few studies have been done on their role in the National Tuberculosis Control Programme.

The study on the 'Impact of Tuberculosis on private for-profit providers' was undertaken with the aim of getting details of the diagnostic and treatment practices of qualified PPs and their willingness to collaborate in the Revised National Tuberculosis Control Programme (RNTCP). The study was conducted at Chennai, one of the major metropolis in the country and its immediate neighbouring rural areas of Chengalpattu district. Fifty percent of the PPs were directly identified, and the other 50% indirectly, based on information received from TB patients earlier managed by PPs.

Questionnaire to elicit information on practice details of PPs was finalised based on Focus Group Discussion (FGD). The interview schedule included the practitioners' personal data, their diagnostic approach, treatment methods, patient retrieval methods, monitoring and record maintenance, the source of information on TB management, their willingness to participate and assist in the RNTCP.

A separate questionnaire was designed and finalised for patient interview, using also the output from FGDs. The name and address of the provider, details of diagnosis, treatment, reasons for attending government facilities and the cost incurred during private treatment were recorded for each patient.

Both the questionnaires were field tested prior to the main interview. The responses to the interview schedule based on the practices as reported by 303 qualified allopathic PPs managing adult pulmonary TB patients, in both rural and urban areas, and the responses of 203 TB patients earlier managed by PPs were analysed.

The practices reported by both urban and rural PPs appear similar. They continue to rely on chest radiograph for diagnosis as they have no reliable facilities for sputum microscopy. They manage newly diagnosed, relapsed and drug resistant pulmonary TB patients and the drug regimens

prescribed are tailored to individual patient needs. They have no mechanism for monitoring patient compliance or patient retrieval and maintenance of patient records.

Most of the PPs are aware of RNTCP and are willing to assist in all aspects of the programme including DOTS. Hence further studies may be undertaken to evolve strategies to involve them in the programme.

OPERATIONAL RESEARCH STUDIES - IN PROGRESS

Feasibility of using tribal literate youths to improve the nutritional status of the vulnerable population in Jawadhu Hills tribal area

(Ongoing study, 1996-98)

The Centre has been involved in tuberculosis activities in the Jawadhu hills tribal area for the past few years. Local literate youths were trained in tuberculosis case finding and case holding activities which they carried out successfully. There was then a demand from the tribal population to provide general health care services as their access to government health facilities is very poor. It was noticed that the nutritional- status of women of child bearing age and children under five years was very poor with high incidence of anaemia and Vitamin A deficiency. Hence it was decided to study the feasibility of providing nutritional services to this population using literate youth.

Sixty-one literate volunteers were trained in methods of nutritional surveillance and rehabilitation at the Tamil Nadu Integrated Nutrition Program's Training Centre in Chennai. Each volunteer is responsible for census taking and regular weighing of the children and antenatal women in his/her area. There are approximately 1250 children under five years and 150 pregnant and lactating women in the study area. Baseline weights of children in the study area as well as from a control (nonintervention) area have been recorded. The team from TRC supervises the youth and random checks of weighing are carried out

regularly. In addition, supplementary food is provided to malnourished children under three years and antenatal and postnatal women by TINP. The youths are responsible for the distribution of food in their respective villages. The children are dewormed once in six months and iron and calcium tablets are provided to anaemic individuals. The youths have also been trained in management of common ailments like diarrhoea and acute respiratory infections. In addition, health education of the tribal women is carried out by the medical social workers of TRC.

At the end of one year, weighing of all children in the study and control areas was repeated. This study is funded by the Tribal Welfare Department (Government of Tamil Nadu) and is being done in collaboration with the Tamil Nadu Integrated Nutrition Project. The study is in progress.

CLINICAL STUDIES - COMPLETED

Quality of life measurements at the end of treatment for patients treated for pulmonary tuberculosis

(Completed study, 1993-96)

Quality of life is a multidimensional concept concerned with the impact of physical symptoms and side effects of treatment on patients' functioning and psychosocial wellbeing. It is generally assumed that treatment of pulmonary tuberculosis with potent chemotherapeutic agents and making them non-infectious will be accompanied by improved health and well being. However, disturbances in physiological functions persist, even though the precipitating event (bacillus) has been eliminated. Activities that may be disturbed by disturbances in physiological functions are physical, social, emotional, intellectual, economic and spiritual. Impairment is an abnormality of physiologic functions and disability is the effect of impairment on patients life. Quality of life measurements utilising general health questionnaires such as the Sickness Impact Profile and Quality of Wellbeing Scale are designed for application to a very wide range of diseases and hence there are limitations regarding their precision and sensitivity. Hence, it has been

suggested that disease-specific questionnaire will provide a more precise and sensitive measurement of quality of life than a general index. Two quality of life measures developed for chronic lung diseases are Chronic Respiratory Questionnaire and St.George's Respiratory Questionnaire

The objectives of the study are to assess quality of life measurements using Disease-specific Questionnaire (Chronic Respiratory Questionnaire) in patients who had undergone treatment for pulmonary tuberculosis and to compare the Disease-specific Questionnaire (Chronic Respiratory Questionnaire) with 6-minute walking test and pulmonary function tests in patients treated for pulmonary tuberculosis.

A group of 150 pulmonary tuberculosis patients treated with short-course chemotherapy will be included in the study if they had no previous history or any other illness and had not received any anti-tuberculosis treatment at the time of initiation of short course chemotherapy. Initial clinical and respiratory symptom assessment including chest x-rays are done using "Questionnaire of the European Community For Coal and Steel on Respiratory symptoms". Disease-specific quality of life measurement is assessed using chronic respiratory questionnaire.

The following objective measurements of impairment are also undertaken:

1. Pulmonary function tests: Flow-volume loops are recorded in each patient using Morgan Transfer Test Model C. At least 3 acceptable readings are obtained from each patient as per American Thoracic Society recommendations.

2. Six-minute walking test: This test is carried out in a level corridor. Each patient is instructed to cover as much ground as he could on foot for 6-minutes and to keep going continuously if possible, but not to be concerned if he had to slow down or stop to rest. The patient's aim should be to feel at the end of the test that he could not have covered more ground in the time. An investigator accompanies the patient, acting as timekeeper and giving encouragement as and when necessary. The actual distance covered is measured.

A total of 154 patients have been admitted and the results are being analysed.

A study to evolve objective criteria for diagnosis and assessing the progress in paucibacillary leprosy

(Completed study, 1993-96)

A pilot study was initiated to evolve an objective criteria for diagnosis and assessing the progress in paucibacillary leprosy. A patient was eligible for the study if he/she was aged 5 years or more, disease was classified as BT/TT clinically, skin smear for AFB was negative and has had no previous specific chemotherapy (DDS for more than one month or even a single dose of rifampicin) in the last 6 months. All the patients were prescribed NLEP regimen for paucibacillary leprosy (rifampicin once a month plus DDS daily for 6 months) and were treated on ambulatory basis.

Of the 60 patients admitted to the study, 7 were excluded and the remaining 53 were analysed. At the end of 6 months, 15 (28%) of the 53 patients were clinically active and 38 (72%) were clinically inactive. As the criteria for clinical assessment of paucibacillary patients is subjective, an attempt was made to give a semi-quantitative measure to assess the status of the patients at various time points during treatment as well as follow-up period. Various factors of clinical signs such as colour, border, infiltration, palpation, nerve tenderness and sensation were considered and a weighted scoring system was developed.

On admission clinical diagnosis was confirmed histopathologically in 80% of the patients. At 6 months 15 patients (28%) had either no evidence or healed leprosy, 22 (42%) had regressing or resolved leprosy, the remaining 16 (30%) still had evidence of leprosy although 50% of them were clinically inactive. Seven out of the 15 active patients had a fall in the antigen level in comparison to 15 of the 38 in the inactive group. Van Gieson staining for the presence of fibrous tissues was done and no difference was found either in the intensity or in the extent of staining.

At the end of 24 months 6 more patients failed to attend for assessment and the remaining 47 were followed up. Of these 47 patients, 4

relapsed and required retreatment and the remaining 43 were clinically inactive.

By doing all these exercises it was found that clinical assessment is still more reliable and consistent for diagnosis (as supported by the independent assessor and 80% histopathologically) and assessing the progress of PB patients. Laboratory parameters especially histopathology definitely helps in confirmation but does not assist much in prognosis.

A randomised double blind multi-centric controlled clinical trial of two regimens in the treatment of paucibacillary leprosy

(Completed study, 1995-97)

A WHO sponsored multi-centric study in the treatment of paucibacillary (PB) leprosy patients was conducted by CJIL field unit, Avadi. The CMD unit of Tuberculosis Research Centre was one of the participants in the study.

Paucibacillary leprosy patients with two or three lesions were eligible for the study. Satellites were counted as separate lesions. Patients with trunk enlargement or positive bacterial index were not eligible.

The objective was to evaluate the efficacy of a combination of rifampicin plus minocycline plus ofloxacin as a single dose. The total duration of the study was 18 months.

The results suggested that the efficacy of single dose regimen is comparable to six months of multi drug therapy (MDT). However, this has to be evaluated on larger number of patients.

Post surveillance follow-up of Multibacillary Hansen's cases

(Completed study, 1996-97)

When multidrug therapy (MDT) was introduced in NLEP, multibacillary(MB) Hansen's cases were started on MDT irrespective of

the smear status. They were given treatment for a minimum period of 2 years or till smear negativity. They were released after 5 years of stopping treatment. An earlier study done at the Leprosy Unit of TRC where inspite of continuing treatment till smear negativity and patients remaing quiescent for 4-5 years, 3 of 55 patients relapsed during 13-14 year of follow-up. This raises a question whether 5 year's surveillance is adequate.

A one time assessment was done on MB patients who were started on MDT during 1986-87 and completed surveillance during 1993-94. The study was initiated in November 1996 in collaboration with the District Leprosy Officer, Leprosy Control Unit, Poonamalle, Chengai M.G.R. District.

A total of 129 patients were assessed and none of them were found to have clinically or bacteriologically active disease. It will be ideal to do a similar follow-up study on a fresh cohort of patients before drawing firm conclusions.

CLINICAL STUDIES - IN PROGRESS

3-5 month regimens containing ofloxacin in the treatment of sputum positive pulmonary tuberculosis

(Ongoing study, 1995-98)

Several effective 6-month short course regimens given either daily or intermittently have been evolved in the treatment of sputum-positive pulmonary tuberculosis. Even with these 6-month regimens, poor treatment completion remain a major problem for implementation under programme.

With the advent of another powerful bactericidal drug, ofloxacin, which is reported to be as powerful as rifampicin and isoniazid and superior to streptomycin in terms of bactericidal activity, the Centre is

currently undertaking a study to find out whether by using this drug the duration of treatment can be reduced further, say 3 or 4 months. An earlier study conducted at this Centre had shown that a 3-month regimen of SHRZ daily was near 100% effective in patients with initially drug-sensitive organisms, and 18% of these had a bacteriological relapse over a 5-year follow-up period. The aim of this study is to find out whether this high relapse rate can be reduced significantly by using ofloxacin instead of streptomycin.

Patients with sputum positive pulmonary tuberculosis with previous chemotherapy not exceeding 2 weeks, are randomly allocated to one of the following 4 regimens:

1. 3OHRZ/-
2. 3OHRZ/1 RH_2
3. 3OHRZ/2RH_2
4. 2OHRZ/2RH_2

All drugs are given under supervision in the clinic. It is proposed to admit about 150 patients to each of the regimen. The study is in progress both at Chennai and the TRC unit at Madurai. So far 392 patients have been admitted to the study. The study is in progress.

Six-month regimen for pulmonary tuberculosis with two double-drug combinations on alternate days for the first two or three months - Follow-up phase

(Ongoing study, 1996-99)

Several highly effective short-course chemotherapy regimens of 6-8 months duration have been evolved for the treatment of pulmonary tuberculosis and in most of these regimens, four drugs, namely, rifampicin, isoniazid, pyrazinamide and streptomycin or ethambutol are given together in a single dose, either daily or intermittently. The number of tablets/capsules to be consumed in a single dose is therefore large and the incidence of adverse reactions such as arthralgia and jaundice is high with daily regimens. The Centre is investigating , both at Chennai and its

unit at Madurai, a regimen of rifampicin and ethambutol on one day and isoniazid and pyrazinamide on the next day, each combination given thrice a week for the first 2 or 3 months, followed by rifampicin and isoniazid thrice a week for the next 4 and 3 months, respectively; so that, the toxicity is expected to be low, while the high level of efficacy is unlikely to be affected. These two regimens are to be compared with a control regimen of rifampicin, isoniazid, pyrazinamide and ethambutol given together in a single dose, thrice a week for the first 2 months, followed by rifampicin and isoniazid twice a week for the next 4 months. The intake to the study has been completed.

The percentage of culture negativity for patients with initially drug sensitive organism showed that the sputum conversion was similar in all the three regimens. Favourable response at the end of treatment was similar in all the three regimens. Adverse reactions were not a serious problem and was similar in all the 3 regimens.

All the patients are being followed-up for five years.

Treatment regimens for patients who fail or relapse on short course chemotherapy - Follow-up phase

(Ongoing study, 1987-2000)

Pulmonary tuberculosis patients who have been treated with short course regimens and who (i) show a serious clinical deterioration, (ii) have a persistent radiographic deterioration, (iii) have an unfavourable bacteriological response or (iv) have a bacteriological relapse requiring retreatment are prescribed an appropriate regimen depending on the last available drug sensitivity test results.

The chemotherapeutic regimens are as follows:

1. $3EmbHRZ_2/6/9RH_2$ for patients with organisms sensitive to rifampicin and isoniazid.

2(a) $6SREmbZ_2/6REmbZ_2$ for patients with organisms resistant to isoniazid.

(b) 6KREmbZ_2/6EmbZ_2 for patients with organisms resistant to streptomycin and isoniazid.

3(a) 3S3EmbEthZ_7/9EmbEthZ_7 for patients with organisms resistant to rifampicin and isoniazid.

(b) 3K3EmbEthZ_7/9EmbEthZ_7 for patients with organisms resistant to rifampicin, streptomycin and isoniazid.

So far 114 patients have completed treatment in Reg.1 , 47 in Reg.2(a), 47 in Reg. 2(b), 35 in Reg. 3(a) and 52 in Reg. 3(b).

Of this, 87 in Reg.1, 32 in Reg.2(a), 41 in Reg. 2(b), 16 in Reg. 3(a) and 36 in Reg. 3(b) have received more than 75% of chemotherapy.

Among patients who had received more than 75% of chemotherapy, 93% in Reg.1, 75% in Reg. 2(a), 73% in Reg.2(b), 37.5% in Reg. 3(a) and 42% in Reg. 3(b) had bacteriologically quiescent disease at the end of treatment. Thus, patients with organisms resistant to rifampicin along with isoniazid alone or isoniazid and streptomycin pose a problem in management. The intake to the study has been completed and patients are being followed-up.

Short course chemotherapy for pulmonary tuberculosis in children - Follow-up phase

(Ongoing study, 1992-2002)

There are many reports available on SCC in adults but information regarding. the value of the same in childhood tuberculosis is limited. Hence this Centre started an SCC study in pulmonary tuberculosis in children, in collaboration with the Institute of Child Health & Hospital for Children (ICH & HC), Egmore, Chennai. In brief, patients aged between 1 and 12 years who had not received more than 2 weeks of previous anti-tuberculosis treatment are admitted to the study. The diagnosis is based on chest radiography.

The patients are randomly allocated to one of the following 2 regimens:

Regimen I: 9HR: Isoniazid and rifampicin daily for 9 months.

Regimen II: $2H_3R_3Z_3/4H_2R_2$: Isoniazid, rifampicin and pyrazinamide thrice a week for the first 2 months followed by isoniazid and rifampicin twice a week for the next 4 months.

So far 142 patients have been admitted to the study, 72 to the daily regimen and 70 to the intermittent regimen. The intake to the study has been completed and patients are being followed-up.

Collaborative controlled clinical trial on treatment of Lymphnode tuberculosis - Follow-up phase

(Ongoing study, 1988-98)

Follow-up of patients admitted to the randomised controlled clinical trial on treatment of lymphnode tuberculosis is being continued in TRC Unit at Madurai. This study is being conducted in collaboration with the Paediatric and Adult Surgery Departments of the Government Rajaji Hospital, Madurai. Patients with biopsy confirmed superficial lymphnode tuberculosis were randomised to either of the two following 6 month regimens of treatment, viz. Regimen 1 : 6 RH_7(tm): an unsupervised daily regimen of isoniazid and rifampicin, the drugs being supplied twice a month for self-administration or Regimen 2: $2RHZ_2/4RH_2$ a supervised twice weekly regimen of isoniazid and rifampicin with a supplement of pyrazinamide for the first two months (2RHZtw/4RHtw). Patients who defaulted for treatment were visited at home or letters were posted. All patients were reviewed monthly upto 12 months, every three months upto 24 months and every six months upto 60 months.

Two hundred and seventy seven patients have been admitted to the study. Patient characteristics and the results at the end of treatment were presented in the Annual report of 1993. After excluding 15 patients, 116 (87%) of 133 in Regimen 1 and 112 (87%) of 129 in

Regimen 2 had a "Favourable" response at the end of treatment; 13 (1 0%) patients in Regimen 1 and 17 (1 3%) in Regimen 2 had "Doubtful" response, i.e., had palpable lymphnodes exceeding 10 mm diameter. Four (3%) patients, all treated with Regimen 1 had an "Unfavourable" response and had their treatment changed.

Patients have now been followed up for 36-60 months after treatment. During this period, of the 13 patients in Regimen 1 who had a "doubtful" response at the end of treatment, in 12 the nodes regressed or disappeared subsequently and the response was reclassified as "Favourable", the other relapsed. In addition, one patient relapsed in Regimen 1 from the initially "favourable" category. In Regimen 2, of the 17 patients with "doubtful" response, 15 were reclassified as "Favourable", one relapsed and one still had large nodes at 60 months. In addition two patients relapsed from the initially "Favourable" category. Two patients died due to non-tuberculous causes, one from each regimen.

In summary, after 36-60 months of follow-up of 277 patients treated with two six months regimens of treatment for superficial lymphnode tuberculosis, 126 (95%) of 133 patients treated with an unsupervised daily regimen, and 124 (96%) of 129 patients treated with a supervised twice-weekly regimen had favourable outcome. Follow-up is continuing.

Collaborative study of abdominal tuberculosis - Follow-up phase

(Ongoing study, 1992-2001)

A collaborative controlled clinical study of abdominal tuberculosis was carried out at the Centre. The objectives of the study are as follows :

(a) to identify the clinical and laboratory profiles of peritoneal, intestinal and mesenteric tuberculosis in South Indian patients and

(b) to compare the efficacy of a short-course regimen with that of a standard regimen in the treatment of abdominal tuberculosis.

Patients with bacteriological, histopathological or radiological confirmation, as well as those with a clinical condition highly suggestive of abdominal tuberculosis, were admitted to the study. Patients were randomly allocated to either of the following regimens.

2RHZ/4RH (R6 - rifampicin series) : Rifampicin, isoniazid and pyrazinamide daily for 2 months, followed by rifampicin and isoniazid daily for the next 4 months.

SEH/EH (E12 - non-rifampicin series) : Streptomycin, ethambutol and isoniazid daily for 2 weeks, followed by ethambutol and isoniazid daily for the next 50 weeks.

Results upto 60 months after admission : Of the 157(84 R6, 73 E12 series) patients who were symptom free or had clinically improved at the end of treatment, 1(R6 series) died due to tuberculosis in the 7th month after start of treatment, following surgery for acute abdomen, 10 (7 R6, 3 E12 series) died due to causes other than tuberculosis and 6 (2 R6, 4 E12 series) required retreatment for other types of tuberculosis. Three patients (1 R6, 2 E12) were lost to follow-up in the 60th, 11th and 18th month, respectively, and all were asymptomatic at that time. None of the remaining 136 (72 R6, 64 E12 series) patients had relapse of abdominal tuberculosis and are being followed-up to 120 months after admission. The follow-up is in progress.

Collaborative clinical study of cutaneous tuberculosis

(Ongoing study, 1992-98)

A collaborative clinical study of skin tuberculosis with an aim to evolve diagnostic criteria and to assess a SCC regimen of 9 months' duration is carried out at the Centre.

Patients diagnosed clinically as having cutaneous tuberculosis by the dermatologist are admitted to the study after a skin biopsy. All patients aged 12 years or more admitted to the study are treated with rifampicin

(450 mg) and isoniazid (300 mg) daily for 9 months and those aged less than 12 years are treated with weight adjusted dosages. The patients are assessed at the Centre and also at the collaborating hospitals every month during treatment and at 3-monthly intervals up to 24 months.

A total of 259 patients were admitted to the study and so far 239 have completed chemotherapy. After excluding 52 patients (non-TB 19, HIV 2, non-TB death 2, change of treatment 3, lost after admission 26), there remains 187 in the analysis. Fifty percent had lupus vulgaris, 34% verrucosa cutis, 10% scrofuloderma and 2% tuberculid. A total of 150 (80%) patients had single lesion, 26 (14%) had 2 lesions and 11 (6%) had multiple lesions.

A diagnosis of cutaneous tuberculosis was confirmed by bacteriology and/or histopathology in 168 (90%) of 187 patients. At the end of 6 months the lesion resolved in 82 (87%) of 94 patients with lupus vulgaris, 52 (81%) of 64 with verrucosa cutis and the over all resolution was 160 (86%). However at the end of 9 months the lesion resolved in 177 (95%) of 187 patients. 1 (lupus vulgaris) did not resolve and treatment was changed; 1 (lupus vulgaris) was lost. In the remaining 8 patients (all verrucosa cutis), the induration subsided subsequently but verrucosity persisted which disappeared after applying keratolytic agent without specific anti-tuberculosis retreatment.

Study of the cellular immune response to mycobacterium tuberculosis in children

(Ongoing study, 1996-98)

Immunologic resistance to mycobacterial infection is thought to be mediated by cooperative interaction between T-cells and macrophages. This interaction is dependent on the interplay of cytokines produced mainly by T-cells of which the Th1 cytokines (gamma interferon, IL-2, IL-12) are considered to be protective.

The study of tuberculous infection in children provides a unique

opportunity to evaluate the primary human immune response to infection. Children infected with tubercle bacilli can remain healthy tuberculin reactors, develop uncomplicated primary tuberculosis or suffer severe complications such as miliary or meningeal TB. This study aims to evaluate the cytokine profile in different manifestations of childhood tuberculosis in order to understand the protective immune response against tuberculosis.

Four groups of children are being studied, namely, a) pulmonary tuberculosis, b) disseminated and miliary TB, c) healthy tuberculin positive children and d) healthy tuberculin negative children. Twenty children in each group from the age group of 2-12 years will be studied. Apart from clinical and routine laboratory investigations, the Th1 and Th2 cytokines (gamma interferon, IL-4, IL-10 and IL-12) will be measured in cell culture supernatants stimulated with different antigens. RTPCR will also be used to confirm the findings. In addition, lymphocyte subsets in blood and bronchoalveolar lavage fluid will be studied using flow cytometry (FACS) and appropriate monoclonal antibodies. So far, 22 children with pulmonary tuberculosis and 17 healthy controls have been studied.

Randomised controlled clinical study of two short-course regimens in Non-insulin Dependent Diabetic (NIDDM) patients with pulmonary tuberculosis - Pilot study

(Ongoing study, 1997-2000)

Pulmonary Tuberculosis has been reported to be approximately twice as common among diabetics as among non-diabetics. Active tuberculosis intensifies Diabetes Mellitus; thus the two diseases constitute a dreaded combination. Studies conducted in TRC had shown that Short-course regimens of 6 to 9 months have been found to be effective both in pulmonary and extra-pulmonary forms of tuberculosis. It has been reported from Japan (Retrospective analysis) that a 12-month "Short-course chemotherapeutic" regimen was effective in diabetic

patients with pulmonary tuberculosis, whereas under RNTCP category I regimen ($2EHRZ_3/4HR_3$) is recommended for patients with DM. it is proposed to compare the efficacy of a 6-month regimen(Cat I regimen) with a 9-month regimen in diabetics in this study.

A collaborative clinical study of DM with tuberculosis with an aim to (a) Assess and compare the efficacy of a 6-month and a 9-month short course regimens in patients with NIDDM and (b) To identify the clinical and radiological profile of pulmonary tuberculosis in patients with NIDDM is carried out at the Centre.

Adult NIDDM patients attending the Government General Hospital, Kilpauk Medical College and the TRC, suspected to have pulmonary tuberculosis and diabetes are investigated at TRC.

It is proposed to admit 120 patients to the study. Patients found eligible will be randomly allocated in equal proportions to the following two regimens.

Regimen 1. $2EHRZ_3/4RH_3$ - Ethambutol 1200 mg plus isoniazid 600 mg with pyridoxine 10 mg, plus rifampicin 450 mg in patients weighing 40 kg or less and 600 mg in patients weighing >40 kg, plus pyrazinamide 1.5 gm in patients weighing 40 kg or less and 2.0 gm in patients weighing >40 kg. three times a week under supervision for 2 months followed by rifampicin plus isoniazid in the same dose, for the next 4 months.

Regimen 2. $2EHRZ_3/7HR_3$ - The drugs and the dosage are the same as above for the first 6 months excepting that the duration is prolonged by 3 more months.

The management of Diabetes Mellitus is also randomised in equal proportions to oral antidiabetic drugs or insulin during the period of anti-tuberculous treatment.

So far, 16 patients have been admitted.

A controlled clinical study of multi-drug therapy for multi-bacillary leprosy

(Ongoing study, 1988-98)

The study was undertaken to find out the feasibility of fixed duration chemotherapy. Treatment was stopped at the end of 24 months irrespective of BI value and the patients were clinically monitored up to 84 months. Chemotherapy was extended for one more year for patients who had reaction and required prolonged steroids and the case reviewed after one year.

Patients were followed-up every month till 48 months, and every 3 months till 60 months and once a year up to 84 months. A total of 80 patients were admitted to the study and all of them have completed 60 months. Twenty-four were excluded from analysis for various reasons (6 died-not due to leprosy, 2 migrated, 1 toxic to dapsone, 5 non co-operation, 2 taking treatment elsewhere, 7 absent for more than 1 year, 1 taking treatment for tuberculosis). Of the remaining 56 patients, 1 had reactivation requiring retreatment, 13 had chronic ENL reaction and as they required high doses of steroids, treatment was extended beyond 24 months. At 60 months all these 13 patients had positive BI though there was a gradual fall. Among the remaining 42 patients who did not have reaction, 13 had negative BI and in 29 there was gradual fall at 60 months. The study is in progress and it is proposed to follow them up till 120 months.

Controlled clinical trial of dapsone as continuation chemotherapy beyond 7 years

(Ongoing study, 1977-2003)

As mentioned in the previous annual reports the Centre undertook a controlled clinical trial of rifampicin and non-rifampicin regimens in the treatment of leprosy at the CMD unit of Tuberculosis Research Centre, at Government Royapettah Hospital, Chennai.

Among 210 patients who were admitted to the study 73 were excluded at the end of 14 years (17 died - not due to leprosy, 18 migrated, 21 failed to attend for more than 1 year, 10 absconded/ discharged against medical advice, 3 discharged, 3 treated for tuberculosis and 1 toxic to DDS) and the remaining 137 were analysed. One patient was retreated at 106 months for reactivation. In more than 95% of the cases, the BI was less than 0.50 at 120, 132 and 144 months and all these patients were clinically inactive.

Four patients relapsed both clinically and bacteriologically (at 162,169,177 and 210 months) and they were managed with multibacillary NLEP regimen. Fourteen patients showed occasional positive BI after attaining negativity but there was no clinical signs suggestive of relapse and their subsequent smears were negative for BI. They are being monitored clinically and bacteriologically.

A double blind study to compare the efficacy of penicillin and DEC in prevention of adenolymphangitis (ADL) - Follow-up phase

(Ongoing study, 1995-98)

The objective of this double-blind, placebo controlled study is to compare the efficacies of four treatment regimens, given over a period of one year, for patients with filariasis, in preventing adenolymphangitis.

Each patient will be initiated to a programme of cleaning of the affected limb. This would be standardised by the investigator and will include for the following:

1) Cleaning of the affected limb every night with soap and water.
2) Keeping the affected limb dry.
3) Clipping the nails and
4) Applying salicylic acid ointment to webs of the toes, nails and sides of the feet every night.

Patients will be allocated to one of the five treatment regimens on the basis of stratified random sampling according to grade of lymphoedema.

Oral penicillin group: Patients in this group will receive two capsules- one containing 800,000 units of penicillin G potassium and the other containing placebo, every day for one year.

DEC group: Patients will receive two capsules - one containing DEC 1mg/kg and the other containing placebo, daily, for one year.

DEC plus antibiotic group: Patients will receive two capsules - one containing DEC 1 mg/kg and the other containing 800,000 units of penicillin G potassium daily, for one year.

Local antibiotic ointment group: Patients will receive two capsules of placebo every day and will apply framycetin ointment locally to the affected limb whenever indicated for one year.

Placebo group: This group will receive two capsules of placebo daily, for one year.

A total of 64 patients satisfying the criteria have been enrolled. All the patients have completed their treatment for the prescribed one year and are being followed-up for the occurrence of ADL attacks every 15 days. Since the study is blinded the results will be available after all the patients have completed their one year follow-up.

LABORATORY STUDIES - COMPLETED

Susceptibility of M.tuberculosis $H_{37}Rv$ and M.bovis BCG to pyrazinamide in $7H_9$ broth

(Completed study, 1994-96)

Pyrazinamide(PZA) is believed to be effective in an acid environment (pH 5.6) such as prevalent inside the macrophage. It has been suggested that the antimycobacterial effect of PZA is mediated through its primary metabolite pyrazinoic acid (PZC), the hydrolysis being carried out by PZA

deamidase, a microsomal enzyme. Susceptible strains such as **M.tuberculosis** $H_{37}Rv$ and **M.microti** are known to produce the enzyme PZA deamidase in the acid environment of the host's phagotysosome. Strains resistant to PZA such as **M.bovis** BCG do not produce PZA deamidase, do not convert PZA to PZC and therefore not killed by PZA.

The mode of action of PZA on tubercle bacilli is not clear. The capacity of **M.tuberculosis** $H_{37}Rv$ to become susceptible to PZA with environmental change suggests that, in the acid medium, an unknown metabolic pathway in the bacilli is activated, or that there is a change in the state of the drug. There is a possibility that the enzyme PZA deamidase present in the susceptible organism may convert the inactive PZA into antimicrobially active metabolite, PZC. So the role of PZA deamidase in the antimycobacterial action of PZA in $7H_9$ culture broth at pH 5.6 and 7.0 was studied.

This study showed that PZA was active only in acid pH and not in neutral pH against **M.tuberculosis** $H_{37}Rv$, a PZA susceptible strain. When PZC was directly added, a double fold reduction in viable count was observed at acid pH. When PZC was added to the $7H_9$ broth culture medium (pH 5.6) containing **M.bovis** BCG, a PZA resistant strain become a susceptible strain confirming that PZC was the active component.

Advantage of early reading of sensitivity tests for detection of drug resistance

(Completed study, 1997)

With increasing incidence of multi-drug resistance among tuberculosis patients there is an urgent need to detect resistance early. For this it would be most useful to see how conventional methods can yield results in a shorter time. Direct sensitivity tests can reduce the time by 4-5 weeks but it is more reliable with heavier sputum positivity. The standard indirect test was therefore read at the end of 2 weeks and again at 4 weeks as normally done, to estimate the yield of early results.

Over a period of 3 months, sensitivity tests for streptomycin (578), INH (672) and rifampicin (589) were read at 2 weeks and at 4 weeks by independent readers with no reference to the earlier reading. Analysis showed that 65-85% of resistant strains could be actually detected by 2 weeks. Only 6-10% tests could not be interpreted at 2 weeks because of inadequate growth.

Therefore 2 weeks sensitivity reading can be reasonably used for detecting resistance early. However, since the majority of strains (> 80%) are sensitive, the 2 weeks reading can be done selectively as and when required by the clinicians.

Application of the BACTEC method in the early diagnosis of pulmonary and extra-pulmonary tuberculosis, childhood tuberculosis and tuberculosis in the HIV-infected individuals

(Completed study, 1997)

Specimens from patients with pulmonary TB, extra-pulmonary TB, childhood TB and HIV TB was tested by the BACTEC system and concurrently tested by the conventional methods. Cultures positive by the BACTEC method were further subjected to the NAP differentiation test and drug susceptibility by the BACTEC methods. While there was no difference in the total number of positive cultures by the two methods in smear-positive pulmonary TB, the BACTEC method yielded a larger number of positives from among the smear-negative specimens. Further, the rate of isolation of positive cultures by the BACTEC method was significantly faster with 87% of the positives being obtained by 7 days. In extra-pulmonary TB, childhood TB as well as in HIV-TB, although the BACTEC method did not yield additional positives, the detection of positives was faster than with conventional methods in which the degree of growth was also scanty. The agreement in drug susceptibility tests was 94% each for streptomycin and isoniazid, 99% for rifampicin and 91 % for ethambutol. Further, a majority of these results by the BACTEC method became available by 8 days.

WHO - sponsored surveillance of drug resistance in tuberculosis in Tamil Nadu, South India

(Completed study, 1997)

This study, as part of the WHO/IUATLD global project on drug resistance surveillance in over 40 countries was undertaken in February-March, 1997 in collaboration with the Government of Tamil nadu. The aims of this study were:

a) To determine the prevalence of initial and acquired drug resistance in the survey area in order to use the level of drug resistance as a performance indicator for the National Tuberculosis Programme and to assess whether the recommended regimens are appropriate.

b) To establish the foundation for routine surveillance of drug resistance with procedures based on defined guidelines in order to observe trends in drug resistance in the survey area.

c) To promote external and internal quality control on laboratory procedure of susceptibility testing, in collaboration with the Supra-national Reference Laboratories.

The entire state of Tamil Nadu formed the survey area. All the District Tuberculosis Centres in the district head quarters and the x-ray centres in the taluk head quarters as well as TB Clinics attached to the medical colleges were identified as the participating centres. All smear-positive and previously untreated cases of tuberculosis registered in February-March, 1977 were included in the study.

A total of 400 cultures from as many patients, comprising of 384 from patients with no history of previous treatment and 16 patients with previous treatment was isolated. Resistance to isoniazid was observed in 15.4% of the former and 50% of the latter. Resistance to rifampicin was seen in 4.4% of untreated patients as against 25% of patients with previous treatment. These included 3.3% of patients with H + R resistance in the untreated group and 24.9% in the treated group. And

thus for the first time authentic basic data on initial and acquired drug resistance was made available from Tamil Nadu.

PCR-SSCP for the detection of rifampicin resistance

(Completed study, 1997)

The conventional bacteriological methods, used to detect drug resistance, take more than 4 weeks after the isolation of the primary culture. Molecular methods such as PCR-SSCP is being considered as an alternative method to detect mutations in the genes associated with drug resistance. A nested PCR was employed to amplify rifampicin resistance determining region (RRDR) of the **rpoB** gene of **M.tuberculosis.** Later the PCR products were subjected to SSCP analysis. Reannealing of DNA strands were found to affect the SSCP profiles. Hence, a novel strategy of generating biotinylated PCR product was explored to overcome this problem. The biotinylated DNA strand was separated from the unbiotinylated DNA strand using streptavidin paramagnetic beads. The individual strands were subjected to PAGE. This strategy was found to ease the identification of mutations in RRDR of **M.tuberculosis.**

Since the above protocol involved a nested PCR, which is expensive and time consuming, a modification was attempted to generate the biotinylated PCR products in a single PCR and the products were subjected to the SSCP analysis as before. This modification was used for the identification of rifampicin resistance in clinical isolates and it was found that 81 per cent of the results were in agreement with the conventional bacteriological tests.

Although this method can be employed in Molecular Microbiology Laboratory as screening procedure, much simpler, inexpensive molecular method need to be explored for that to be used in Diagnostic Laboratory.

HLA phenotyping in pulmonary tuberculosis patients and control subjects

(Completed study, 1994-97)

The main objective of the HLA study is to analyse the HLA-A, B, C, -DR and -DQ phenotype of a number of individuals to find out whether there exists an association between HLA antigens and the occurrence of pulmonary tuberculosis.

Serological determination of HLA-A, B, C, -DR and -DQ antigens was carried out in 209 pulmonary tuberculosis patients and 122 control subjects (50 patients' contacts, 72 healthy volunteers of the Centre)

The study revealed a significant increase of HLA-DR2 and -DQ1 antigens in pulmonary tuberculosis patients than the control subjects. ($P<0.01$ and $p =0.003$, respectively). The present study suggests that HLA-DR2 as such or in combination with other HLA and/or non-HLA genes may be involved in the susceptibility to pulmonary tuberculosis.

Non-HLA gene polymorphism studies in pulmonary tuberculosis patients

(Completed study, 1996-97)

The completed HLA studies carried out at this Centre suggested a significant increase of HLA-DR2 ($P<0.001$) and -DQ1 with pulmonary tuberculosis. However, HLA-DR2 association is a minor association and suggests the possible association of other Non-HLA genes in the susceptibility to pulmonary tuberculosis.

Human genome analysis revealed several candidate Non-HIA genes. Due to point mutations most of these Non-HLA genes occur as diallelic polymorphic forms. Such polymorphic genes have been shown to be associated with the susceptibility to a number of infectious and non-infectious diseases.

To find out whether Non-HLA genes are associated in the

susceptibility to pulmonary tuberculosis. The following Non-HLA gene polymorphisms were studied in 202 pulmonary tuberculosis patients and 109 control subjects.

IL-1 Receptor Antagonist (IL-1RA) gene: IL-1 RA is a cytokine which competes for IL-1 binding site and regulates the production of IL-1 . 86 base pair tandem repeat mini satelite polymorphism was studied.

Mannose-Binding Protein genes: Mannose binding protein plays an important role in the host defence against pathogens. Mutations in the genes of mannose binding protein results in low plasma level of this protein which leads to susceptibility to infection. Wild type and mutant alleles frequency of MBP 52, 54 and 57 was studied.

Vitamin-D Receptor (VDR) gene: Vitamin-D3 (1,25 dihydroxy vitamin-D3) is an immunoregulatory hormone and activates monocytes and stimulates cell-mediated immune response. These effects are exerted by interaction with the Vitamin-D receptor. VDR is a nuclear hormone receptor. Mutation in the gene leads to low expression of VDR which results in increased circulating Vitamin-D level.

Tumor Necrosis Factor alpha gene (TNFa): Tumor Necrosis factor alpha is an inflammatory cytokine mainly produced by monocytes and macrophages. This cytokine plays an important role in the pathogenesis of severe infectious diseases. Mutation at the promoter region of this gene affects the production of TNFa. Promoter region polymorphisms at -308 and -238 region were studied.

Natural Resistance associated macrophage protein-1 (NRAMP-1) gene: Identification of BCG gene in mice that affects resistance to several intracellular pathogens including some strains of Bacille Calmette Guerin (BCG). The equivalence for the BCG gene is the NRAMP-1 gene, which codes for the natural resistance associated macrophage protein which is involved in macrophage activation. CA repeat microsatellite polymorphism was studied in the human homologue of NRAMP-1 gene.

Inducible Nitric Oxide Synthase (iNOS) gene: Nitric Oxide has been shown to be microbicidal. Inducible nitric oxide synthase (iNOS) is

transcriptionally regulated enzyme that synthesise nitric oxide from L-arginine that has a key role in the pathophysiology of systemic inflamamtion. CA repeat microsatellite polymorphism was studied.

The above polymorphism studies were carried out as part of a Department for International Development(DIFD) Project and in a DFID fellowship at the Laboratory of Prof. Adrian V.S. Hill, Wellcome Trust Centre for Human Genetics, Oxford, U.K. Of the six non-HLA gene polymorphisms studied, Functional Mutant Homozygotes (FMH) of mannose binding protein gene is significantly (P = 0.008) associated with pulmonary tuberculosis (10.9%) patients when compared to control subjects (1.8%). No association has been found with the other non-HLA gene polymorphisms studied.

RFLP of isolates from different regions of India using DR probe

(Completed study, 1995-97)

In continuation of the RFLP studies carried out with DR probe to fingerprint the South Indian strains as reported last year we have also attempted to fingerprint the tuberculosis strains from various regions of India.

RFLP studies were carried out to determine the geographic variation or strain predominancy, if any, among **M.tuberculosis** clinical isolates from 6 different regions of India. In addition we have also compared the fingerprints of pulmonary tuberculosis isolates and extrapulmonary tuberculosis isolates using the direct repeat (DR) element as a probe.

A total of 98 strains of **M.tuberculosis** were obtained from the same number of patients with newly diagnosed pulmonary/extra-pulmonary tuberculosis. Each strain was coded with a random number so that no information regarding its geographic origin was available during RFLP typing. The 68 isolates from 6 different geographic regions were grouped into 26 RFLP types based on the number and molecular size of the bands obtained. The 18 extrapulmonary tuberculosis isolates typed showed 11 different RFLP types. The number of hybridizing bands

ranged from 2 to 7, and most of the isolates showed 5 to 6 bands. The isolates from Amritsar showed predominantly a specific RFLP type which is a interesting finding. It should be substantiated by analysing more strains.

Development of an experimental model for fibrosis

(Completed study, 1993-96).

This study was started with the aim of understanding the fibrogenic mechanisms associated with mycobacterial disease. Sensitized guinea pigs were challenged with live **Mycobacterium tuberculosis** and then sacrificed at various time points. In addition to culturing the bacilli, histological examination of the injected site, spleen, liver and lung for the presence of granuloma, organisms and fibrosis was made. Further, the levels of collagen, elastin and hexosamine were also estimated in these organs. Briefly, the following were noted:

1. Maximum numbers of **M.tuberculosis** were recovered from the spleen at the fourth week after infection and after the thirtieth week, only minimal organisms could be cultured from the spleen.

2. There was an initial decrease in the collagen, elastin and hexosamine levels in the lung, liver and the spleen upto 10th or 12th weeks after infection. They returned to pre-infection levels thereafter and between the twentieth and the fortieth weeks, there was an increase in collagen and elastin concentrations though these were not significantly different from those before infection. The changes were maximal in the lung and minimal in the liver.

3. Granulomata were seen maximally in the spleen followed by the lung and these were minimal in the liver. Changes indicative of fibrosis could be discerned using Van Gieson's staining of these tissues.

It is concluded that this model could be used to address further questions regarding basic fibrogenic mechanisms and to use antifibrotic drugs to modulate the development of fibrosis in tuberculous infections.

Immunopathology of cutaneous tuberculosis

(Completed study, 1992-97)

The aims and objectives of this study have been outlined in the previous annual reports. During the year under review, biochemical parameters of fibrosis such as collagen and elastin were estimated in the active and healed lesions of cutaneous tuberculosis. Further, the biopsy sections were stained for the presence of fibrosis using Van Gieson's staining and for the presence of fibronectin using immunohistochemical staining. It was found that the levels of collagen, elastin and fibronectin positive cells were significantly higher in the active compared to the healed lesions. Clinically also, the lesions healed with minimal scarring.

It was noted earlier that more than one third of the 250 patients with cutaneous tuberculosis who were tested with PPD showed an induration of 10mm or more on day 21. Therefore, biopsies of the Mantoux test sites on days three and twenty one were obtained from some of the patients. The biopsy on day 3 revealed infiltration predominantly by lymphocytes and macrophages while on day 21 , well-developed epithelioid cell granulomata were found. The lymphocytes in the biopsies on both days stained predominantly with anti- CD3 (pan T cell) antibody.

Evolution of dermal granuloma induced by antigen-antibody complexes containing M.tuberculosis in the guinea pig

(Completed study, 1996-97).

Immune complexes containing **M.tuberculosis** and anti **M.tuberculosis** antiserum in various proportions were injected intradermally into guinea pigs. The lesion was studied histologically from 6hrs to 84 days. It was found that while polymorphs and lymphocytes were the first cells to appear in the lesion, epithelioid cells started appearing from day 5-7 and reached a peak by 21 days. Histologically, the lesions started

clearing by the 42nd day and complete resolution of the granuloma occurred by the 84th day.

The sections were stained for the presence of acid fast bacilli and mycobacterial antigen also. It was found that intact bacilli disappeared first followed by stainable antigen and finally only the granuloma itself resolved. This finding has potential clinical application.

LABORATORY STUDIES - IN PROGRESS

WHO-assisted multicentric study of early bactericidal activity (EBA) of ofloxacin

(Ongoing study, 1994-98)

A WHO assisted multicentric study, of which this Centre is one of the participants is in progress from 1994. Laboratories from other countries which participate in this exercise include Kenya, South Africa and Hong Kong. The purpose and design of this study is given in 1995 annual report. This study on completion would un-equivocally prove the role of ofloxacin during the early phase of tuber-culosis treatment. And also for the first time a study on enumeration of number of acid fast bacilli in sputum smear collected at different time points is being evaluated following a standard method in order to evaluate its value against the well established culture method by viable count. This study is expected to be completed in 1998.

Action of pyrazinamide alone and in combination with isoniazid and rifampicin on M.tuberculosis in vitro under different growth conditions

(Ongoing study, 1994-98)

As reported earlier (annual reports 1994 & 1995) a study is being carried out to determine the activity of pyrazinamide alone and in combination with isoniazid and rifampicin and metronidazole alone on **M.tuberculosis** in vitro under different growth conditions.

The earlier experiment carried out in acidic $7H_9$ liquid medium (pH 5.6) at 37°C, showed that the growth at this pH is more or less similar to that of the $7H_9$ liquid medium at normal pH. Therefore it was planned to repeat the experiment using the BACTEC radiometric system at a pH of 4.8. The activity of the drug will be measured by the growth index (G.I) at an interval of 3 days and viable count will be set up at an interval of 7 days.

Simultaneously in another set of experiments it was found that pyrazinamide did not show any bactericidal activity on **M.tuberculosis** grown under conventional anaerobic condition for up to 12 days. The experiment is being repeated with a test model of dormancy as developed elsewhere and also exposing the **M.tuberculosis** strain for a longer period of time (22 days).

Evaluation of the activity of metronidazole alone and in combination with other anti-tuberculosis drugs

(Ongoing study, 1996-98)

As mentioned in the previous reports (annual reports 94 and 95) a study was carried out to look at the bactericidal and sterilizing action of metronidazole(M) alone and in combination with isoniazid(H) and rifampicin(R) on a drug sensitive strain of **M.tuberculosis** in vivo using the murine model.

The preliminary experiment yielded some interesting findings. Of the 8 groups included, the first 4 groups of animals were treated with H, R, M and HR respectively for a period of 3 months and the remaining 4 groups of animals were treated for 3 months with the same schedule as above but metronidazole was included in the beginning of the 3rd month of treatment.

The viable count(VC) of lungs and spleens was less in the group of mice treated with RM when compared to the group treated with R alone. Similarly, the spleen VC of animals treated with HM was less than H alone, thereby clearly showing the contributory role of M when added to the individual drugs at the end of second month. The initial decline in CFU observed with both spleen and lung in animals treated with H and R alone was not sustained and an increase in CFU was seen in both these organs at the end of 3rd month. However, as expected, the animals treated with HR combination was effective and no organism was detected from both these organs. Although a positive contribution of M was observed in containing the dormant mutants that are likely to emerge at the end of 2nd month and as seen in groups of mice treated with individual drugs, the significance of this finding could be ascertained in full only by including more number of animals in each of these groups. The work is being continued.

Slide culture in the determination of susceptibility of M.tuberculosis to anti-tubercular drugs

(Ongoing study, 1994-98)

The aim was to determine the criteria of resistance for isoniazid and rifampicin in slide culture technique. A total of 113 specimens were processed for slide culture sensitivity of **M.tuberculosis.** Of them, 35 with 3+ grade in either of the control cultures were included in the analysis. They included 28 rifampicin resistant and 7 rifampicin sensitive cultures as classified by the conventional indirect sensitivity test. A 3+ grade in 0.4 ug of rifampicin per ml was chosen as the definition of resistance, as this classified 19 of 28 rifampicin resistant cultures and 6 of 7 rifampicin sensitive cultures correctly. Similarly 3+ grade in 0.2 ug of isoniazid per ml classified 15 of 29 isoniazid resistant cultures and 4 of 5 isoniazid sensitive cultures correctly. Modification of the culture conditions are being studied to achieve better agreement between the two methods. The study is in progress.

Evaluation of bactericidal action of sultamicillin in comparison with rifampicin and isoniazid in the murine tuberculosis

(Ongoing study, 1997-2000)

Sultamicillin is a mutual prodrug of sulbactam and ampicillin that was developed to overcome the poor oral absorption of sulbactam when administered orally. Sultamicillin is readily absorbed and rapidly hydrolysed to provide high levels of its two constituents in equimolar concentrations and both ampicillin and sulbactam are widely distributed among various body fluids and tissues. Hence, a study has been undertaken to evaluate the activity of sultamicillin on **M.tuberculosis** in the murine model. The aim of this investigation is to study the bactericidal and sterilising action of different dosages of sultamicillin in comparison with rifampicin and isoniazid, given five days a week for 3 months on a drug sensitive strain of **M.tuberculosis** in vivo.

Utility of smear grading in the bacteriological assessment of tuberculosis patients

(Ongoing study, 1996-98)

The lowest grade used for grading positive sputum smears by fluorescent microscope comprises a very wide range in the number of bacilli present and therefore poses limitations in predicting the culture result of the same specimen. A study has been initiated to classify further the 1-plus smears in order to evaluate its utility in predicting culture positivity and also to help in determining the bacteriological status of the patients at different points during their treatment period. This study if completed, may provide additional information on smear positive culture negative phenomenon which is usually observed with short course chemotherapy treatment.

Protective effect of 65 kDa HSP vaccine against M.tuberculosis infection in the guinea pig model

(Ongoing study, 1997-98)

The earlier studies carried out elsewhere had shown that mice injected intramuscularly with 65 kDa HSP encoding mycobacterial DNA offered protection against challenge with **M.tuberculosis** infection and it was greater than the protection offered by the BCG.

The same vaccine is being tested at present using guinea pigs as a model, because the course of disease in guinea pigs is fairly similar to that in humans with remarkable similarities in the granulomatous and hypersensitive response between guinea pigs and humans.

The guinea pigs immunized with BCG and 65 kDa HSP are being challenged with **M.tuberculosis** $H_{37}Rv$ and a drug sensitive strain of South Indian Variant (SIV). The protective response of hsp 65 kDa HSP is being compared with that of BCG, based on the viable count enumerated from the spleens of animals sacrificed at the intervals of 12, 14 and 16 weeks after immunization. The experiment is in progress.

Quality assurance studies:

(i) External quality assurance studies in drug susceptibility testing of mycobacterial isolates

(Ongoing study, 1997-98).

One of the aims of the WHO/IUATLD global project on drug resistance surveillance undertaken in Tamil Nadu State was to promote external and internal quality assurance on laboratory procedures of susceptibility testing, in collaboration with a supranational Reference Laboratory. As a continuation of this, the Bacteriology Laboratory of this Centre was evaluated by the WHO collaborating Centre for Tuberculosis at Brisbane, Australia. This involves periodical testing of strains received from Brisbane for drug susceptibility to S, H, R and E at Chennai. Similarly, positive cultures isolated at Chennai were subjected to drug

susceptibility testing at Brisbane. So far, two rounds of this exercise have been completed and the agreement between the two laboratories has been nearly 100%.

(ii) Quality assurance programme in sputum smear microscopy

(Ongoing study, 1997-2000).

As part of the WHO-sponsored study on drug resistance surveillance study in Tamil Nadu, the WHO collaborating Centre for Tuberculosis at Brisbane, Australia sent a set of coded smears to be stained and examined at Chennai. The agreement between the laboratories at Brisbane and Chennai were found to be excellent.

Further, to assist in Govt. of India in the implementation of the RNTCP programme, this Centre has been assigned the task of carrying out Quality Assurance in sputum microscopy at different state TB demonstration and training centres as a continuing exercise. To start with a set of 100 stained smears have been despatched to each of 8 different laboratories. The results are awaited.

Pharmacokinetics of rifampicin and isoniazid in patients with renal failure on continuous ambulatory peritoneal dialysis

(Ongoing study, 1996-98)

The dosages of drugs employed for the treatment of tuberculosis in patients with renal failure are largely empirical, being about half of those administered to normal individuals. Such an empirical reduction could still lead to toxic plasma levels of the drugs in patients with severe renal failure.

To study the single-dose pharmacokinetics of isoniazid 7.5 mg/kg and rifampicin 12 mg/kg in patients with continuous ambulatory peritoneal dialysis and on the basis of these findings, to estimate appropriate dosages of these drugs to be employed in such patients.

This study was undertaken in collaboration with the Nephrology Department of the Apollo Hospitals, Chennai

Patients with renal failure who were on continuous ambulatory peritoneal dialysis were admitted to the pharmacokinetic study. Patients with impaired hepatic function were not admitted to the study. Further, patients on active chemotherapy including steroids in any form for other ailments or have received anti-tuberculosis drugs previously were also not eligible.

Plasma and peritoneal fluid concentrations of isoniazid and rifampicin were determined at 1, 2, 3, 6 and 8 hours following administration of isoniazid 7.5 mg/kg and rifampicin 12 mg/kg. Based on these findings, for plasma and peritoneal fluid, several pharmacokinetic variables, such as peak concentration, the time at which peak concentration is attained, coverage, exposure and half-life will be calculated for both the drugs.

The association between the mean concentrations of the 2 drugs in plasma and peritoneal fluid at different time-points will be examined and attempts will be made to find out a suitable dosage to be administered to these patients.

A total of 22 patients were admitted to the study and the investigations pertaining to the study has been completed. The results are being analysed.

Microsomal mixed function oxidases in experimental tuberculosis

(Ongoing study, 1994-98)

The role of cytochrome P-450 in the development of drug resistance is well established in bacteria and insects. Attempts were made to isolate cytochrome P-450 initially in **M.smegmatis** and later in **M.tuberculosis H_{37}Rv.**

It was then decided to purify the protein in **M.smegmatis** to homogeneity and further characterise it. The cytochrome P-450 that was isolated in the pellet is tightly bound to the membrane proteins. Cytochrome P-450 was then purified by passing the solubilised protein through octyl amino sepharose column and eluting it with 100mM

phosphate buffer pH 7.4 containing 1% sodium chelate and 0.1% emalgen 913. The P-450 enriched fraction was run on SDS-PAGE. Upon silver staining a prominent band with an approximate molecular weight of 50,000 daltons was obtained. Work is now in progress to purify completely the protein in **M.smegmatis** and **M.tuberculosis H_{37}Rv.**

The activity of cytochrome P-450 was compared between **M.tuberculosis** strains, sensitive and resistant to isoniazid. The drug resistant bacteria contained 1.60 nmol/mg protein of cytochrome P-450 while the corresponding sensitive ones had 0.73 nmol/mg protein of cytochrome P-450.

In **vivo experiment:** Male rats weighing about 150g, were used for this purpose. The animals were divided into 5 groups each group containing 4 animals.

Group 1 received 20mg/kg body weight of isoniazid
Group 2 received 15mg/kg body weight of rifampicin
Group 3 received a combination of isoniazid and rifampicin in the same dose.
Group 4 received saline and served as the control group.
Group 5 received a combination of saline and diluted HCI & served as control for the rifampicin group.

The drugs were given intraperitoneally for 3 days. The animals were sacrificed 48 hrs after the last injection. Cyt.P-450 content was determined in the liver by standard method. The amount of Cyt.P-450 increased to 0.49 nmol/mg protein in the presence of rifampicin compared to the corresponding control group which had 0.21 nmol/mg protein of Cyt.P-450. There was a slight decrease in the P-450 content when isoniazid was given. The values were 0.28 and 0.38 for the groups receiving isoniazid and the controls respectively. However, in the group which received rifampicin and isoniazid together there was no change in the values between the test and the control groups, the amounts being 0.35 and 0.38 nmol/mg prot. respectively.

Role of iron in intracellular growth of M.tuberculosis

(Ongoing study 1997-99)

Recent published data support the old hypothesis that some degree of nutritional deprivation of the macrophage, particularly iron, might be necessary for bactericidal action **in vivo** and **in vitro.**

Macrophages play an important role in iron recirculation as they are responsible for the catabolism of effete erythrocytes and subsequent release of iron to the circulation.

Study of the role of iron in the pathogenesis of tuberculosis, transferrin receptor expression, iron uptake, intracellular ferritin concentration and 'labile iron pool' might offer some insight in understanding the antimycobacterial activity of macrophages. The assay methods are being standardised.

Serum concentrations of neopterin in relapse and failure cases of pulmonary tuberculosis

(Ongoing study, 1996-98)

It was earlier shown that concentrations of neopterin in serum was significantly higher in patients than in healthy subjects and there was a steady decline during treatment.

Whether serum neopterin level in pulmonary tuberculous patients correlates with treatment failure or with relapse following bacteriological quiescence.

Among the patients admitted to current main study at the Centre, the following will be considered for this study; namely, those who have unfavourable response (failures) or those who relapse.

Matched controls for the above cases will also be included. Patients who remain negative upto the same time as the relapse / failure case will be taken as controls. As many controls as the cases will be selected. Controls will be matched for regimen, severity of disease on admission based on radiographic extent of disease and age.

Neopterin level will be determined at 0, 7, 3 months at the end of treatment (with samples collected for routine biochemical investigation) and at the time of relapse. Neopterin assay will be done by HPLC in these samples along with previously stored samples.

Ten confirmed relapse cases with matched controls have been admitted to the study till March 1997.

Monitoring bioavailability of rifampicin in fixed dose combination of drugs

(Ongoing study, 1997-99)

The Tuberculosis Research Centre has been chosen to participate in this network. Evaluate to bioavailability indices of fixed dose combination will be according to the protocol outlined by WHO.

The bioavailability of anti-TB drugs in triple drug formulations and to find out the feasibility of using these preparations in controlled clinical trials for pulmonary tuberculosis are being assessed.

In vitro quality appraisal: The first phase of **in vitro** measurement of rifampicin and dexacetyl rifampicin concentration of an initial 30 coded test serumlplasma and to 15 test urine samples to assess the standard of the laboratory's analytical expertise had been successfully completed.

The next phase of bioavailability assessment of anti-tuberculosis drugs either in volunteers or patients is under progress.

HLA-DR2 phenotype and plasma lysozyme in pulmonary tuberculosis

(Ongoing study, 1997-99)

Our earlier work on HLA-DR2 phenotype and plasma lysozyme, beta-glucuronidase and acid-phosphatase levels in pulmonary tuberculosis revealed a decreased level of lysozyme in the plasma of HLA-DR2 positive pulmonary tuberculosis patients when compared to DR2 negative patients. It is planned:

a. to find out whether binding of lysozyme on live H37Rv **M.tuberculosis** strain affect the viability of **M.tuberculosis.**

b. to find out whether the bacillary load (live **M.tuberculosis** affect the level of lysozyme.

c. to find out whether live H37Rv treated plasma lysozyme of H37Rv treated HLA-DR2 and DR2 negative patients with active disease has any effect on the spontaneous lymphocyte response and **M.tuberculosis** culture filtrate antigen induced lymphocyte response.

This study will be carried out in the plasma samples and lymphocyte response in 10 normal subjects.

HLA genotyping: DNA typing in pulmonary tuberculosis patients and control subjects

(Ongoing study, 1997-99)

Studies on the serological determination of HLA-A, -B, -C, -DR and -DQ antigens revealed an increased phenotype frequency of HLA-DR2 antigen ($P<0.001$). To find out the sub types of DR2 antigen at the DNA level, genotyping has been planned. HLA-DR2 positve patients' DNA samples will be subjected to Polymerase Chain Reaction using specific primers. HLA-DR2 genotyping will be carried out by dot-blot technique using specific oligonucleotide probes. The study will be carried out in HLA-DR2 positive patients (n = 100) and control subjects (n = 50).

Studies on HLA and Non-HLA gene polymorphism in tuberculous spine (Extrapulmonary tuberculosis)

(Ongoing study, 1997-99)

Studies on antigen profile in pulmonary tuberculosis revealed a significant increase of HLA-DR2 in PTB patients. To find out whether HLA-DR2 or any other antigen (both HLA-Class I and Class II antigens) is/are associated with extrapulmonary forms of tuberculosis. A study on HLA and tuberculous spine has been initiated. The study will be carried

out in 50 to 75 TB-spine patients and 50 to 75 control subjects consisting of spouses (family contacts) of TB-spine and PTB patients. During this period serological determination of HLA-A, B, DR and DQ has been typed in 25 patients and 10 family contacts of the patients.

Non-HLA gene polymorphism such as IL-1 receptor antagonist, Vitamin-D receptor and Tumor necrosis factor and mannose binding protein gene polymorphism will also be studied.

Identification of the promoter of amidase gene for expression of useful mycobacterial genes

(Ongoing study, 1996-2000)

Identification of a regulatable mycobacterial promoter is essential to drive the expression of important mycobacterial genes. We chose to identify the promoter of mycobacterial amidase because it is the first inducible promoter identified in mycobacteria. The amidase gene has been cloned and the sequence have been published. We designed primers from the sequence of the upstream region of the amidase gene. The fragments were constructed by PCR technology. These were used as probes to identify the size of the amidase transcript-on a nothern blot. The RNA was isolated using two different methods from induced and uninduced cultures of **M.smegmatis.** This RNA was found to be suitable for northern blot and primer extension analysis.

The northern blot revealed 2 products of 3kb and 1.2kb. These 2 bands were recognised by all four probes of the upstream amidase gene. These results suggests that the whole amidase transcript is 3kb in size and is then processed further.

In order to identify the transcription start sites, primer extension analysis was carried out. Oligonucleotides (18mers) complementary to the various regions upstream of the coding region were used in the primer extension reactions. The primers were designed to hybridise 200 bases apart since this provides optimal sensitivity in the primer extension reaction. Of 12 oligonucleotides used, gave consistent products. The two primer extension products were confirmed and the transcription start sites were deciphered by running sequencing ladder generated by using the respective primers and the template. From primer extension studies

and northern blot studies, it is clear that amidase is regulated by polycistronic message. A full product of 3.0kb is formed which is further processed at a single site to give amidase gene product of 1.2kb and the other product coding for ORF 1,2 and 3.

The exact region of the promoter has been reduced and this will be used to express the important mycobacterial proteins.

Cytokine profiles in pre and post BCG vaccinated adult population- Analysis by PCR detection of cytokine mRNA and ELISA

(Ongoing study, 1993-98)

In continuation of the annual report of 1995, the methodology for reverse transcriptase polymerase chain reaction (RT-PCR) was standardised. The optimal conditions for each cytokine PCR with their respective primer pairs were set. Only IL12 failed to give any ‘amplification while IL6 and IL10 gave a lot of nonspecific background and the conditions were further optimised by using Invitrogen optimiser kits.

Having optimised the assay conditions, it was then necessary to carry out preliminary experiments on a limited number of samples in order to (i) determine the optimal time point at which to look for cytokine gene expression, and (ii) determine which mycobacterial antigens gave the optimal response. As a result of these experiments we decided to concentrate an the 48 hrs time point and to restrict the antigens to PHA, PPD and heat killed **M.tuberculosis.** It was also decided from these initial screening experiments, to focus first on the T cell cytokines, i.e. IFNr, IL4 and IL10 to see whether the vaccination has influenced a Th1 or Th2 type response.

Totally 12 pairs of BCG vaccinated subjects were screened for IFNr, IL4 and IL10 cytokine expression. Also 12 subjects from the PPD positive group were screened for the same cytokines. All the results of these experiments were expressed in relation to expression of the house-keeping gene B-actin using a semi- quantitative scoring systems. Quantitative techniques for cytokine gene expression are currently underway.

In order to support the results on cytokine gene expression using RT-PCR, we also investigated cytokine protein production by ELISA. These results demonstrated increased levels of IFN-r production following stimulation with mycobacterial antigens in the PPD + ve population, reflecting a Th1 type of response.Interestingly, there was no evidence of a Th1 response in the PPD-ve population even following vacicnation with BCG.

Evaluation of the indigenously developed probe (pTRC4) for diagnosis

(Ongoing study 1996-98)

Nucleic acid amplification technique or PCR is a promising approach for rapid diagnosis of **Mycobacterium tuberculosis** infections. Several procedures have been described to detect **M.tuberculosis** genome, the target sequence being the main difference. PCR has been found to be promising for diagnosing extra pulmonary tuberculosis because even culture for **M.tuberculosis** which is considered the gold standard is low in sensitivity in these forms of the disease.

We have developed a DNA probe by constructing a genomic library of **M.tuberculosis** in a plasmid pGEM4Z vector. Selection of the recombinant clones was by hybridisation with 32P labelled **M. tuberculosis.** As mentioned in the previous years the clone pTRC4 has been selected on the basis of its specificity for the **M.tuberculosis** complex only and not for any other non mycobacterial species or atypical mycobacterial species. This mycobacterial fragment TRC4 is a 2.1 kb insert has been sequenced and primers for PCR have been designed. One set of primers which amplifies 173 bp of the target has been chosen for evaluation.

Fifty pleural effusion samples, 100 CSF samples and 40 lymphnode samples from patients suspected to have tuberculosis and normals have been included for evaluation of PCR using both IS6110,the most widely used primers and the indegeneous primers developed by us (pTRC4). Among the 50 pleural effusion samples 33 were from patients who had clinical evidence of tuberculosis and 17 samples did not have clinical evidence of tuberculosis. Since culture is less sensitive to detect extra-pulmonary tuberculosis especially, TB meningitis and pleural tuberculosis, we used the clinical evidence including response of patients to anti-

tuberculosis drugs as a gold standard. When clinical criteria and response to ATT were used as standard the sensitivity and specificity obtained by PCR, culture and adenosine deaminase activity are 100% and 85%; 55% and 100% and 77% and 72% respectively.

These studies highlighted that PCR can be used as an additional test to detect **M. tuberculosis** from extra-pulmonary tuberculosis in a shorter time. Quality control is very essential while using PCR for diagnosis because of its frequently reported false positivity. The evaluation of the pTRC4 primers in detecting **M.tuberculosis** in TB lymphadenitis and TB meningitis samples is in progress.

Characterization and purification of antigenic components of M.tuberculosis

(Ongoing study, 1988-98)

Under this Project, the laboratory is now interested in purification of 3 antigens from **M.tuberculosis** $H_{37}Rv$, namely 17, 30 (Antigen 85 Complex) and 38 KDa. It has been reported in previous Annual Report (1995, Page 46) that 30 KDa antigen has been purified by a combination of Preparatory IEF and SDS-PAGE Preparatory electrophoresis. It was also observed that a combination of Anion exchange chromatography, followed by phenyl sepharose hydrophobic interaction would separate 30 and 38 KDa from the rest of the antigens and from each other as well.

Purification of individual components 85A, B and C of the secreted Antigen 85 Complex from culture filtrate and 17 KDa from $H_{37}Rv$ cell membrane were completed during this year. In addition, peptide mapping of Antigen 85 C was also carried out.

For further characterization of the identity of the antigens, their reactivity pattern with available monoclonal antibodies were studied and Western Blot results are shown below.

Mab (WHO)	Isotype	Antigen recognised
IT 23	IgG 2b	38 KDa
IT 49	IgG	30 KDa
IT 49	IgG	31 KDa
IT 4	IgG 1	17 KDa
IT 20	IgG 1	17 KDa

Purified Antigen 85°C was further cleaved into smaller fragments, so that the most specific peptide can be evaluated in diagnostic assays. A panel of 4 proteolytic enzymes were employed with the following specificity:

Endoproteinase Asp-N	-	-Asp-X-
Endoproteinase GIu-C	-	Glu- -X-
Trypsin	-	Arg/Lys- -X-
Endoproteinase Lys-C	-	Lys- -x-

The digests was immunoblotted with pooled sera from tuberculosis and endemic normal subjects.

The tryptic digest shows a peptide of mol.wt 13 KDa app, which is specifically recognised only by pooled tuberculous sera and not by pooled endemic normal sera. The predicted sequence of the peptide is as follows:

GVSPTGNAAVGLSMSGGSALILAAYYPQQFPYAASLSGFL
NPSEGWWPT
LIGLAMNDSGGYNANSMWGPSSDPAWK

In future experiments, this peptide will also be evaluated in diagnostic assays, along with purified antigens.

Role of natural killer cells in tuberculosis

(Ongoing study, 1997-98)

Natural killer (NK) cells were first discovered about 20 years ago during studies on cell mediated cytotoxicity. It is believed that the natural killer cell system is a NATURAL RESISTANCE MECHANISM of the host, providing an immediate and nonspecific line of defense against tumor cells and also against a wide range of bacterial, viral, fungal and parasitic infections. NK cells have been characterised as a subset of lymphocytes that are able to lyse target cells even without prior sensitisation or major histocompatibility restriction. In addition to their cytotoxic functions, noncytotoxic functions such as the ability to produce cytokines in response to various stimuli have also been demonstrated. Hence they assume importance as immunoregulatory cells. A number of possible mechanisms of natural killer cell activity have been suggested. Some studies have shown a direct bactericidal/ ungistatic activity by NK cells against organisms like Salmonella

typhimurium and Cryptococcus neoformans. Alternatively NK cells could bind and be cytotoxic to target cells by releasing secretory factors like perforins, NKCF, etc. A third possibility is that they could activate macrophages and neutrophils to release cytokines thereby killing intra-ellular pathogens. The pathogen causing tuberculosis is predominantly found intracellularly within macrophages. Existing literature in this area has resulted in a lot of controversies and contradictions as regards the role played by the NK cell populations in diseases such as tuberculosis. Some authors have suggested that NK cells can play an important role by causing lysis of autologous monocytes infected with Mycobacterium tuberculosis and exposing the intracellular pathogen to the more hostile host environment but some researchers feel that NK cells can stimulate the macrophages to bring about killing of the intracellular pathogen & this activity is augmented by cytokines like IL2, IL12, IFN, TNF, etc.

Hence, a study was undertaken to elucidate the role of NK cells in tuberculosis. The first part of the objective was to compare the NK status between normal and tuberculosis individuals. From the experi-ental data analysed so far the number of NK cells (CD16 + CD56 positive) is 14.1 ± 4.3 percent in normals and 11.3 ± 5.5 percent in tuberculosis patients. The NK activity of normal individuals is 28.2 ± 12.4 percent whereas it is 9.1 ± 6.5 percent in tuberculosis patients. Hence, although the number of NK cells appears to be the same the NK activity is lower in TB patients than in normal individuals.

Since the cytokines IL2 and IL12 form an important part of the cytokine network that are involved in the cross-regulation of various acessory molecules of the immune system like macrophages its effect on NK activity was studied. IL2 stimulation augments NK activity on dat 0 as well as day 3 in case or normal individuals whereas in the case of TB patients there is no response to IL2 on day 0; however when the cells were cultured in vitro for 3 days the NK activity increases almost upto normal levels. However no response to IL12 stimulation can be observed and IL12 does not augment the IL2 response.

The conjugate study data indicate that, of the total number of conjugate cells 65% of them are CD3 conjugates and 34% are NK conjugates, but since the proportion of CD3 cells far exceed the NK cells, it is found that only 34% of the CD3 cells form conjugates while 69% of NK cells form conjugates. By this method it will be possible to define at what stage there is a defect in the cytotoxic mechanism in certain

individuals that is, whether it is at the recognition and binding stage or at the subsequent steps involved in triggering lysis of the target cell. The study is in progress.

Mycobacterial promoters - a molecular study

(Ongoing study, 1997-98)

Despite the medical importance of members in the genus Mycobacterium, the knowledge of mycobacterial gene expression signals has been poorly understood. This difficulty has mainly arisen because of the slow growth rate, undeveloped method of genetic exchange and paucity of genetic tools to manipulate the organism. Understanding gene regulatory mechanisms in pathogenic mycobacteria may aid in understanding the virulence mechanisms and the development of new vaccines and more effective medical interventions.

Recently, a few mycobacterial promoters coding for structural genes have been studied. It is difficult to assess the nature of transcriptional signals in mycobacteria based on the studies of specifically regulated genes. Hence, a better understanding of constitutive gene expression will be made possible by the analysis of randomly isolated promoters by using a promoter probe vector.

We have employed a promoter probe vector carrying a promoterless **lacZ** gene capable of replicating in **E.coli** and mycobacteria and encoding kanamycin resistance as a selection marker. About 25 promoter clones expressing Beta-galactosidase to varying levels have been cloned. The size of the cloned inserts have been sequenced completely and the transcription start point (STP) of at least three promoters have been determined using primer extension analysis.

The Beta-galactosidase levels of the cloned promoters showed interesting features. A few **M.tuberculosis** promoters expressed high levels of Beta-galactosidase in **E.coli** than in **M.smegmatis.** Studies are underway to determine the transcription start point of these promoters and to explore the possibility of driving the gene encoding the 38 kDa protein of **M.tuberculosis** in both **E.coli** and **M.smegmatis** host systems.

Preliminary studies based on TSP have revealed high homology with the -10 consensus sequence of **E.coli** and very little homology with the -35 region. More promoters are been characterised in order to have a better understanding of the transcritpional regulation in mycobacteria.

It is believed that understanding transcriptional signals of mycobacteria will improve our knowledge about gene regulation in mycobacteria and pathogenic mechanisms employed by these organisms at the molecular level. These studies will also help in the development of better expression systems capable of driving mycobacterial genes of immunological importance in a homologus system.

Studies on fibrosis using in vitro fibroblast culture

(Ongoing study, 1996-99)

The molecular mechanisms underlying fibrosis may be better understood by using an **in vitro** system in which cultured fibroblasts are subjected to fibrogenic stimuli. Further, the modification of the response of the cells by drugs also can be studied using this system. Fibroblasts obtained from guinea pig are cultured and after the occurrence of confluent growth, these will be transferred to a 24 well plate and treated with supernatants of primed guinea pig spleen cells stimulated with **M.tuberculosis.** Further, it is proposed to look at the expression of fibrogenic cytokines secreted using in situ hybridization procedures and production of collagen and elastin using 14 C-labelled hydroxyproline by these cells.

EPIDEMIOLOGICAL STUDIES - COMPLETED

Testing of children for comparison of 1TU - RT23 and 3IU of PPD-S

(Completed study, 1995-97)

The purified protein derivative from strain RT23 (PPD- RT23) has replaced that from Seibert strain PPD-S as the antigen for tuberculin testing due to the non-availability of the latter. There is no information

in the literature on the comparability of the PPD-S with PPD-RT23. This comparison was undertaken in the surveillance study area in Thiruvalangadu Panchayat Union, during the first re-survey. In this study, reaction sizes to 1TU RT23, given simultaneously with distilled water or 31U of PPD-S, were compared. In all, 3345 children aged 0-9 years were registered. Of these, 1673 were test read for the both PPD-S and PPD-RT23, the others being a comparison of 1TU RT23 with distilled water. Among the 1673 children, 919 had a BCG scar and 754 had no scar. The mean size of reaction to PPD-S among those without scar was 4.91mm in the 0-4 age group and 6.75 mm in the 5-9 age group. Correspondingly, it was 4.48 mm and 6.61 mm to 1TU RT23 in the 0-4 and 5-9 year age groups, respectively. The distribution was very similar and the antimode by either antigen was 12 mm. The percentage of reactors was 8.15 in the 0-4 age group for PPD-S and 5.56 for 1TU-RT23. Among the 5-9 age group this was 20.66% for both the antigens. Thus PPD-1TU RT23 is comparable with PPD-S in estimating the prevalence of infection.

Site preparation for the evaluation of vaccines for typhoid fever

(Completed study, 1995-96)

A survey was undertaken to obtain epidemiological information for a proposed Typhdid Vaccine Trial in Tiruvellore Taluk of Chingleput District. Twice-weekly surveillance was established in 4 of the eight villages for active surveillance from the 20 villages selected from Poondi Panchayat Union covering two PHIs at Katchur and Poondi and a Govt. Hospital at Uthukkottai. The remaining villages were covered for passive surveillance through these centres.

During the survey period, 390 visits were made in the 4 active surveillance villages, comprising a population of 4406 and individuals satisfying the criteria for blood collection were directed for clinical examination and blood collection. Out of the 400 persons eligible for blood collection, blood specimens were collected from 331 (83%) persons and 5 have become culture positive. In passive surveillance, 451 visits were made to the 3 health facilities. In the meantime, GH-Uthukkottai was excluded for passive surveillance due to lack of referrals from the study area. Blood specimens were collected from 312 (68%) persons from

among the 461 referred from these health facilities and 5 have become culture positive.

It was observed that the contamination rate was more in children (25%) as compared to that of adults (11%). Of the 10 culture positives, 3 cases were clinically typhoid only, 39% of the eligible cases were diagnosed as clinically typhoid.

All the procedures for the survey including referral for clinical examination, blood collection and processing of the specimen using the BACTEC method were standardised. The clinical findings of typhoid fever does not appear to be typical. So we need to define criteria for blood collection and clinical diagnosis of typhoid.

Surveillance of more than a year has yielded a very low prevalence in the rural area. Random visits made to the remaining four villages has not changed the trend of the disease. Hence the rural area may not be a suitable site for vaccine trials.

Surveillance of individuals infected with the Human Immuno-deficiency Virus (HIV) for the development of tuberculosis

(Completed study, 1989-97)

A longitudinal cohort study was started in July 1989 with the objective of monitoring the occurrence of tuberculosis among patients with HIV infection.

Patients identified to be positive for HIV infection of ELISA testing from the various surveillance centres (Chennai, Vellore and Pondicherry) are included. They are registered in the Centre and followed up at 6-monthly intervals with clinical examinations, comprehensive sociological assessment and detailed investigations.

The family members including the spouse and other sexual partners are also registered and followed-up to study the pattern of transmission of HIV infection.

The study cohort contains 241 HIV positive patients of whom 105 had tuberculosis (84 at registration and 21 during follow-up). They were treated with $2EHRZ_7$ - $7RH_7$ or routine 8-month short course regimen at the nearest centre. Twelve of these had multi-drug resistance.

Sixty-five patients had died over a period of 78 months follow-up, of whom 38 had tuberculosis. Tuberculosis was the commonest cause of death.

Seven year follow-up has been concluded for all patients recruited to the study. Two percent of HIV infected individuals developed tuberculosis every year. The mortality in individuals with HIV and TB was 5 times that in HIV infected persons without TB.

EPIDEMIOLOGICAL STUDIES - IN PROGRESS

Development of surveillance methodology for tuberculosis

(Ongoing study, 1997-2000)

This is a long term epidemiological study undertaken in BCG Trial area with high non-specific sensitivity, with a view to identify a simple, inexpensive tool for the surveillance of tuberculosis in the community. The following are the parameters being studied:

1. Age specific prevalence of infection and its trend.
2. Age-sex specific rates of disease prevalence and trend.
3. The proportion of chronic excreters among prevalence cases and other drug sensitivity, status.

The methodology has been described in detail in the annual reports of 1990 and 1991. The planned intake of about 100,000 could not be completed due to lack of X-ray units even during the year reported. So, also the 30th month Selective Follow-up (SFU) could not be taken up. The selective follow-up for 48 and 60 months was completed in the remaining villages of Kadambature Panchayat Union and 48 month follow-up in Thiruvalangadu. The first resurvey was completed in all the remaining villages of Thiruvalangadu Panchayat Union.

The coverages obtained for the follow-up round and resurvey are found to be maintained at high levels for all examinations like X-ray, putum and tuberculin testing. However, the time schedule could not be kept up due to various technical reasons.

In all, 114 sputum positive cases were diagnosed during the year 1996. Of these, 49 were from follow-up round and the remaining from resurvey. Ninety-two cases were positive only on culture; 22 were positive on smear and negative by culture. Of the total culture positives, drug sensitivity results were available only for 71 cases; of these, 64 (90%) cultures were sensitive, 7(10%) had a history of previous treatment.

Management of cases: Sputum positive cases were referred for anti-tuberculous treatment with Short Course Chemotherapy (SCC) at the nearest Primary Health Centre. Information on their symptomatic status and drug regularity status was obtained along with two specimens of sputum.

Passive case finding: A total of 2349 symptomatics were registered in all PHCs and sputum collected. Of these, 248(11%) became sputum positive and were put on treatment with SCC by the medical officer of the health facility.

Multicentric study for diagnostic criteria in childhood tuberculosis

(Ongoing study, 1995-98)

The diagnosis of childhood tuberculosis has remained one of the most controversial issues. Since bacteriologic confirmation is possible only in 20-30% of cases, the diagnosis is usually based on clinical, epidemiologic and radiologic features and the tuberculin test results. Although several diagnostic schemes have been suggested and tried out, reliable and objective criteria are yet to be developed. To be applicable on a wide scale, the criteria developed should be simple, reliable and valid and as specific for tuberculosis as possible. The task force convened by the ICMR, examined the available information and made the following recommendations for diagnostic criteria:

1. Persistent radiological lesion even after 4 weeks of adequate non-specific antibiotic treatment.

2. X-ray lesion with a positive Mantoux test or with AFB on smear.
3. Histopathology proved in cases of glandular swelling.

It was therefore proposed to do a multicentric study to evaluate the task force criteria and the study was started in January 1995.

The centres involved in the study are the Institute of Child Health, the Pediatric departments of the Stanley Medical College, Sri Ramachandra Medical College and the Child Trust Hospital, Chennai. Children aged 6 months to 12 years will report to these centres and those considered referable to TB clinic will form the study population.

So far, 2749 children have been registered and all examinations were completed in 90%. All symptomatic children are followed up at 2 weeks, 4 weeks, 8 weeks and at 3 monthly intervals upto 1 year and are examined clinically and chest X-ray, Mantoux test and gastric lavage for AFB smear and culture are done. In all, 15379 specimens were collected and among them 311 were found to be bacteriologically positive. Among 112 biopsies done, 55 were reported as positive on histopathology.

So far, 433 children have been started on anti-tuberculous drugs on the guidelines of ICMR; among them 172 based on clinical features and 220 on bacteriological basis. A panel of doctors will review the case status and reason for anti-TB treatment once a month.

Improving the specificity of diagnosis of smear negative, X-ray positive pulmonary tuberculosis

(Ongoing study, 1997-98)

The main objective of this study is to improve the specificity of X-ray cases by minimising the false positives by following methods: (a) X-ray to be read by three independent readers and considering as X-ray cases only those read by at least two readers as cases (b) giving a course of broad spectrum antibiotics for 10 days and reviewing the X-ray lesions. (c) gastric lavage for culture (d) sputum culture for all x-cases (e) follow-up of X-ray cases with X-ray and culture (sputum) every month upto 3 months and thereafter once in 3 months upto one year. This study is being conducted at Government Royapettah Hospital's TB Clinic.

This study was started in Sep tember 1997. Upto December 1997, 578 individuals were referred to the TB Clinic from Medical O.P., of which 237 symptomatics were registered for this study. 184 symptomatic were smear negative and X-ray negative. 34 symptomatics became bacteriologically positive (smear or culture). 19 symptomatics were smear negative X-ray positive.

Correlation of population characteristics with disease frequency

(Ongoing study, 1997-98)

Prevalence data are available for 30 panchayat in Kadambathur and Tiruvelangadu panchayat unions from the surveillance study. Population characteristics like mean literacy level, occupation and population density will be collected from all these panchayats and the relationship between these population characteristics and the prevalence rates will be studied. This study was started in September 1997. Upto December 1997, 5821 individuals were interviewed to collect data on population characteristics.

Tobacco smoking and pulmonary tuberculosis

(Ongoing study, 1997-98)

Bacteriological cases were diagnosed during the l-resurvey of the surveillance study. From among these cases, males aged 20 to 50 years were included in this case-control study. Male controls aged 20-50 years were selected from the study area in the ratio of 1 :5. Both cases and control groups were interviewed fro assessing tobacco smoking habits among them. This study was started in September 1997. Upto December 1997, 447 individuals were attempted for interview.

Surveillance of HIV infection in tuberculosis patients

(Ongoing study, 1997-98)

This study was initiated with the aim of studying the following objectives:(1). to study the prevalence of HIV infection among new

tuberculosis patients, (2). to study the trend of HIV infection in tuberculosis patients and (3). to identify those tuberculosis patients most at risk of having HIV infection.

The study was started with the collaboration of and funding from the State AIDS Control Society, at four centres i.e. Vellore DTC, Pennathur Sanatorium, Kancheepuram DTC and Government Thiruvotteswarar Tuberculosis Hospital, Otteri, Chennai. Kancheepuram DTC was started in September 1997, Otteri TB Hospital in November 1997 and Vellore (2 Centres) in December 1997. So far, 247 patients have been registered in Otteri TB Hospital of which 5 were HIV positive by ELSA on two occasions, 244 from Kancheepuram DTC of which 2 were HIV positive, 138 from DTC Vellore of which 2 were Positive and 212 were registered in Pennathur of which 20 were HIV positive by ELISA on two occasions.

LIBRARY & INFORMATION SERVICES

The Library and Documentation Centre continued to serve the doctors, researchers and faculty of most of the medical colleges and medical research institutions in Chennai. In addition, several other services such as Tuberculosis Alert, a fortnightly computerised Selective Dissemination of Information (SDI); MEDLINE searches on the CD-ROM Diskettes (1991 + 1); Online Search of Bibliographic databases at NIC; E-Mail & Bulletin Board and other network facilities through the SIRNET, CSIR and the RENNIC, NIC, New Delhi; Updating and maintenance of Bibliographic databases, viz., Library Book Catalogue (LIBCAT), the TUBERCULOSIS, TRC publications (TRCPUB), journal holdings (SERHOLD), MAIL LIST etc., CC on Disc : Life Science services, Resource sharing with Vector Control Research Centre, Pondicherry, Institutional membership facility at the British Council Library, Chennai and publication of the quarterly TRC Bulletin were also carried out.

The Centre is connected to the Gateway Internet Access Services (GIAS) of the Videsh Sanchar Nigam Limited (VSNL) for full fledged Internet facility.

The National Database Project on Tuberculosis and Allied Diseases, a joint venture by TRC and NIC, is now available on the Information Superhighway. One of the first of its kind in India.

APPENDICES

TRAINING PROGRAMMES

Post-graduate students, medical and para-medical personnel and technicians from other institutions in Chennai and outside underwent one- to four- week training in various departments of the Centre.

In addition, one- or two-day training programmes were arranged at the Centre for batches of medical students, post-graduates, nursing students and para-medical personnel.

STAFF DEVELOPMENT PROGRAMME

1. Dr. V.K. Vijayan was awarded the D.Sc. Degree by the Tamil Nadu Dr. MGR Medical University, Chennai, during 1996.

2. Dr.D. Vijayabhaskara Rao was awarded Ph.D. in "Some contributions to reliability systems" by the Venkateswara University, Tirupathi, A.P., during 1996.

3. Dr.D. Sulochana Das was awarded a 6-month fellowship under the British Government ODA programme to work on BCG project at the National Institute for Medical Research, Mill Hill, London, from March, 1996.

4. Dr. K. Sadacharam, Mr. R. Selvaraj, Mr. S. Sathyamoorthy, Mr.S. Janakiraman, Mr.J.S. Visuvasan, Mr.P. Nagarathinam, Mrs.K. Jaggarajamma and Mrs.Mohanarani Suhadev underwent training in "Methods of nutritional surveillance and assessment" at the Communication and Training Wing of the Tamil Nadu Integrated Nutrition Project, Taramani, Chennai, during March, 1996.

5. Mr. N. Rajendran and Mr. D. Thangaraj underwent a 1-year training in Laboratory Technician Course at the Central Leprosy Teaching and Research Institute, Chengalpattu, from April, 1996.

6. Dr.Daniel Herbert underwent a 5-week INDO-US VAP training programme at the Centre for Disease Control, Atlanta, from April, 1996.

7. Mr.M.G.Sreekumar was awarded a 6-month U.S. Fulbright Visiting Research Fellowship in Library and Information Science for 1996-97 at the National Library of Medicine of National Institute of Health, Maryland, USA, from July, 1996.

8. Dr.Alamelu Raja participated in a 3-month Indo-US Vaccine Action Programme at the Laboratory of Dr. Patrick J. Brennan, Dept. of Microbiology, the Colorado State University, Fort Collins, USA from August, 1996.

9. Mrs. Geetha Ramachandran underwent a 1 -month training in "Studies related to cytochrome P-450 in experimental animals and **M.tuberculosis"** at the Department of Biochemistry, the Indian Institute of Science, Bangalore, from September, 1996.

10. Dr.P. Selvaraj was awarded a 6-month British Government ODA Fellowship to carry out Non-HLA gene polymorphism studies/training in pulmonary tuberculosis at the Wellcome Trust Centre for Human Genetics, Oxford, UK, from November, 1996.

11. Dr. Soumya Swaminathan was awarded a 3-month scholarship in the Division of Infectious Diseases, Department of Medicine, the USC, Los Angeles, USA as an Exchange Scholar of the NIH Funded study on "Cellular immune response to **M.tuberculosis** in children", from November, 1996

12. Mr. S.I. Eusuff underwent a 1-week training in "Computer software course in FoxPro", at the Triumph Information Technology Ltd., Chennai, during November, 1996.

13. Dr. P. Venkatesan was awarded the "Master of Population Studies (MPS) degree" by the International Institute of Population Sciences, Mumbai, during 1997.

14. Dr Rema Mathew, Assistant Director was awarded a WHO Fellowship on "Modern concepts of biomedical ethics in human and animal research" for 3 months from 25th August, 1997, at the Kennedy Institute of Ethics, Georgetown University, Washington DC, USA.

15. Dr.Vanajakumar was awarded a 9-month WHO Fellowship for training in 'Molecular Biology of Mycobacteria' at Albert Einstein College of Medicine, Bronx, New York, USA.

16. Dr.C.N.Paramasivan was awarded D.Sc. degree by the University of Madras, Chennai, during 1997.

17. Dr. N.Selvakumar was given ICMR Award for BioMedical Research For Scientist Belonging to under privileged Communities for the Year 1995.

18 Mrs. Geetha Ramachandran, underwent training at the Biochemistry Department of the Indian Institute of Science, Bangalore, during August 1 997 in connection with the project entitled "Microsomal mixed function oxidases in experimental tuberculosis"

SPECIAL TRAINING PROGRAMMES/WORKSHOPS/SYMPOSIA ORGANISED BY THE CENTRE JOINTLY WITH NATIONAL / INTERNATIONAL AGENCIES

SYMPOSIUM ON THE RECENT ADVANCES IN DIAGNOSIS OF TUBERCULOSIS

A Symposium sponsored by Becten Dickinson, India, was organized to highlight the recent advances in the diagnosis of tuberculosis by Tuberculosis Research Centre, Chennai and Becten Dickinson, India at Tuberculosis Research Centre, Chennai on 26th April, 1996. Presentations were made by experts in the field. This was followed by open house discussion.

Two demonstrations, one on "MGIT" and the other on "Bactec 460" were given.

TRAINING FOR LEPRA INDIA

A 4-day training programme on TB was organised at the Centre for the staff of Lepra India between 26.11.96 and 29.11.96. The participants were medical officers and paramedical staff of Lepra India. Components of the National Tuberculosis Control Programme viz., epidemiology, clinical features, case finding, case holding, chemotherapy, documentation and social aspects were discussed.

TRC/DANIDA/WHO PARTICIPATORY TRAINING FOR MEDICAL OFFICERS AND LAB TECHNICIANS

A 10-day participatory training programme on RNTCP was organized at the Centre by TRC/DANIDA/WHO in May, 1997. Eight senior technical officers, 6 from Orissa and 2 from Andhra Pradesh and 5 laboratory technicians from Orissa participated in the programme. Instead of conventional class room teachers, for the first time, a participatory training was organised using 10 training modules on different aspects of RNTCP. This helped to refix the training modules with inputs from teaching programme and research personnel.

TRC/NACO WORKSHOP ON "HIV/TB"

A workshop on HIV/TB was held at the Tuberculosis Research Centre, Chennai on July, 9-10, 1997. The purpose of this Workshop was to network and integrate HIV/TB researchers and to develop uniform criteria so that within a short while the nation will have definite authentic and reliable information that will be most essential for the planners of National Health Programmes. Scientists and policy planners from several parts of the country participated. Scientists formed 3 small groups and discussed proposed research studies. The deliberations of each group were then presented to the House during the last session. Protocols for HIV/TB was developed based on these deliberations in the two days and action plan was finalized.

EXPERT GROUP MEETING ON "DRUG RESISTANCE SURVEILLANCE IN TUBERCULOSIS" - TRC/CENTRAL TB DIVISION (DGHS)

An Expert Group Meeting for developing guidelines for Surveillance of MDR-TB was organised by Central TB Division at Tuberculosis Research Centre, Chennai, on September 25-26, 1997. Scientists from all parts of the country participated. Study reports were presented by the scientists arid the paucity of data relating to treatment history, lack of representativeness and standardization (laboratories/epidemiological) were observed. A draft recommendation on the methodologies to be employed for internal and external quality control was finalized and it was agreed that TRC, NTI and New Delhi TB Centres will be Referral Institutes for the Quality Control.

TRC/TAMIL NADU INTEGRATED NUTRITION PROJECT (TINP) / DEPARTMENT OF ADIDRAVIDAR AND TRIBAL WELFARE(TN) JOINT REVIEW MEETINGS

A Joint Review Meeting of the Tuberculosis Research Centre, Department of Adi-Dravidar and Tribal Welfare (Government of Tamil nadu) and TINP was held on October 8, 1997 at the Tuberculosis Research Centre, Chennai. The meeting reviewed the past findings of TRC relating to the prevalence of TB among tribals in Jawadhu Hill area. It also reviewed the present ongoing project of TRC, "Feasibility of utilising literate youth volunteers to improve the nutritional status of antenatal and postnatal women and children under five years in Jawadhu Hill Tribal Area". The Secretary, Department of Adi-Dravidar and Tribal Welfare, Govt. of Tamil Nadu expressed his desire that a balanced diet for children be provided to all the tribal groups in the state by providing a good school lunch. As there are only 6 primitive tribes with a 6 lakh population in the entire state, there is a need to develop a scheme to cover 100% of the tribal population.

NATIONAL SCIENCE DAY

National Science Day (NSD) was celebrated at this Centre on 28.2.97. Basing the theme "India of our Dreams", for the NSD activities in 1997, an exhibition with a participatory component was organised at

the Centre highlighting the importance of science. Pamphlets were distributed among school students to increase the awareness and know about the impact of science in the diagnosis and treatment of various diseases. A total of 860 students from seven schools in Chennai, in the age group of 12-16 benefited by this. A film show and a quiz programme was also conducted in which the students participated with great enthusiasm and prizes were awarded for the winners.

Two training programmes on tuberculosis were conducted by the staff of TRC; one for the gross-root level workers of the Gems Foundation, a NGO, consisting of teachers, animators, leaders and members of Madhar Sangam and the other for the Arivoli Iyakkam, a Total Literacy Campaign launched by the Corporation of Chennai consisting of 23 slums. There was an informal lecture on the medical aspects of TB. followed by a role play enacted by social workers of TRC highlighting social problems associated with TB.

In the scientific session of the Anti-tuberculosis Week' programmes organised by the Southern Railway, Madras division, presentations were made by the TRC Madurai unit staff on medical and sociological aspects of TB. A total of 88 people consisting of physicians, para-medical workers, volunteers of St. John Ambulance Brigade, Trade union members, Scouts and members of Women's Club participated.

WORLD TB DAY

The Tuberculosis Research Centre, in collaboration with Tamil Nadu Slum Clearance Board, organised a Workshop on the ' Role of NGOs in Tuberculosis Control' as part of the celebrations to commemorate the World TB Day at the TRC Complex on 24th March, 1997. Dr. C.J. Paul, Chief Community Development Officer, Tamil Nadu Slum Clearance Board. Mr. Karikalvalavan, Deputy Commissioner, Corporation of Chennai and Dr.(Capt.)K.Bhaskaran, Additional Director of Medical Services(TM), Govt. of Tamil Nadu, participated in the function along with senior scientist. There were 40 participants representing various NGOs. The session was interactive. The school children, trained by a NGO presented a 'Villupattu' on TB. Volunteers from another NGO presented a role play on TB.

PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

Name of conference, venue and date	Title of paper	Name of staff member
National Conference of Indian Academy of Paediatrics, Mangalore, 4-7 January, 1996	Newer diagnostic tests for tuberculosis (Guest lecture)	Dr.Soumya Swaminathan
35th National Conference of the National College of Chest Physicians, Jaipur, 2-4 February, 1996	Bronchoalveolar lavage studies in pulmonary tuberculosis (Guest lecture)	Dr.V.K. Vijayan
- do - (Session on tuberculosis)		Dr.V.K. Vijayan
International Conference on Phenothiazines and Structurally related Psychotropic Drugs, University of Rajasthan, Jaipur, 26-29 February, 1996	Mycobactericidal activity of a phenothiazine compound: Trifluoperazine	Dr.N. Selvakumar
Indian Association for Clinical Pharmacology and Therapeutics: Mid Conference, Pondicherry, 24-25 August, 1996	Management of chronic obstructive pulmonary diseases (Guest lecture)	Dr.V. K.Vijayan
XV Conference of the International Working Group on Mycobacterial Taxonomy (IWGMT), New Castle Upon Tyne, United Kingdom, 28-3 1 August, 1 996	Characterization of **M.avium** complex (MAC) isolates obtained from clinical samples and the environment of the South Indian BCG trial area	Dr.C.N. Paramasivan

Name of conference, venue and date	Title of paper	Name of staff member
8th National Paediatric Pulmonary Conference, Mumbai, 28-29 September, 1996	Multi-drug resistantTB	Dr.Soumya Swaminathan
Conference on Global Lung Health and Annual Meeting of the IUATLD, Paris, 2-5 October, 1996	Currently available information on anti-tuberculosis drug resistance in India	Dr.C.N.Paramasivan
51 st National Conference on TB & Chest Diseases, Bangalore, 3-6 November, 1996	Immunopathology of cutaneous tuberculosis	Dr.V.D.Ramanathan
- do -	A 5-year follow-up study of children treated for tuberculous meningitis with short course chemotherapy	Dr.Padma Ramachandran
- do -	Operational research studies of TRC	Dr.V.K. Vijayan
- do -	-	Dr. T. Santha Devi
- do -	Controlled clinical trial on treatment of lymphnode tuberculosis	Dr. M.S. Jawahar
- do -	Management of multi-drug resistant tuberculosis - A 10 year experience at TRC, Chennai	Dr.R. Balambal

Name of conference, venue and date	Title of paper	Name of staff member
51 st National Conference on TB & Chest Diseases,Bangalore, 3-6 November, 1996	A feasibility study of utilization of traditional birth attendants in TB case finding and drug delivery	Dr.Rani Balasubramanian
- do -	Neopterin as a marker for cell-mediated immunity in patients with pulmonary tuberculosis	Mrs.Chandra Immanuel
- do -	Pharmacokinetics of rifampicin, ethambutol, isoniazid and pyrazinamide given alone and in combination in healthy volunteers	Mrs. Geetha Ramachandran
- do -	Role on Non-Governmental Organizations in TB control	Mrs. Beena E.Thomas
- do -	Anti-mycobacterial activity of cephalosporins	Dr.N. Selvakumar
XIV Annual Conference of the Indian Society for Medical Statistics and National CME on Research Methodology and Clinical Epidemiology, Pune, 30 November-2 December, 1996	Inference on J-shaped risk response relationship in medical data	Dr. P.Venkatesan
- do -	Marginal analysis of recurrent events in longitudinal data	Dr. P.Venkatesan

Name of conference, venue and date	Title of paper	Name of staff member
XIV Annual Conference of the Indian Society for Medical Statistics and National CME Research Methodology and Clinical Epidemiology, Pune, 30 November-2 December, 1996	Symptomatic status among bacteriologically quiescent cases	Mr.P.G.Gopi
- do -	Predictor of compliances among TB patients under DTP condition	Mrs. M.P. Radhamani
- do -	Sample size estimations using repeated measurements as biomarkers as outcome in a study of providing comprehensive health care to the tribal community using resident literate youth volunteers	Mr. R. Selvaraj
37th Annual Conference, Association of Microbiologists of India, Chennai,4-6 December, 1996	Pathogenic mechanisms in tuberculosis and recent advances in anti-tubercular therapy	Dr.C.N.Paramasivan
XVI National Congress on Respiratory Diseases, Bangalore, 7 December, 1996	Pulmonary function tests (Meet the professor session)	Dr.V.K.Vijayan (Moderator)
- do - (Scientific session)	-	Dr.V.K.Vijayan (Chair person)

Name of conference, venue and date	Title of paper	Name of staff member
36th National Conference of the National College of Chest Physicians, JIPMER, Pondicherry, 24-26 January, 1997	Pulmonary function tests (Guest lecture)	Dr.V.K. Vijayan
- do - (Session on "ARDS")	-	Dr.V.K. Vijayan (Chair person)
6th Tamil Nadu Conference on Tuberculosis & Chest Diseases, Chennai, 1-2 February, 1997	Acceptability of drug delivery at sub-centres by tuberculosis patients in West Godavari district, Andhra Pradesh	Mrs.K.Jaggarajamma
- do -	Pulmonary tuberculosis	Dr.C. N. Paramasivan (Chair person)
- do -	Awareness about tuberculosis in South Indian rural community before and after health education	Mrs.Niruparani Charles
- do –	Feasibility of involving student volunteers in City TB programme in Madurai	Mr.Victor Mohan
- do -	Economic impact of TB on patients and family	Dr.Geetharamani Shanmugam
- do -	Single strand confirmation polymorphism and detection of rifampicin resistance in M.tuberculosis	Dr.N.Selvakumar

Name of conference, venue and date	Title of paper	Name of staff member
6th Tamil Nadu Conference on Tuberculosis & Chest Diseases, Chennai, 1-2 February, 1997	The molecular mechanisms of drug resistance in mycobacteria	Dr. N .Selva kumar
- do -	-	Dr.T.Santha Devi
- do -	Feasibility of health education on TB at schools involving parents and their awareness on TB - a preliminary report	Mrs.Mohanarani Suhadev
- do -	Extra-pulmonary tuberculosis	Dr.V.Kumaraswami (Chair person)
- do -	Tuberculosis prevalence survey	Mr.P.G.Gopi
- do -	Sensitising an urban community on TB - TRC experience in Madurai	Mr . Rajasakthivel
- do -	Utilization of sub-centres for drug delivery and its impact on case holding	Mr.V.Chandrasekaran
- do -	HIV infection in patients with tuberculosis	Dr.Ranjani Ramachandran
- do -	A feasibility study of utilization of traditional birth attendants in TB case-finding and drug delivery	Dr.Rani Balasubramanian

Name of conference. venue and date	Title of paper	Name of staff member
The Teleconference in connection with TB Net 1997 and Conference in NGO involvement in TB control, Katmandu, 12-14 February, 1997	-	Dr.T.Santha Devi Dr.Manjula Datta Dr .V. Kumaraswami Dr. M.S. Jawahar Mr.S.Sivasubramanian Mrs.Sudha Ganapathy Dr. Geetharamani Shanmugam
National Congress on Diseases of the Chest organised by The American College of Chest Physicians, South India Chapter, Chennai, 25-28 February, 1997	Tropical eosinophilia (Guest lecture)	Dr. V.K.Vijayan
- do -	-	Dr.V.K.Vijayan (Organizing Chairman)
- do -	-	Dr. P. Venkatesan (Organising Secretary)
- do -	Surgical treatment for myasthenia gravis	Dr .V. K.Vijayan (Chair person)
- do -	Chemotherapy of pulmonary tuberculosis in children (Guest lecture)	Dr. Padma Ramachandran
- do -	Tuberculosis in HIV positive individuals	Dr.Usha Ramanathan

Name of conference, venue and date	Title of paper	Name of staff member
National Congress on Diseases of the Chest organised by The American College of Chest Physicians, South India Chapter, Chennai, 25-28 February, 1997	-	Dr.C.N.Paramasivan (Faculty member)
- do -	Bacillary prevalence estimation from the tuberculosis prevalence surveys - a critical review of the methodology	Dr.C.Kolappan
- do -	Pharmacokinetics of rifampicin,ethambutol, isoniazid and pyrazinamide following administration of the drugs individually or in different combination in healthy volunteers	Mrs. Geetha Ramachandran
- do -	Management of patients with pulmonary tuberculosis excreting drug resistant bacilli: a 30-year experience	Dr.Rema Mathew
23rd Middle East Regional Conference of the International Union against TB & Lung Diseases at Teheran, 21 -24, April, 1997	"Prescription practices of Medical Practitioners for TB in India"	Dr Rani Balasubramanian

Name of conference, venue and date	Title of paper	Name of staff member
23rd Middle East Regional Conference of the International Union against TB & Lung Diseases at Teheran, 21 -24, April, 1997	-	Dr Rani Balasubramanian (Chair person)
11th Karnataka State Conference on TB & Chest Diseases, Gulbaraga, 25th-26th Sept. 1997	Management of Multi-Drug Resistance - A 10 year experience at TRC, Chennai	Dr R.Balambal
-do-	RNTCP	Dr.T.Santha Devi
-do-	-	Dr.M.S. Jawahar
20th Biennial National Conference of Indian Association of Leprologists, Gandhi Medical College, Bhopal, 28-30, November, 1997	. Objective parameters to monitor the response to therapy in pauci-bacillary leprosy	Dr A Thomas
-do-	Value of the Midfinger smears in Multibacillary leprosy patients treated with fixed duration therapy	Mrs Lalitha Hari

Name of conference, venue and date	Title of paper	Name of staff member
XV Annual conference of Indian Society for Medical Statistics, SMS Medical College, Jaipur, 12-14, December, 1997	Reconstruction and future trends of AIDS epidemic	Dr. P.venkatesan
- do -	Joint estimating equation in the Covariate analysis of missing data	Dr. P.Venkatesan
52nd National Conference on TB&CD, Ahmedabad, from 19-22nd December, 1997	Short Course Chemotherapy for pulmonary tuberculosis in children	Dr.Padma Ramachandran
- do -	Bioavailability of anti-TB drugs from a triple drug formulation Blood vs. Urine	Dr. Prema Gurumurthy
- do -	Ten-year findings. of a comparison of ambulatory short course chemotherapy with medical surgery plus chemotherapy for tuberculosis of the spine in Madras	S. Sivasubramanian

Name of conference, venue and date	Title of paper	Name of staff member
XIV Annual Conference of Indian Immunology Society and Symposium on "Immunomodulation in Health and Disease", Calcutta 22-24 December, 1997	Influence of HLA-DR genes/gene products on immunity to pulmonary tuberculosis	Dr.P.Selvaraj
-do-	Cytokine response to BCG vaccination in South India	Dr.D.Sulochana

LIST OF PUBLICATIONS

Papers published

1. Vijayan, V.K. and Sankaran, K. Relationship between lung inflammation, changes in lung function and severity of exposure in victims of Bhopal tragedy. **European Respiratory Journal,** 1996, **91,** 1977-1982.

2. Vijayan V.K., Paramasivan, C.N. and SanKaran, K. Comparison of bronchoalveolar lavage fluid with sputum culture in the diagnosis of sputum smear negative pulmonary tuberculosis. **Indian Journal of Tuberculosis,** 1996, **43,** 4, 179-182.

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2. Vijayan, V.K. Pulmonary tuberculosis. **In: Text Book on Tuberculosis. Ed: Sharma, S.K: (AIIMS),** New Delhi.

3. Vijayan, V.K. Tropical pulmonary eosinophilia. **In: Recent Advances in Pulmonary Medicine. Ed: Sharma, S.K. and Behera, D.,** New Delhi.

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20. Sulochana D Das, Narayanan,P.R., Kollappan,C. and Colston, M.J. The cytokine response to BCG vaccination in South India. **The International Journal of Tuberculosis and Lung Disease.**

21. Vijayan, V.K. Reactive airways dysfunction syndrome (Editorial). **Lung India.**

JOURNAL CLUB

Journal Club meetings were held each week, at which published scientific articles covering different areas of research were reviewed by staff members of various departments in turn. A synopsis of the paper(s) to be presented and the reference details were circulated in advance, to facilitate better participation by the audience in the discussion that followed the presentation. In all, 95 such meetings were conducted during the two years including 11 guest lectures and 5 lectures by the staff of IRMS.

GUEST LECTURES

Dr. Deepak Gopal, Chief Resident, Internal Medicine, the Sunny Brook Hospital, Toronto, Canada, delivered a lecture on "Idiopathic mucosal inflammation in AIDS responds to cortico steroids".

Dr. K. Ganapathy, Neurosurgeon, Consultant, Apollo Cancer Institute, Chennai, delivered lectures on "Life after death" and "Computers in health care and neurological sciences".

Dr. S. Sandhyamani, Addl. Professor, Sri Chitra Thirunal Institute for Medical Sciences and Technology, Trivandrum, delivered a lecture on "Mucoid vasculopathy: A new degenerative vascular disorder".

Dr. Saradha Suresh, Health Economist, the Clinical Epi-demiology Unit, Madras Medical College, Chennai, delivered a lecture on "Cost effectiveness analysis of three short course anti-TB programmes compared with standard regimens in Thailand".

Dr. Ravi I. Jayakaran, Zonal Associate Director (West Zone), the World Vision of India, delivered a lecture on "The use of PZA methodology in social science research".

Dr. M. J. Colston, Head,Mycobacteriology, National Institute of Medical Research (NIMR), London, "Recent advances in mycobacterial research".

Dr. Adrian V.S. Hill, Principal Research Scientist, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, U.K., "Genetic factors in susceptibility to malaria and tuberculosis".

GUEST LECTURE SERIES - 50th YEAR OF INDIA'S INDEPENDENCE CELEBRATIONS

DATE	TOPIC	SPEAKER
20-09-97	Tuberculosis outstripping mankind	Prof. V. Ramalingaswamy, National Research Professor
15-10-97	Challange of HIV Epidemic	Prof.C. N. Deivanayagam, Superintendent, Govt. Hospital of Thoracic Medicine, Tambaram.
25-11-97	Women and AIDS	Dr. Suniti Solomon, Director, YRG Care, 1 , Raman Street, T'Nagar, Chennai - 600 017.
29-12-97	Alternate therapy in HIV/ AIDS:Validation guidelines and Indian experience	Dr.S. P. Thyagarajan, Prof. and Head, Dr.ALMPGIBMS, University of Madras, Taramani, Chennai - 600 113.

DISTINGUISHED VISITORS

Dr. Adrian V.S. Hill, Principal Research Scientist, Wellcome Trust Centre for Human Genetics, University of Oxford, U.K.

Dr. M.J. Colston, Head, Microbacteriology, National Institute of Medical Research (NIMR), London & Consultant, the British ODA - Phase III.

Dr. Nirbhey Kumar, Professor of Immunology, the Johns Hopkins University, Maryland, USA.

Dr. J.N. Pandey, Professor & Head, Dept. of Medicine, the AIIMS, New Delhi.

Dr. S. Srinivasan, Senior Vice-president, Medical Services, the ROUSSEL India Limited, Bombay.

PRIZES AND AWARDS RECEIVED BY THE STAFF MEMBERS

Dr. V.K. Vijayan was awarded the Amrut-Mody. Unichem Prize in Chest Diseases by ICMR.

Mrs. Geetha Ramachandran was awarded "cash prize" for the best paper entitled " Pharmacokinetics of rifampicin, ethambutol, isoniazid and pyrazinamide following administration of the drugs individually or in different combinations in healthy volunteers" at the National Congress on Diseases of the Chest, organised by the American College of Chest Physicians, South India Chapter, Chennai, during February, 1997.

ACKNOWLEDGEMENT

The Director acknowledges the efforts of Mr.S. Sivasbramanian, Dr.P. Venkatesan and Mr.M. Nagarajan in editing and organising the publication of this report. The enthusiatic and untiring efforts of Mr. R. Segaran, Mr.V. Sundaram and V. Chandrasekaran in compiling, processing and preparing this report using computer and Mrs.V. R. Vijayalakshmi for secretarial assistance is greatly appreciated

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