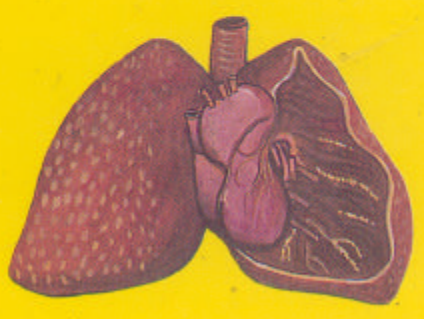
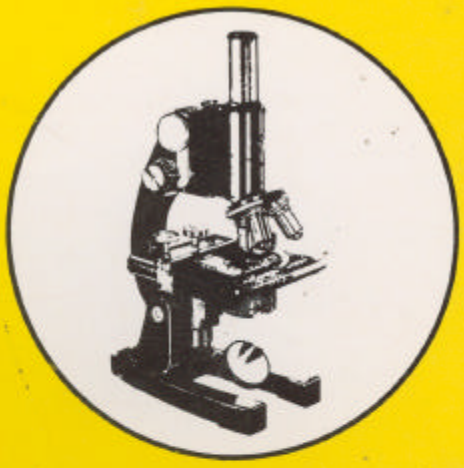
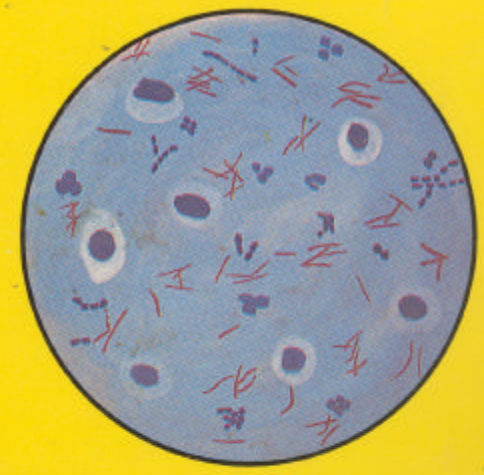
TUBERCULOSIS RESEARCH CENTRE

Chetput Madras - 600 031

REPORT ON RESEARCH ACTIVITIES DURING 1993







INDIAN COUNCIL OF MEDICAL RESEARCH
NEW DELHI

TUBERCULOSIS RESEARCH CENTRE

CHETPUT MADRAS - 600031

REPORT ON RESEARCH ACTIVITIES DURING 1993

CONTENTS

PREFACE

The Centre has been continuing to undertake operational research studies in tuberculosis to evolve methodologies relevant for application in the National Tuberculosis Programme. Different strategies are being evaluated, covering the two important components of the programme, case-finding and case-holding.

An operational research study, utilising literate youths for case finding and Village Health Nurses for drug delivery, in a tribal area in Jawadhu Hills in North Arcot district is in progress. In West Godavari district, utilization of sub-centres for drug delivery and ICDS workers and Village Health Guides for motivating patients for regular drug intake was initiated in six PHCs. A study to find out the attitudes towards illness, utilization of available health facilities and awareness of tuberculosis was undertaken in tribal families belonging to Buttayagudem mandal in West Godavari district. A feasibility study using male NSS students (volunteers) for drug supply and defaulter retrieval was also undertaken during the year, in a city TB programme in Madurai city. A pilot study to investigate the feasibility of patient-to-patient motivation to improve compliance, by utilising a patient who has been regular for treatment, has been completed at the Centre. A bacteriological study to assess the effectiveness of SCC under programme conditions has been planned to be conducted in Raichur district.

A controlled clinical trial of 6-month fully oral short course chemotherapy (SCC) regimens, in the treatment of sputum-positive pulmonary tuberculosis with two split-dose double-drug combinations on alternate days (rifampicin and ethambutol on one day followed by isoniazid and pyrazinamide on the next day), each combination given thrice a week for the first 2 or 3 months, followed by isoniazid and rifampicin twice a week in the continuation phase for the next 4 and 3 months respectively, is being continued both at Madras and Madurai. If the findings are promising, this will be a major step towards the possible use of blister packs for drug delivery in tuberculosis programmes.

A controlled clinical trial of short course chemotherapy in pulmonary tuberculosis in children, comparing a 9-month regimen with a 6-month regimen is continuing. 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 in progress. A study of paucibacillary leprosy has

been initiated with an aim to evolve objective criteria to assess the diagnosis and progress in paucibacillary leprosy and to differentiate between relapse and reversal reaction. A study to assess the "quality of life" measurements in sputum-positive pulmonary TB patients who had undergone treatment is in progress.

A study on the use of cetylpyridium chloride (CPC) for storage of sputum samples at ambient temperature is in progress for possible application under field conditions, to transport sputum specimens without making special arrangement. The study on the use of vancomycin in selective Kirchner's liquid medium (SKLM) has shown that the modification resulted in considerable reduction of contamination rate which would enable higher yield of cultures of **M. tuberculosis.** A direct sensitivity test for rifampicin has been evolved using L-J medium, on similar lines as for streptomycin and isoniazid reported earlier. For possible shortening of the duration of drug susceptibility tests, a study based on bioluminescence assay of Adenosine Triphosphate (ATP) has been undertaken and further, a luciferase reporter phage assay is also being evaluated. Successful outcome of these studies could be of help in better management of patients.

Studies to elucidate the immuno-modulatory effect of **M. avium intracellulare** and **scrofulaceum (MAIS)** complex and **M. fortuitum** are being conducted, which also include susceptibility patterns of these organisms to anti-TB drugs and heavy metals. **In vitro** studies to evaluate the bactericidal and sterilising actions of Ofloxacin and sulbactam/ampicillin on **M. tuberculosis** isolates are in progress and could yield valuable information on these newer anti-tuberculosis drugs.

Two independent assays (microbiological and fluorimetric) have been standardised to estimate Ofloxacin in biological fluids which are considered useful in clinical research. Studies on immunopathological aspects of skin tuberculosis, using an animal model to study fibrogenesis are in progress.

Diagnostic kits for tuberculosis, developed nationally and internationally have been evaluated for their possible use in immunodiagnosis of tuberculosis. DNA finger printing has been successfully established with IS6110 and DR probes, which has helped to undertake further studies for typing clinical isolates from various regions in India. HLA typing of tuberculosis patients and their families is in progress to investigate the possible association of genetic factors with this disease.

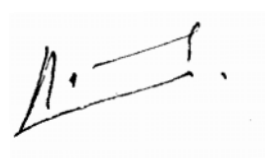
The Centre has planned a multicentric study to develop diagnostic criteria in childhood TB. The longitudinal follow-up of HIV infected individuals and surveillance of HIV infection among tuberculosis patients is continuing. The study has revealed that about 6% of HIV infected individuals develop tuberculosis over a period of 30 months. It has also been observed that mortality in HIV infected individuals who also have TB is more than double compared to those who do not have TB. A community-based study of the socio-economic impact of lymphatic filariasis funded by WHO-TDR unit is in progress. Preliminary work for a community-based study on the effectiveness of DEC-medicated salt in the prevention of Adeno Lymphangitis (ADL) attacks and cutting transmission of the disease is underway. Studies for estimating attack rates of typhoid in the community are also being planned as a preamble to an Indo-US collaborative research project with a new anti-typhoid vaccine.

The Medical Library Information System (MLIS) has been expanded considerably with the inclusion of CD-ROM, SIRNET which is a Computer Network of CSIR with E-Mail facility. This will immensely benefit the scientific community by easy accessibility to medical literature and also for faster communication between scientists nationally and internationally.

During the year, the Centre had initiated efforts to bring out a quarterly publication, 'TRC Bulletin', for highlighting and disseminating different aspects of tuberculosis research undertaken by the Centre.

The Scientific Advisory Committee, which met on the 6th January 1994 under the chairmanship of Dr.S.P.Pamra, gave very useful suggestigns and guidance relevant to the different research activities of the Centre.

Finally, I wish to gratefully acknowledge with pleasure, the untiring efforts by my colleagues at the Centre, to nurture and ensure high quality research and also I express my sincere thanks to the technical and administrative staff for their able support.



(R. Prabhakar)
Director

STAFF MEMBERS

AS ON 31.12.93

Director

R. Prabhakar, M. D., F.C.C.P.

Division of Chemotherapy

T. Santha Devi, M.B.B.S., D.T.C.D.
V.K. Vijayan, M.D., D.T.C.D., M.A.M.S., Ph.D.(Med.)., F.N.C.C.P., F.C.C.P.
Padma Ramachandran, B.Sc., M.D., D.C.H.
A. Thomas, M.D., Dip. in Leprosy
V. Kumaraswami, M.D., M.N.A.M.S.
Rajeswari Ramachandran, M.D., D.M.(Neuro.)
Rani Balasubramanian, M.D., D.G.O.
M.S. Jawahar, M.D.
Soumya Swaminathan, M.D., Dip. N.B.
K. Rajaram, B.Sc., M.B.B.S., D.T.R.D.
Rema Mathew, M.B.B.S., D.C.H.
A.M. Reetha, M.B.B.S., D.C.H.
Paulin Joseph, M.B.B.S., D.D.
R. Balambal, M.D.
K.C. Umapathy, M.B.B.S.
Usha Ramanathan, M.B.B.S., D.P.M.
Ranjani Ramachandran, M.B.B.S.
M. Parvathy Raghavan, R.N., R.M., C.P.H.
Sudha Ganapathy, M.A.
K.V. Kuppu Rao, Ph.D.
Ambujam Ganesh, R.N., R.M., C.P.H.
Rajamanohari Dason, R.N., R.M., C.P.H.
K.N. Gopilingam, C.R.A.

Division of Bacteriology

C.N. Paramasivan, Ph.D.
N. Selvakumar, Ph,.D.
Vanaja Kumar, Ph.D.

P. Venkataraman, BSc., A.I.C.
B.N. Gopalan, B.Sc., D.M.T.
Sara Mathew, BSc.
Lalitha Hari, M.Sc.
M. Nazeema, Ph.D.
Daniel Herbert, Ph.D.

Division of Biochemistry

Rajiswamy, M.D., Ph.D.
Prema Gurumurthy, Ph.D.
M. Kannapiran, Ph.D.
Chandra lmmanuel, M.Sc.

Division of Immunology

P.R. Narayanan, Ph.D., D.I.I.Sc.
Alamelu Raja, Ph.D.
Sujatha Narayanan, Ph.D.
A. Ravoof, BSc.
P. Selvaraj, Ph.D.
D. Sulochana, Ph.D.

Division of Pathology

V.D. Ramanathan, M.B.B.S., Ph.D.

Division of Epidemiology

Manjula Datta, M.D., D.C.H., M.Sc.(D.M.E.)
C. Kolappan, M.B.B.S., M.Sc.(Epid.)
K. Sadacharam, M.B.B.S.
A.M. Diwakara, MSc.
P.G. Gopi, MSc.
M.P. Radhamani, MSc.
D.L. Sathyanarayana Rao, BSc.
B.N. Appe Gowda, BSc.
R. Selvaraj, MSc.
R. Subramani, MSc.

Division of Statistics

P.V. Krishnamurthy, M.Sc.(Stat.), M.Sc.(D.M.E.)
A.S.L. Narayana, B.Sc.
S. Sivasubramanian, B.A.
Fathima Rahman, B.Sc., Stat. Dip.(I.S.I.)
P. Venkatesan, M.Phil., Ph.D.,D.S.Q.C.O.R.(I.S.I.)

Library

M.G. Sreekumar, B.Sc., M.L.I.S.

Administration

J.N. Tandon, B.A.
V. Lakshminarayanan, B.Com., D.Com., A.C.S.
M. Subramanian, B.Com.
C.J.Arunachalam

Emeritus Medical Scientist

Debidas Ray, F.R.C.P.(London), F.R.C.P.(Glasgow),
F.C.C.P.(Chicago), F.N.C.C.P.

SCIENTIFIC ADVISORY COMMIITEE

Chairman

Dr. S.P. Tripathy	Director-General, Indian Council of Medical Research, New Delhi.

Members

Dr. S. Ananthakrishnan	Director of Medical Services and Rural Health, Government of Tamilnadu, Madras.
Dr. G.V.J. Baily	Former Director, National Tuberculosis Institute, Bangalore.
Prof. J.C. Bhatia	Professor of Health Services Management and Chairman, Research and Publications, Indian Institute of Management, Bannerghatta Road, Bangalore.
Dr. K.K. Datta	Adviser-in-TB and Deputy Director-General of Health Services (TB), Directorate General of Health Services, New Delhi.
Dr. M.D. Gupte	Project Officer, Central JALMA Institute of Leprosy Field Unit, Avadi, Madras.
Prof. K. Jagannath	Director, Institute of Thoracic Medicine, Madras.
Dr. S.P. Pamra	(Q-5, Model Town, Delhi.
Dr. S. Radhakrishna	Director,Institute for Research in Medical Statistics (Madras Chapter), Madras.
Dr. A. Ramalingeshwar Rao	Director of Public Health, Government of Tamilnadu, Madras.

Dr. Rama Mukherjee	Head, Microbiology Division, National Institute of Immunology, New Delhi.
Dr. S. C. Sehgal	Director, Regional Medical Research Centre, Port Blair.
Prof.(Mrs.) Sivakumar	Professor of Social Sciences, University of Madras, Madras.
Prof. S.S. Sivakumar	Professor of Econometrics (Health Economics), University of Madras, Madras.
Dr. M. Susila Raj	Director of Medical Education, Government of Tamilnadu, Madras.
Prof. K. V. Thiruvengadam	Former Professor of Medicine, Madras Medical College, Madras.
Dr. B.T. Uke	Director, National TB institute, Bangalore.
Dr. K.C. Mohanty	Professor, Grant Medical College and Sir JJ Group of Hospitals, Bombay.
Dr. R. Prabhakar (member-secretary)	Director, Tuberculosis Research Centre, Madras.

EPIDEMIOLOGY SUB-COMMITTEE

Dr.G.V.J.Baily,
Former Director,
National Tuberculosis Institute,
Bangalore.

Dr.P.Chandrasekar,
Former Epidemiologist,
National Tuberculosis Institute,
Bangalore.

Dr.G.D.Gothi,
A3,Lanu Villa,79-B, Tagore Road,
Santacruz(West),
Bombay.

Dr.S.Radhakrishna,
Director,
Institute for Research in Medical
Statistics (Madras Chapter),
Madras.

Dr.A.Prabhakar (member-secretary),
Director,
Tuberculosis Research Centre,
Madras.

ETHICAL COMMIITEE

Chairman

Shri N.Krishnaswarny Reddy,
Justice (Retired),
Madras.

Members

Prof. M.V. Chari,
Consultant Physician,
V.H.S. Hospital,
Madras.

Prof. K.N. George,
Director,
Madras School of Social Work,
Madras.

Dr. (Mrs.) Lalitha Kameswaran,
Former Vice-Chancellor,
The Tamilnadu Dr. M.G.R. Medical University,
Madras.

HONORARY CONSULTANTS

Name of consultant	Field of specialisation	Designation and Institution
Prof.K.Jagannath	Medicine	Director, Institute of Thoracic Medicine, Madras.
Dr.I.Kandaswamy	Radiology	Professor of Vascular Radiology, Government General Hospital, Madras.
Dr.R.Parthasarathy	Medicine	Former Deputy Director, Tuberculosis Research Centre, Madras.
Dr.S.Radhakrishna	Statistics	Director, Institute for Research in Medical Statistics (Madras Chapter), Madras.
Dr.P.S.Seshadri	Leprosy	Former Assistant Director, Central Leprosy Teaching and Research Institute, Chengalpattu.
Mr.P.R.Somasundaram	Statistics	Former Deputy Director (Sr.Gr), Tuberculosis Research Centre, Madras.
Prof.K.V.Thiruvengadam	Medicine	Former Professor of Medicine, Madras Medical College, Madras.
Dr.S.Thyagarajan	Ophthalmology	Former Professor of Ophthalmology, Government Rajaji Hospital, Madurai.
Dr.N.S.Venugopal	Ophthalmology	Former Superintendent, Government Ophthalmic Hospital, Madras:

OPERATIONAL RESEARCH STUDIES - COMPLETED

Patient-to-patient motivation - an additional effort to improve compliance

(Completed study, 1991-93)

A pilot study to investigate the feasibility of patient-to-patient motivation, by utilising a patient who has been regular for treatment to talk to a new patient was initiated during 1990. As the study progressed, more treated patients were trained and after ensuring that they could motivate without difficulties, a controlled study was started in 1991.

Only those patients who were found unsuitable for admission into our ongoing controlled clinical trial were admitted into this investigation. Patients are allocated randomly, to either routine motivation (RM) or patient-to-patient motivation (PM) and all are treated with a 6-month fully supervised regimen with twice-weekly attendance.

Routine motivation (RM): Motivation is done by clinic staff only.

Patient-to-patient motivation (PM): Motivation is done by treated patients in addition to clinic staff motivation, on admission and at 1 and 4 months.

As compliance is likely to be influenced by the distance to be travelled to reach the clinic, previous treatment and personal habits' like drinking, stratified random allocation procedure has been adopted. The medical officers, clinic nurses and health visitors are not aware of the group to which the patient is allocated.

Defaulter retrieval action is taken for both groups, in accordance with the DTP manual, i.e., a letter is posted on the day following the default and again on the 8th day. No home visits are made. Patients defaulting continuously even after defaulter actions for a month, are considered 'lost'.

All the 297 patients admitted to the study have completed 6 months of treatment. Excluding 16 patients (4 died and 12 had change of treatment) there were 281 patients in the analysis. Of these, 106 were lost to treatment.

The drug regularity pattern in the PM and RM groups is given in table 1.

Table 1

Distribution of patients according to treatment received

Treatment received (%)	Patient-to-patient motivation(PM)		Routine motivation (RM)	
	No.	%	No.	%
≥90	57	**40**	55	**40**
75-89	22	**15**	24	**17**
<75	6	**4**	11	**8**
Lost	58	**41**	48	**35**
Total Patients	143	**100**	138	**100**

Forty percent of the patients have had more than 90% of treatment in both groups. The results show that there is not much of a difference in the regularity pattern in both groups.

Table 2

Distribution of 'Lost' patients according to month of treatment

Month lost	PM		RM		Total	
	No.	%	No.	%	No.	%
1	17	**29**	8	**17**	25	**24**
2	17	**29**	21	**44**	38	**36**
3	9	**16**	12	**25**	21	**20**
4	4	**7**	5	**10**	9	**8**
5	10	**17**	2	**4**	12	**11**
6	1	**2**	0	**0**	1	**1**
All	58	**100**	48	**100**	106	**100**

The distribution of patients 'lost' for treatment in the RM and PM groups is given in table 2.

It can be observed that nearly 60% of patients 'lost' to treatment were in the first phase of treatment in both groups.

This study shows that Patient-to-patient motivation has not resulted in any greater improvement in patient compliance. However these findings may have to be confirmed by replicating the study in a different setting before drawing any conclusion.

*****

Health seeking behaviour among tribal community and their acceptability of health facilities in West Godavari district of Andhra Pradesh

(Completed study, 1993)

Tribal communities are much different from other communities because of their traditional and cultural background. Before launching any programme it is necessary to find out the health seeking behaviour of the tribals and their acceptability of the available health facilities. Therefore a study on these aspects was conducted in a tribal community at Buttayagudem Mandal in West Godavari District of Andhra Pradesh.

The population of Buttayagudem Mandal is 46,489, of which 27,000 (58%) belong to tribal communities. The total number of villages in this mandal is 53, of which 34 villages predominantly occupied by tribal communities, covering a population of 18,000 were selected. A random sample of 429 households (about 10%) was selected for the study. The head of the household or the next responsible person was interviewed using a questionnaire in local language (Telugu), with the help of I.T.D.A. (Integrated Tribal Development Agency) school teachers. These teachers were trained by the Centre's staff for this work.

It is seen (Table 1) that 227 (53%) of 429 respondents are males and of these 184 (81%) are aged more than 25 years. Of 202 females, 155 (77%) are aged more than 25 years. Of the 429 interviewed, 288(67%) were illiterates.

Table 1

Age and sex distribution of the respondents

Sex	Age (years)				Total
	≤25	26-40	41-55	>55	
Male	43	120	51	13	227
Female	47	111	33	11	202
Total	90	231	84	24	429

Table 2 presents the reason for sickness given by the 429 respondents. A "sick person" for the purpose of this study is a person, who is unable to attend to his daily routine work. Sixty-one percent of respondents have attributed superstitious belief as reason for sickness.

Table 2

Reasons attributed for sickness bv tribal community

Reasons attributed for sickness	Respondents	
	No.	%
Superstitious beliefs	261	61
Use of fertilizers and pesticides	78	18
Unhygienic conditions	46	11
Change of food habits	44	10
Total respondents	429	100

It can be seen (Table 3) that 355 (86%) of 413 respondents have said that they approached the available health facilities when they fell ill. Only 13% of the respondents resorted to 'faith healing'.

Table 3

Health facilities approached by the tribal community when they fell sick*

Health facilities approached	Respondents	
	No.	%
Health facilities (modern medicine)	355	**86**
Faith healing	53	**13**
Tribal leaders	21	**5**
Others (indigenous medicine, home remedies, etc.)	24	**6**
No. of respondents	413	-
Not answered	16	**4**
Total respondents	429	-

*More than one answer was given by some of the respondents

It may be observed that even though 67% of the respondents were illiterates and 61% had superstitious beliefs, yet 86% were approaching available health facilities (personnel) when they fell sick. This appears to be contrary to the general belief that tribals favour indigenous medicines, faith healing etc. This could probably indicate change of attitudes and beliefs among tribals towards the available health facilities.

*****

OPERATIONAL RESEARCH STUDIES - IN PROGRESS

Pilot study in Jawadu hills for augmentation of District Tuberculosis Programme among Tribals

(Ongoing study, 1990-96)

The DTP has been in operation for the past 3 decades. However, the case-finding activity has been practically "nil" in Jawadu hills tribal area, though the prevalence of bacillary disease is as high as it is in the plains. Being a hilly region, a majority of hamlets have to be reached by jungle trails. Hence, a pilot study was initiated in 1990 to find out the feasibility of involving youths (who can read and write) from the same community for case-finding in 19 road-side hamlets. (For details, refer to 1992 Annual Report.)

Attempts made to supply anti-tuberculous drugs through literate youths could not succeed due to several administrative problems. Alternatively, issue of antituberculous drugs by village health nurses (VHNs) is being investigated since 1992.

Initially, an assessment of awareness about TB was done in 3 hamlets, in a total of 245 households. The salient findings are: 71% had not heard of TB; 19% had said that it was an infectious disease; 15% had mentioned the symptoms of TB properly and only 6% had knowledge of BCG vaccination even though this area is covered by the Universal Immunisation Programme.

In order to sensitize the community regarding TB, various health education methods such as exhibitions on tuberculosis, village meetings, message on TB through audio-visual aids and briefing the villagers on basic facts on TB by literate youths had been undertaken at periodic intervals in 1992.

A random sample of 4 sub-centres in Jamnamarathur Primary Health Centre area was chosen, so that the sample included both interior and roadside hamlets. All the 61 hamlets in the 4 sub-centres were covered.

Briefly, one or more literate youths per hamlet were chosen and trained in census taking; identification of chest symptomatics was undertaken as per DTP definition. From each chest symptomatic sputum

is collected and transported to PHC with proper labelling. Sputum results are communicated back to chest symptomatics through literate youths/TRC staff. Treatment card is opened at PHC for sputum positive patients. For these patients, treatment is initiated by TRC Medical Officer/PHC Medical Officer.

The patients with a positive sputum smear result are prescribed an 8-month short course chemotherapeutic regimen. The regimen consisted of rifampicin 450mg, isoniazid 300mg and pyrazinamide 1.5g daily for two months, followed by thioacetazone 150mg and isoniazid 300mg daily for a further period of six months. Drugs were supplied once a fortnight by VHN to patients.

Monitoring of the study was done by TRC team, consisting of a Medical Officer(MO), a Medical Social Worker(SW) and a team of para medical workers(PMWs). Monthly meetings with youths and VHNs were organised. Systematic validation of the performance of the study was done every month by TRC team. Surprise drug checks for patients started on treatment were done by pill count to check on drug regularity.

The total population screened by literate youths was 9,383 persons in 61 hamlets; of them, the eligible population (viz.; 15 years or more) was 5,755. One literate youth per hamlet was selected for 59 hamlets. Two youths were selected for one hamlet, as the population of that hamlet was above 1000. In the remaining hamlet, no literate person was available. The youths from the neighbouring hamlet, agreed to do the work in that hamlet. The educational status of the youths varied from 5th standard to 12th standard. All were males and unemployed.

One sputum specimen was collected from each chest symptomatic and the sputum smear was examined by Ziehl-Neelsen method. The sputum microscopy was done at the PHC by the lab. technician or at the Centre. Attempts were made to set up sputum cultures at TRC but the rate of contamination was unacceptably high, as there was a delay in transportation.to TRC.

The case-finding by literate youths is given in table 1. In an eligible population of 5755 persons, the number of chest symptomatics identified by literate youths was 338 (5.9%) and among the sputum specimens examined, the number of patients with sputum smear positive for AFB was 12 (3.6%) of 338 specimens. Treatment was initiated for all the 12 patients by TRC team.

Table 1

Case finding activity by literate youths

	No.	%
Total population screened	9383	
Population aged ⩾ 15 years	5755	**61.3**
Chest symptomatics identified by LY	338	**5.9**
Patients identified as smear positive	12	**3.6**

In order to ascertain whether the youths had identified all the chest symptomatics in the community and also to find out whether they had made proper selection of the chest symptomatics, a 10% random sample of all the hamlets and the chest symptomatics identified by the literate youths were interviewed by TRC MO/PMW and the details are given in table 2. The time lag between the identification of the chest symptomatics by the literate youths and the interview by the MO/SW vaned from 15 to 35 days.

Table 2

Validation of the work done by literate youths

Chest symptoms *		Assessed by literate youths		Total
		Present	Absent	
Assessed by MO/SW	Present	318	3	321
	Absent	20	590	610
Total		338	593	931

* as per DTP definition of "Chest symptomatic"

A total of 931 persons were screened. There were 321 chest symptomatics in the community. Of which, 318 were identified by the youths and 3 were missed. Out of 338 chest symptomatics identified by the youths, 318 were found to be true chest symptomatics and 20 were non chest-symptomatics as per DTP definition. The salient findings are that the youths have missed only three chest symptomatics and 20 were wrongly labelled as chest

symptomatics. To sum up, misdiagnosis by literate youths was only 23(2%) out of 931 patients.

During a period of one year, VHNs issued the drugs to patients once in 15 days for the first 9 months. They did not issue on two occasions, as the hamlets became unapproachable due to heavy rains. Subsequently on a trial basis, literate youths were entrusted with the responsibility of handing over the drugs to patients. They successfully distributed the drugs to patients for the remaining 3 months. Surprise drug check by TRC team revealed that patients were issued the drugs regularly at periodic intervals. As per pill count, 11 of 12 patients were regular in drug consumption.

The study is in progress.

*****

Feasibility of utilisation of village Dais in improving DTP - A pilot study

(Ongoing Study, 1989-94)

"Dais" are the traditional birth attendants, conducting deliveries at home in the villages. A pilot study was undertaken in Sriperumbudur to explore the feasibility of utilising the services of Dais for the improvement of case finding and case holding in the District Tuberculosis Programme (see 1992 Annual report). There are 44 villages with a population of 26,413 in Sriperumbudur taluk, Chingleput district, Tamilnadu. A voluntary health organisation ('Prepare') functioning in Sriperumbudur taluk, trains the village Dais to provide primary health care to the village community.

The Dais in these villages were given practical training by a team consisting of a medical officer, a social worker and a clinic nurse in identifying chest symptomatics and in collecting sputum specimens from them, for transportation to the Centre. One sputum specimen was collected from each chest symptomatic.

Case finding by village Dais: A total of 598 sputum specimens were collected prior to treatment from chest symptomatics identified so far and examined by smear for AFB and by culture for **M. tuberculosis** (Table 1). Of these specimens, 62 (10%) were found to be positive by smear for AFB and 14 (2%) were negative by smear but positive by culture for

M. tuberculosis. Sensitivity tests to isoniazid and rifampicin were done on all positive cultures. Results are available for 76 cultures. Thirteen (17%) cultures were resistant to isoniazid and 3 (4%) were resistant to isoniazid and rifampicin.

Chemotherapy: All patients positive by smear or culture were prescribed ethambutol 800 mg plus isoniazid 300 mg daily for 12 months. Treatment was initiated by a Medical Officer of the Centre, and monthly supplies of drugs were issued to the Community Health Assistants, to be handed over to the respective Dais,-who supplied the drugs to the patients with instruction to take the drugs daily at night after food.

Out of 76 patients who were positive by smear or culture, treatment could be initiated only for 66 patients. Of the remaining, 3 died before starting treatment, 2 patients refused chemotherapy in spite of repeated motivation (by TRC Medical Officer, Health Visitor and 'Prepare' Medical Officer), 1 patient belonged to an area outside the taluk, 1 patient migrated, 2 patients received treatment from other hospitals and

Table 1

Case finding activity by village Dais

	No.	%
Population catered by Dais (according to 1991 census)	26413	
Population aged ≥ 15 years	16740	**63**
Total symptomatics identified by Dais	598	**4**
Sputum positives:		
by smear alone	22	**10**
by smear and culture	40 } 62	
by culture alone	14	**2**
Over-all positivity	76*	**13**

* Excluding one specimen from non-prepare area

1 patient could not be identified. During treatment, 5 patients died and 3 migrated. Two patients complained of giddiness in the first month of

treatment but with reassurance, one felt better and the other refused to take treatment in spite of repeated motivation.

So far, 47 patients have completed one year of treatment and 7 patients are still under treatment. At the end of treatment, sputum was collected from 36 patients by the Dais. Seven specimens were positive by smear and culture; the organisms were sensitive to isoniazid and rifampicin and retreatment was initiated with ethambutol, Isoniazid, rifampicin and pyrazinamide thrice a week, the Dais administering the drugs under supervision.

A Health Visitor does surprise drug check at least once a month for all patients on treatment. Efforts are also being made to identify and collect sputum samples from chest symptomatics missed by the Dais, by visiting a sample of households from each village where treatment is initiated for sputum smear-positive patients, and also interview a sample of chest symptomatics identified by the Dais whose sputum smears were negative for AFB.

Table 2

Detection of chest symptomatics by TRC Health Visitor

	No.	%
Total no. of house-holds visited	559	
Total population covered	2728	
Population aged ≥15 years	1730	63
No. of chest symptomatics detected	61	4
Sputum positive by smear	1	2

Table 2 gives the details regarding detection of chest symptomatics by TRC health visitor. A total of 559 households were visited and 2728 persons were screened, of whom 1730 were aged 15 years or more. The total number of chest symptomatics identified was 61 (4%) of 1730. One sputum specimen, from each chest symptomatic was collected and examined by smear and culture for **M. tuberculosis.** Sputum specimen from one patient was positive by smear and treatment was initiated for him.

The study is in progress.

Encouraged by the findings of the study, it was proposed to extend the study to a few other areas in Sriperumbudur. The village Dais are not on the pay roll of the government. In order to develop a methodology for approaching the Dais in the villages, about 32 Dais serving the villages in Sriperumbudur Taluk were addressed in 3 sessions by the staff of the Centre to find out their willingness to participate in the programme. Majority of them were willing and they suggested that village headman be approached first for permission. In order to find out the feasibility of trying this strategy, one village was chosen and health education was conducted to improve the general awareness of the villagers. The Dai was trained in identification of chest symptomatics and collection of sputum from them. The Dai had collected 15 sputum specimens from chest symptomatics all confirmed to be true over a period of 3 months and one of them was positive by smear for AFB. Subsequently no sputum specimens were collected by the Dai. The reason was that the villagers expected comprehensive primary health care. The initial enthusiasm was not sustained in this village.

In two other villages, health education and health camps were held. About 600 to 700 villagers gathered. About 132 persons collected symptomatic drugs. Attempts were made to collect 3 specimens from each chest symptomatic. A total of 76 sputum specimens were collected from 27 chest symptomatics. All sputum specimens examined were negative for AFB by smear and one yielded positive for **M. tuberculosis.** The Dais asked for remuneration for continuing this work in the villages, and they did not collect sputum from the chest symptomatics (identified during the camp) subsequently.

The study has shown that by training the traditional birth attendants (Dais) and utilising their services, it is possible to improve the case-finding component of DTP.

*****

Utilisation of NSS volunteers to augment the case-holding component of Madurai city TB programme

(Ongoing study, 1993-94)

In an earlier study conducted in Madurai, it was found feasible to use National Service Scheme (NSS) student volunteers in the TB programme (see 1992 annual report). Encouraged by the findings in this study a second study has been started utilising the male students to improve case-holding component of Madurai city TB programme by (a) distribution of anti-TB drugs by students to pulmonary tuberculosis patients through centres situated closer to patients' home and (b) defaulter retrieval by students.

NSS student volunteers of American College, Madurai, were briefed on general aspects of tuberculosis and TB programme. These students were also trained in home visits and in the art of motivating a patient for drug compliance. Later these students were assessed for their ability to communicate with the patient. In addition, their willingness to participate in the programme was ascertained.

A camp was conducted in Sellur Madurai Corporation Dispensary (Sellur) to identify the chest symptomatics belonging to Urban Health Post (UHP) areas of Sellur. Initial clinical screening was carried out in the camp to identify the chest symptomatics. Twenty-seven active pulmonary TB cases were identified from among these symptomatics, by sputum examination and X-ray chest.

Patients found suitable for admission to the study were started on ethambutol, isoniazid, rifampicin and pyrazinamide daily for 2 months followed by ethambutol and isoniazid daily for 6 months ($2EHRZ_7/6EH_7$).

The drugs are pre-packed in polythene covers and are distributed at Sellur dispensary by NSS student volunteers once a week during the treatment period. Defaulters are visited and motivated by the NSS students during the same week and also in the successive week, if the patient continues to default.

The study is continuing.

*****

CLINICAL STUDIES - COMPLETED

Role of bronchoalveolar and gastric lavages in the diagnosis of pulmonary tuberculosis in children

(Completed study, 1992-93)

Bacteriological diagnosis of pulmonary tuberculosis in children is difficult because of the paucibacillary nature of the disease and difficulty in obtaining sputum specimens from children. Most patients are now treated on the basis of clinical presentation and chest roentgenographic appearances. This usually results in overdiagnosis and unnecessary treatment of children with anti-tuberculous drugs. Since a reliable immunodiagnostic test for tuberculosis is not currently available, a study has been planned to evaluate the role of bronchoalveolar (BAL) and gastric lavages (GL) for bacteriological diagnosis of childhood pulmonary tuberculosis. Although bronchoalveolar lavage is not at present a standardised procedure in children, the bronchial and alveolar specimens obtained during flexible fibreoptic bronchoscopy are sufficient for bacteriological examination. This study was carried out in collaboration with the Institute of Child Health and Hospital for Children, Madras (Professor N. Somu, Principal Investigator).

Table 1

Comparison of culture results of BAL specimens with Gastric Lavage specimens

'BAL specimens	Gastric lavage culture		
	Positive	Negative	
Positive	5	1	6
Negative	11	33	44
Total	16	34	50

Children aged 1-12 years attending the Institute of Child Health and presenting with respiratory symptoms and persistent chest radiographic findings suggestive of pulmonary tuberculosis were evaluated. Bronchoalveolar lavages were done at the Institute of Child Health, while

bacteriological investigations were carried out at the Centre. Fifty suspected pulmonary tuberculosis patients were admitted to the study.

Out of the 50 suspected patients, 17 had tuberculosis as revealed by positive culture of **M. tuberculosis** in gastric and/or bronchoalveolar lavages. Of these, 5 (29%) had both BAL and GL specimens positive. Eleven (65%) were positive by GL alone compared with only one patient who had culture positivity by BAL alone (Table 1). Therefore, GL is superior to BAL for diagnosis of pulmonary tuberculosis in children.

*****

Late sequelae in tuberculous meningitis children during 5-year follow-up

(Completed study, 1988-93)

In the 1992 annual report, the clinical status at the end of 5-year follow-up of 99 surviving TB meningitis patients (32 with moderate sequelae, 14 with mild sequelae and 53 with complete recovery) was presented. This report gives the results of the following investigations carried out, in collaboration with the various departments of the Institute of Child Health (ICH), Egmore, Madras (Director: Dr. A. Chandrabhushanam), to find out the occurrence of late sequelae in these children:

1. Electro-encephalography (EEG)
2. Psychometric evaluation
3. Skull radiography for evidence of calcification
4. Hearing assessment (Audiometry).

Except the hearing assessment which was done at the 8th, 14th, 20th (6, 12 & 18 months after stopping streptomycin) and 60th monthly examinations, all the others were done between 48 and 60 months. The results are as follows:

Electro-encephalography: EEG could be done in 96 of the 99 patients. Of these, the tracing was normal in 73 (76%) patients, even though 8 of them had developed fits. The EEG was abnormal in 23 (24%)

including 11 who had clinical epilepsy. All the 3 patients for whom EEG could not be done, had clinical epilepsy.

Psychiatric evaluation: Evaluation was done in 96 of the 99 patients. Of these, 48 (50%) patients were found to be normal while 25 (26%) patients were classified as mild, 18 (19%) as moderate and 5 (5%) patients as severely retarded. In all, 16 (17%) patients developed behaviour problems and had to be referred to the child guidance clinic for treatment.

Skull radiography: Skull X-ray was taken to find out whether there was calcification. Of the 98 patients who had been X-rayed, calcification was found in 3 (3%) patients. In one patient, it was suprasellar and in the other parasellar. The third patient had multiple calcified spots.

Hearing assessment: Audiometry was done in 98 of the 99 patients. Hearing was found to be normal in 96 (98%) patients. One patient developed bilateral conductive loss of hearing which is unlikely to be related to streptomycin and the other had unilateral sensorineural loss of hearing but the exact cause of deafness could not be assessed as the patient did not report for repeat testing.

In conclusion, the results stress the need for long-term follow-up of tuberculous meningitis patients after completion of treatment in order to find out the development of long-term sequelae.

*****

CLINICAL STUDIES - IN PROGRESS

Six month regimen for pulmonary tuberculosis with 2 double-drug combinations on alternate days for the first two or three months

(Ongoing study, 1990-94)

Several highly effective rifampicin-containing short course chemotherapy regimens of 6-8 months' duration have been evolved for the treatment of pulmonary tuberculosis. In almost all 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. One of the measures that might help to overcome these difficulties is to split the four oral drugs into two 2-drug combinations, each combination given on alternate days, thus making each 2-drug combination intermittent.

The Centre is investigating, both at Madras 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 given twice a week for the next 4 and 3 months, respectively. Since both the drug combinations will be given intermittently, the toxicity is expected to be low while the high level of efficacy is unlikely to be affected. If the findings are promising, this will be a major step towards the possible use of blister packs for drug delivery in TB Programmes. 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. This will provide information as to whether the regimen will be equally effective when all 4 drugs are given together or when they are given as two 2-drug combinations on alternate days.

Patients are randomly allocated, irrespective of their previous chemotherapy, to one of the following regimens.

1. **$2RE_3HZ_3$ (alt.) /$4RH_2$: (2alt)** : Fully supervised regimen of 6 months' duration, consisting of rifampicin and ethambutol on one day and isoniazid and pyrazinamide on the next day, thrice a week for

2 months, followed by rifampicin and isoniazid twice a week for the next 4 months.

2. **$3RE_3HZ_3$ (alt.)/$3RH_2$: (3alt) :** This is similar to regimen 1, but the initial and continuation phases are each of 3 months' duration. (For regimens 1 and 2, Sunday is a drug-free day).

3. **$2REHZ_3/4RH_2$: (Thrice) :** All the 4 drugs are given together under supervision in one dose thrice a week for 2 months, followed by rifampicin and isoniazid under supervision twice a week for the next 4 months.

The dosages are the same for all the 3 regimens in both the phases, namely, rifampicin 450 mg, ethambutol 1000 mg and pyrazinamide 1.5 g for patients weighing 40.0 kg or less, and 600mg, 1200 mg and 2.0 g, respectively, for patients weighing 40.1 kg or more. The dosage of isoniazid is 600 mg, irrespective of body-weight. The dosages are increased for gain in weight, but not reduced for loss of weight.

Table 1

Number of patients allocated to different regimens

Centre	2alt.	3alt.	Thrice	Total
Madras	149	146	148	443
Madurai	145	147	145	437
Both	294	293	293	880

A total of 880 patients (443 in Madras and 437 at Madurai) have been admitted up to December 1993 (Table 1). Of these, 750 patients (390 at Madras and 360 at Madurai) admitted up to June '93 were expected to complete the prescribed chemotherapy by the end of the year. Twenty-three patients were excluded from analysis because of, early death (3), pretreatment culture negativity (8), only one smear positive (1), infection with non-tuberculous mycobacteria on admission (5), non-tuberculous death (2), chemotherapy modified for adverse reaction (2) and HIV positives (2). Of the remaining 727 patients, 519 (71%) patients received 90% or more of chemotherapy during phase I, i.e., 177 (74%) patients, in "2 alt", 169 (70%) patients in "3 alt" and 173 (71%) patients in "thrice" regimen (Table 2).

Table 2

Percentage of chemotherapy received during phase I

Rx. received (%)	Number of patients		
	2 alt.	3 alt.	Thrice
100	60	46	87
95-99	68 } **74%**	82 } **70%**	48 } **71%**
90-94	49	41	38
80-89	29	39	35
70-79	16	15	13
60-69	7	11	6
50-59	5	2	7
< 50	3	2	6
Continuous FTA >1m	1	4	3
Total	238*	242	243

* excluding 4 patients who died of TB.

Table 3

Percentage of chemotherapy received during phase I and II

Rx. received (%)	Number of patients		
	2 alt.	3 alt.	Thrice
100	31	28	52
95-99	85 } **67%**	91 } **68%**	70 } **68%**
90-94	43	44	43
80-89	37	41	35
70-79	14	16	12
60-69	10	6	8
50-59	4	2	2
< 50	1	0	1
Continuous FTA >1m	13	13	20
Total	238	241*	243

* excluding 1 patient who had change of treatment.

Regarding chemotherapy during phases I and II combined, out of the 722 patients, 487 (67%) patients received 90% or more of chemotherapy i.e., 159 (67%) patients in ,"2 alt", 163 (68%) patients in "3 alt" and 165 (68%) patients in "thrice" regimen (Table 3).

Excluding 101 patients who had inadequate chemotherapy (i.e., missed 25% or more of total doses, or missed 50% or more in either phase or missed drugs continuously for 1 month or more in either phase), there were 626 patients for the efficacy analysis. Of these, 504 (81%) patients had organisms initially sensitive to isoniazid and rifampicin. Of the remaining, 101 were resistant to isoniazid only, 2 to rifampicin only and 19 to isoniazid and rifampicin.

Culture results during treatment: The percentage of culture negativity, based on 3 specimens per month, during 1-6 months for patients with initially drug sensitive organisms is given in table 4. The proportion with all cultures negative was 28-30% in the first month, 80-83% in the 2nd month and 95-99% in the 3rd and subsequent months. The sputum conversion was very rapid by 2 months.

Table 4

Percentage of culture negativity among patients with initial drug-sensitive organisms

Month after start of chemotherapy	Percentage culture negative		
	2 alt.	3 alt.	Thrice
1	28	30	29
2	80	83	82
3	97	96	96
4	97	99	98
5	95	98	96
6	97	95	98
Total patients (range)	171-173	168-169	161-162

Bacteriological status at the end of treatment among patients with sensitive organisms is given in table 5. A patient was classified as having

had an unfavourable bacteriological response at the end of chemotherapy, if he had a total of 2 or more positive cultures in the last 2 months including at least one in the last month of treatment, and at least one of these having a growth of 20 colonies or more. Patients who had a change of treatment for persistent bacteriological positivity or radiographic deterioration or clinical deterioration, and those who died of tuberculosis are also classified as having an unfavourable response.

Favourable response (all cultures negative in the last two months of chemotherapy) was observed in 163 (94%) patients in "2 alt" regimen,158 (93%) patients in "3 alt" regimen and 152 (94%) patients in "Thrice" regimen.

Table 5

Bacteriological status at the end of treatment among patients with initial drug-sensitive organisms

Response at the end of Rx.	2 alt.		3 alt.		Thrice	
	No.	%	No.	%	No.	%
Favourable	163	**94**	158	**93**	152	**94**
Doubtful	6		9		10	
Unfavourable	4		2		0	
Total	173		169		162	

Among patients with bacilli resistant to 'H' alone, favourable response was observed in 21(78%) patients in "2 alt", 29(81%) patients in "3 alt" and 28(74%) patients in "Thrice" regimen. Two patients with organisms resistant to rifampicin alone had a favourable response at the end of treament. There were 19 patients with organisms resistant to R and H and of these, 18 patients had an unfavourable response and one patient had a favourable response at the end of chemotherapy.

Regarding adverse reactions (Table 6), among 733 patients who completed chemotherapy, 27 (11%) patients in "2 alt", 43 (18%) patients in "3 alt" and 41 (17%) patients in "Thrice" regimen had some complaint(s). Fourteen patients (4 in "2 alt", 4 in "3 alt" and 6 in "Thrice") had modification of chemotherapy; other minor reactions were managed symptomatically.

Table 6

Adverse reactions

Regimen	Total No.of patients	Patients with complaints						
		Any No.	%	Gastro-Intestinal	Arthralgia	Cutaneous	Giddiness	Hepatitis	Others*
2 alt.	243	27	11	9	7	7	3	2	1
3 alt.	244	43	18	13	19	6	4	4	3
Thrice	245	41	17	20	7	9	6	1	4

* Includes 'flu' syndrome, purpura, hypersensitivity reaction, peripheral neuropathy and glossitis stomatitis.

The study is in progress.

*****

Controlled clinical trial of fully oral short course regimens in Madras and Madurai: follow-up phase

(Ongoing study, 1986-95)

As described in previous annual reports (1988-92), a controlled clinical trial is in progress to investigate three fully oral regimens of 6 or 8 months' duration, with different rhythms, varying frequencies of attendance and full, partial or no supervision of drug intake. Patients with positive sputum smears were randomly allocated, irrespective of previous chemotherapy, to one of the following regimens.

1. $2EHRZ_7$ (ow)/$6EH_7$ (tm) : This is a fully self-administered daily regimen of 8 months' duration. Ethambutol 600 mg, isoniazid 300 mg, rifampicin 450 mg and pyrazinamide 1.59 daily are prescribed for the first 2 months, followed by ethambutol 600 mg and isoniazid 300 mg daily for the next 6 months. The patients are required to attend the clinic once a week during the first 2 months and twice a month during the next 6 months for drug collection.

2. $2EHRZ_2$/$4EHR_2$ (tw) or $2EHRZ_2$/$4EHR_2$ (ow) : This is a twice-weekly regimen of 6 months' duration. The patients receive ethambutol 1200 mg, isoniazid 600 mg, rifampicin 450 mg and pyrazinamide 2.09 during the first

two months and ethambutol, isoniazid and rifampicin in the same dosages during the next 4 months. Half the patients, by random allocation, receive fully supervised chemotherapy at the clinic, necessitating twice-weekly attendance throughout. The other half attend once a week, when one dose is given under supervision and the other dose is supplied for self-administration.

3. $2HRZ_2/4HR_2$ (tw) or $2HRZ_2/4HR_2$ (ow) : This is similar to regimen 2, but without ethambutol.

The study was undertaken at the Centre and its unit at the Government Rajaji Hospital, Madurai (Dean: Dr.Rajappa). Patients at Madurai were admitted on the basis of smear examination done at the Madurai Unit, and multiple sputum specimens were transported to Madras for culture and sensitivity tests. Close liaison is being maintained by the Centre with the Madurai Unit by periodic visits by the Centre's staff.

The intake to the study was completed in October, 1990. A total of 1204 patients (601 in Madras and 603 in Madurai) were admitted to the study. Of these, 113 patients (62 in Madras and 51 in Madurai) have been excluded for various reasons. The response to chemotherapy of the remaining 1091 patients had already been presented in the 1991 annual report.

Relapse requiring treatment: Bacteriological relapse requiring treatment is defined as 2 or more positive cultures in a 2-month period, at least one of which has a growth of 20 colonies or more, associated with a positive smear. A total of 772 patients with initially drug-sensitive organisms have completed 28/30 months of follow-up after stopping treatment. Relapse requiring treatment occurred in 18 (6.2%) of 289 patients in regimen 1, 28 (11.0%) of 255 patients in regimen 2, and 24 (10.5%) of 228 patients in regimen 3 (Table 1). A high proportion (76%) of these relapses has occurred during the first 3 to 6 months after stopping treatment.

The relapse requiring treatment during 28/30 months of follow-up of 144 patients with initially isoniazid-resistant organisms is given in table 2. Six (8%) of 73 patients in regimen 1, 11 (25%) of 44 patients in regimen 2 and 4 (15%) of 27 patients in regimen 3 have relapsed. Nineteen of these 21 relapses have occurred within 3 months after stopping treatment. There is a significant difference in the proportion of relapses between regimen 1 and regimen 2 ($P = 0.03$).

Table 1

Bacteriological relapse during 28/30 months follow-up in patients with initially drug-sensitive organisms

Regimen	Total assessed	Relapse requiring Rx. No.	%	Month of relapse after stopping treatment					
				1-3	4-6	7-12	13-18	19-24	25-28/ 30
1) $2EHRZ_7/6EH_7$	289	18	**6.2 (3.6-9.3)***	11	2	1	1	1	2
2) $2EHRZ_2/4EHR_2$	255	28	**11.0 (7.6-15.8)**	15	7	4	2	0	0
3) $2HRZ_2/4HR_2$	228	24	**10.5 (6.3-14.0)**	13	5	3	1	1	1

* Figures in parentheses are 95% confidence limits

Table 2

Bacteriological relapse during 28/30 months follow-up in patients with initially isoniazid-resistant organisms

Regimen	Total assessed	Relapse requiring Rx. No.	%	Month of relapse after stopping treatment			
				1-3	4-6	7-12	13-28/30
1) $2EHRZ_7/6EH_7$	73	6	**8.2**	5	0	0	-1
2) $2EHRZ_2/4EHR_2$	44	11	**25.0**	11	0	0	0
3) $2HRZ_2/4HR_2$	27	4	**14.8**	3	1	0	0

Follow-up of the patients is continuing.

*****

Treatment regimens for patients who fail or relapse on short course chemotherapy

(Ongoing study, 1987)

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:

Patients with bacilli sensitive to isoniazid and rifampicin: These patients are admitted by random allocation to either $3EHRZ_2/6HR_2$, or $3EHRZ_2/9HR_2$, namely, ethambutol 1200 mg plus isoniazid 600 mg plus rifampicin 450 mg plus pyrazinamide 2.09 twice a week for the first 3 months, followed by isoniazid plus rifampicin in the same dosages twice a week for either the next 6 months (9m regimen), or the next 9 months (12m regimen). Every dose is administered under supervision.

So far, 117 patients have been admitted to these two regimens. Of 88 eligible patients for whom information up to one year is available, 26 are excluded for various reasons (Table 1). Of the remaining 62 (30 in 9m regimen and 32 in 12m regimen), 58 (29 in 9m regimen and 29 in 12m regimen) had a favourable bacteriological response. Four patients (1 in 9m regimen and 3 in 12m regimen) showed an unfavourable bacteriological response, without any associated clinical or radiographic changes, and the 3 in 12m regimen had a change of chemotherapy. Treatment was intensified to daily drug therapy in 2 patients in the 7th and 12th months respectively; the former had produced negative cultures at the first month and the latter, from the 2nd to the 8th month. The regimen was changed in the 6th month for one other patient whose cultures were persistently positive and who had developed isoniazid resistance from the 2nd month. One patient (9m regimen) had been persistently culture positive and had developed resistance to isoniazid at the 3rd month and he had change of chemotherapy at the end of the specified period of chemotherapy.

Patients with bacilli resistant to isoniazid: These patients are admitted to $6SERZ_2/6ERZ_2$, or $6KERZ_2/6ERZ_2$ and prescribed streptomycin 0.75g, or if the bacilli are resistant to streptomycin, kanamycin 1.0g, plus ethambutol 1200 mg plus rifampicin 450 mg plus pyrazinamide 2.0g

twice a week for the first 6 months followed by ethambutol plus rifampicin plus pyrazinamide in the same dosages twice a week for the next 6 months (total 12 months). Every dose is administered under supervision.

Table 1

Distribution of patients with sensitive organisms to H and R according to their status during and at the end of treatment

	No. of patients	
	9m regimen	12m regimen
No. with information up to 1 year	43	45
Change of Rx. due to adverse drug reactions	2	0
Early death due to tuberculosis	0	1
Non-TB death	0	1
Received < 75 % of chemotherapy	11	9
Others (extended phase I chemotherapy)	0	2
No.of patients in analysis	30	32
Favourable response	29	29
Unfavourable response	1	3

$6SERZ_2/6ERZ_2$: So far, 58 patients have been admitted to this regimen. Of 42 eligible patients for whom information up to 1 year is available, 15 are excluded for various reasons (Table 2). Of the 27 in analysis, 20 had a favourable bacteriological response. Of the 7 who had an unfavourable bacteriological response, five patients required a change of chemotherapy. Of these, 1 had positive cultures persistently; his treatment was changed at the 6th month, from intermittent to daily chemotherapy; One patient had produced negative cultures at the 2nd and 3rd months, positive cultures from the 4th month onwards and developed streptomycin resistance at the 5th month, the treatment was changed at the 11th month. One other patient had positive cultures persistently, and his cultures were reported resistant to streptomycin and rifampicin at 8 and 9 months respectively and his treatment was changed at the end of the specified period of chemotherapy. The other 2 patients had produced

positive cultures from the beginning and underwent change of chemotherapy at 5 and 7 months respectively, .and the former had developed rifampicin ,resistance as well. The other 2 patients had produced negative cultures from the 1st and 2nd month and positive cultures from the 5th and 10th month onwards, respectively. None of these 7 patients had associated clinical or radiographic deterioration.

Table 2

Distribution of patients with resistant organisms according to regimen and their response to treatment

	H resistant	SH resistant	RH resistant	SHR resistant
	6 $SERZ_2$/ 6 ERZ_2	6 $KERZ_2$/ 6 ERZ_2	3 $S_3EmbEthZ_7$/ 9 $EmbEthZ_7$	3$K_3EmbEthZ_7$/ 9$EmbEthZ_7$
No. of patients with information up to 1 year	42	44	21	37
Change of Rx. due to adverse drug reactions	3	1	3	3
Non-TB death	1	1	0	2
Early TB death	0	0	0	1
Received <75% of chemotherapy	11	4	8	8
No. of patients in analysis	27	38	10	23
Favourable response	20	28	5	10
Unfavourable response	7	10	5	13

$6KERZ_2/6ERZ_2$: So far 56 patients have been admitted to this regimen Of the 44 eligible patients, 6 are excluded (Table 2). Of the 38 in analysis, 28 had a favourable bacteriological response. Of the remaining 10 patients who had an unfavourable bacteriological response, 8 had a change of treatment between 5 and 10 months for persistent culture positivity; and 6 had developed rifampicin resistance. One other patient had his treatment changed to daily chemotherapy at the 6th month as he got himself admitted in a hospital; he also had persistent culture positivity. The other patient who completed prescribed treatment was culture negative from the 2nd to the 10th month, but produced two positive cultures in the last 3 months of treatment.

Patients with bacilli resistant to isoniazid and rifampicin: These patients are admitted to $3S_3EmbEthZ_7/9EmbEthZ_7$ or $3K_3EmbEthZ_7/9EmbEthZ_7$, and prescribed streptomycin 0.75 g thrice a week or, if the bacilli are resistant to streptomycin, kanamycin 1.0g thrice a week, plus daily ethambutol 600 mg plus ethionamide 500 mg plus pyrazinamide 1.59 for the first 3 months, followed by daily ethambutol plus ethionamide plus pyrazinamide in the same dosages for the next 9 months (total 12 months). Throughout the 12 months, the patients attend thrice a week, when they receive that day's dose under supervision and are supplied with drugs for the following day(s) for self-administration.

$3S_3EmbEthZ_7/9EmbEthZ_7$: So far, 30 patients have been admitted to this regimen. Of the 21 eligible patients, 11 are excluded (Table 2). Of the remaining 10 patients, 5 had a favourable response. Of the 5 with an unfavourable response, 3 persistently had positive cultures and had a change of treatment at the 7th, 7th and 10th months, respectively. Two patients had negative cultures from the 1st month, but produced positive cultures from the 5th month onwards; one of these had a change of treatment at the 8th month and the other after the specified period of chemotherapy.

$3K_3EmbEthZ_7/9EmbEthZ_7$: So far, 42 patients have been admitted to the regimen. Of the 37 eligible cases, 14 are excluded (Table 2). Of the 23 in analysis, 10 had a favourable bacteriological response. Of the remaining 13 who had an unfavourable response, 11 had persistent positive cultures and had a change of treatment between 4 and 12 months. One patient had negative cultures from the 1st to the 3rd month, produced positive cultures from the 4th month, and had a change of treatment at the 6th month. One patient died of active pulmonary tuberculosis during the 2nd month of chemotherapy.

The intake to all the regimens is continuing.

*****

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

(Ongoing study, 1993-95)

Quality of life is a multidimensional concept concerned with the impact of physical symptoms and side effects of treatment on patients' functioning and psycho-social well-being. So far, quality of life measurements have not been carried out in patients with pulmonary tuberculosis. 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 Well-being Scale (J. chronic Dis 1984, 37: 85-95) 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 recently developed for chronic lung diseases are Chronic Respiratory Questionnaire and St. George's Respiratory Questionnaire (Thorax 1987,42:773-78). It had been shown previously that walking distance and subjective disability are well correlated, and there was poor correlation between spirometry and disability.

The objectives of the study are:

1. to assess quality of life measurements using Disease-specific questionnaire (Chronic Respiratory Questionnaire) in patients who had undergone treatment for pulmonary tuberculosis.

2. to compare the General (Quality of Well-being Scale) and Disease-specific (Chronic Respiratory Questionnaire) questionnaire with 6-minute walking test and pulmonary function tests in patients treated for pulmonary tuberculosis.

A group of 200 pulmonary tuberculosis patients treated with short-course chemotherapy will be included in the study if they had no previous

history of 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." General Health measurements that included mobility, physical and social activities of patients are assessed using Quality of well-being scale. 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 (Am Rev Respir Dis 1987,136:1285-96).

2. **Six-minute walking test:** This test (JE Cotes, Lung Function, 5th Edition 1993) 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.

So far 46 patients have been admitted .

The study is in progress.

*****

Short course Chemotherapy for pulmonary tuberculosis in children

(Ongoing study, 1992-96)

The Centre is currently engaged in a short course chemotherapy study of children with pulmonary tuberculosis (see 1992 annual report) in collaboration with the Institute of Child Health (ICH), Egmore, Madras (Director: Dr.A. Chandrabhushanam). In brief, patients aged between 1

and 12 years who had not received more than 2 weeks of previous anti-tuberculosis treatment with an abnormal chest radiograph are admitted to the study. The children are classified into two categories, definite cases (Category A) and probable cases (Category B). Patients in category A are directly started on anti-tuberculosis treatment. Patients in Category B are administered antibiotics for a period of 10-14 days and the chest X-ray is repeated. If the abnormality persists they are admitted to the study. Patients in each category are randomly allocated to one of the 2 following regimens.

Regimen I: 9 HR: Rifampicin and isoniazid daily for 9 months. Patients will attend the clinic once a week; the drugs are administrated together under supervision on the day of attendance and supplied for the rest of the week.

Regimen II: $2H_3R_3Z_3/4H_2R_2$: Rifampicin, isoniazid and pyrazinamide thrice a week for the first 2 months followed by isoniazid and rifampicin twice a week for the next 4 months. All the drugs are administered together under supervision.

The dosages are as follows:

Isoniazid: 6 mg/kg daily and 15mg/kg thrice/twice-weekly.

Rifampicin: 12 mgkg thrice/twice-weekly.

Pyrazinamide: 45mg/kg thrice-weekly.

It is proposed to admit a total of 200 patients (100 in each regimen). So far 61 patients have been admitted (30 to Regimen I and 31 to Regimen II).

The intake is continuing.

*****

Collaborative controlled clinical trial of tuberculous lymphadenitis.

(Completed study, 1988-93)

The Centre has carried out a randomised controlled clinical trial on treatment of lymph node tuberculosis at Madurai in collaboration with the Paediatric (Dr. A. J. Thiruthuvathas) and Adult Surgery (Dr. D. Anantharaj

and Dr. M.N. Kamaludeen) Departments of the Government Rajaji Hospital (see annual report 1992). Patients residing in or near Madurai with biopsy proved tuberculous lymphadenitis were admitted to the study. Previous anti-TB therapy was not a contra-indication for admission to the study. The histopathology of the lymph node biopsy was read by the Professor of Pathology (Dr. V. Ananthalakshmi), Madurai Medical College and bacteriological investigations were done at TRC, Madras.

All patients admitted to the study were randomly allocated to either a 6-month daily regimen of rifampicin and isoniazid ($6RH_7$) supplied twice a month for self-administration, or a 6-month fully supervised twice-weekly regimen of rifampicin and isoniazid, supplemented with pyrazinamide for the first two months ($2RHZ_2/4RH_2$). The drugs were prescribed in uniform dosage for adults, while weight-adjusted dosage was used for children. Patients were treated on an out-patient basis. Surprise home visits were done for pill counts for patients allocated to the self-administered regimen ($6RH_7$).

Patients are assessed clinically every month up to 12 months, every 3 months up to 24 months and every 6 months up to 60 months. At the end of chemotherapy, the clinical response is assessed by an independent assessor. The response was defined as follows:

(a) **Favourable,** if the nodes have regressed in size to 10 mm or less and if any sinus/abscess present on admission has healed,

(b) **Doubtful,** if significant residual nodes(>l0mm diameter) are palpable, or

(c) **Unfavourable,** if change of treatment required due to clinical deterioration and/or post treatment node biopsy culture positive.

Repeat lymph node biopsy is done for patients with significant residual lymphadenopathy. Patients will be followed up for 5 years from the start of treatment.

Results: In all, 277 patients were admitted to the study. After excluding 15 patients for various reasons (received <75% of allocated treatment: 7; lost: 7; non-TB death: 1), 262 patients are available for analysis. Of these, 175 (67%) were adults, 180 (69%) were females and 141 (54%) were in the 13-30 years age group (Table 1).

Table 1

Distribution of patients by sex and age

	Age (years)	Male	Female	Total	
				No.	%
Children	≤6	23	10	33	**13**
	7-12	31	23	54	**21**
Adults	13-30	20	121	141	**54**
	31-40	5	21	26	**10**
	>40	3	5	8	**3**
Total patients		82	180	262	**101**

The results of the bacteriological examination of the lymph node biopsy specimens on admission are shown in table 2. Lymph node culture was positive for **M. tuberculosis** in 69% of the children and 67% of adults prior to start of chemotherapy. Twenty one patients were resistant to either one, two or three drugs (S/H/SH:18; R:1; HR:1; SHR:1).

Table 2

Bacteriological examination of lymph node biopsy on admission

	No. of patients	Culture results on admission*					
		Positive		Negative		NTM	
		No.	%	No.	%	No.	%
Children	84	58	**69**	22	**26**	4	**5**
Adults	173	116	**67**	49	**28**	8	**5**

*Cultures were not done for 3 children and 2 adults.

Response to treatment: The response at the end of treatment is shown in table 3. Thirty-six (86%) of 42 children in $6RH_7$ series and 37 (82%) of 45 in the $2RHZ_2/4RH_2$ series had a favourable response at the end of treatment. In adults,the favourable response was 79 (87%) of 91 and 74 (88%) of 84 in the two regimens respectively.

Table 3

Response at the end of treatment

	Total patients	Response at the end of treatment					
		Favourable		Doubtful		Unfavourable	
		No.	%	No.	%	No.	%
Children							
$6RH_7$	42	36	**86**	6	**14**	0	-
$2RHZ_2/4RH_2$	45	37	**82**	8	**18**	0	-
Adults							
$6RH_7$	91	79	**87**	8	**9**	4	**4**
$2RHZ_2/4RH_2$	84	74	**88**	10	**12**	0	-

Four adult patients (all $6RH_7$) had an unfavourable response and treatment was changed for these cases. In 32 (14 children and 18 adults) patients, the response was classified as doubtful at the end of treatment. Of these, in 23 (12 children and 11 adults) the nodes regressed subsequently. Of the remaining 9, repeat biopsy was done in 7 patients. In 2 patients, the lymphnode culture was positive and the response was reclassified as unfavourable and the patients retreated. In 4 patients, the lymph node culture was negative though histopathology was suggestive of tuberculosis. In 1 patient, the culture yielded NTM and the histopathology was suggestive of tuberculosis. Of the remaining 2 patients, one had completed 60 months follow-up and still had residual nodes and the other is on follow-up.

The follow-up is continuing.

*****

Collaborative study of abdominal tuberculosis: follow-up phase

(Ongoing study, 1983-2001)

As mentioned in previous annual reports, the Centre has carried out a collaborative study of abdominal tuberculosis. The objectives of this

study were:

(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 one of the following regimens.

2RHZ/4RH (rifampicin series): Rifampicin 10 mg/kg plus isoniazid 300 mg plus pyrazinamide 30 mg/kg daily for 2 months, followed by rifampicin 10 mg/kg plus isoniazid 300 mg daily for the next 4 months.

SEH/EH (non-rifampicin series): Streptomycin 0.75 g plus ethambutol 25 mg/kg plus isoniazid 300 mg daily for 2 weeks, followed by ethambutol 15 mg/kg plus isoniazid 300 mg daily for the next 50 weeks.

The details regarding characteristics on admission, confirmation of diagnosis, population in analysis, adverse reactions to drugs and response at the end of treatment, were presented in the 1991 annual report. The results up to 5 years from the time of start of treatment are presented in this report.

Results up to 60 months: Of the 157 patients (84 rifampicin series, 73 non-rifampicin series) who were either symptom free or had clinically improved at the end of treatment period, 135 patients (72 rifampicin series, 63 non-rifampicin series) have completed 48/54 months of follow-up. Six of them, (3 rifampicin series, 3 non-rifampicin series) required retreatment. In the rifampicin series, one patient was retreated following surgery for intestinal obstruction in the 7th month of follow-up, as advised by the surgeon even though there was no histopathological or bacteriological evidence of relapse, one for renal tuberculosis in the 12th month of follow-up and the other one for suspected tuberculous lymphadenitis and TB spine in the 55th month of follow-up, which was later on proved to be squamous cell carcinoma with secondaries in the spine. In the non-rifampicin series, one patient was retreated for renal TB in the 54th month, one for TB lymphadenitis in the 43rd month and the remaining one for multiple brain tuberculoma in the 21st month of follow-up and took treatment elsewhere. One patient (rifampicin series) died due to

tuberculosis and 8 patients (6 rifampicin series, 2 non-rifampicin series) died due to causes other than tuberculosis. One patient (non-rifampicin series) was lost to follow-up at the 11th month; she was asymptomatic at that time. One patient (rifampicin series) did not attend for the 60th monthly review, but was doing well when examined at 48th. month. None of the remaining 118 (61 rifampicin series, 57 non-rifampicin series) patients had relapse. However, long-term follow-up results are essential for drawing definite conclusions.

Follow-up of the patients up to 120 months after admission is continuing.

*****

Collaborative study of brain tuberculoma: follow-up phase

(Ongoing study, 1992-96)

As mentioned in previous annual reports (1990-92), the Centre is carrying out a collaborative study with the Institute of Neurology, Government General Hospital (Prof. Ramachandran), Madras and Railway Hospital (Prof: Zaheer Ahmed Sayeed), Perambur on brain tuberculoma.

The objectives of this study are:

1. to evaluate the efficacy of short course regimens for treating tuberculoma of the brain and

2. to study the CT scan appearance, before, during and at the end of chemotherapy and up to 60 months after the start of treatment.

A secondary objective was to study the role of surgery in the treatment of brain tuberculoma.

The patients admitted to the study were randomly allocated to one of the following 9-month regimens:

Regimen I: $3RHZ_7/6RH_2$ (Daily)

Regimen II: $3RHZ_3/6RH_2$ (Intermittent)

Chemotherapy consisted of 3 drugs, rifampicin, isoniazid and pyrazinamide for 3 months daily in Regimen I and thrice weekly in Regimen II, followed by 2,drugs rifampicin and isoniazid for 6 months, twice-weekly in both the regimens.

A total of 144 patients were admitted to the study and 36 patients were excluded for various reasons. The 1991 annual report described the response to SCC in the remaining 108 patients and in the 1992 annual report, the 24th monthly follow-up findings were presented. All these patients are under follow-up and during 36 to 60 months period none had a relapse requiring treatment.

The five year follow-up is continuing.

*****

Collaborative clinical study of cutaneous tuberculosis

(Ongoing study, 1992-95)

There have been only a few trials on cutaneous tuberculosis using multiple drug regimens. Hence a pilot study on cutaneous tuberculosis, was started in collaboration with Departments of Dermatology, Government General Hospital, Madras (Prof.P. Yesudian) and Government Stanley Hospital, Madras (Prof.S. Premalatha) with the following objectives:

(a) to evolve and establish suitable criteria for diagnosis and

(b) to assess the feasibility of conducting controlled clinical trials to evolve efficacious treatment regimens.

Patients diagnosed clinically as having cutaneous tuberculosis by the dermatologist are admitted to the pilot study. The following investigations are carried out prior to admission:

1) Complete haemogram,

2) Biochemical investigations,

3) Dermatological assessment,

4) Clinical photography,

5) Biopsy of the lesion for histopathology and bacteriological examinations and

6) Mantoux test with 1-TU of PPD (RT 23 with Tween 80) read at 1, 2, 3, 4, 7, 14 and 21 days.

Adult patients (aged 12 years or more) admitted to the study are treated with rifampicin (450 mg) and isoniazid (300 mg) daily for 9 months, while children are treated with weight adjusted dosages of rifampicin (10-12 mg/kg) and isoniazid (5mg/kg). The drugs are supplied once a week for self administration at home. The patients are assessed at the Centre and also at either collaborating hospital at monthly intervals during treatment and at 3-monthly intervals up to 24 months.

A total of 139 patients have been admitted to the study so far. The distributions according to pre-treatment characteristics of the patients are shown in tables 1 & 2.

Table 1

Pre-treatment characteristics

Factor on admission	Patients	
	No.	%
Age(years):		
<12	31	**22**
12-24	59	**42**
25-34	19	**14**
35-44	16	**12**
⩾45	14	**10**
Type of disease:		
Lupus vulgaris	60	**43**
Verrucose cutis	57	**41**
Scrofuloderma	11	**8**
Tuberculid	5	**3**
LV & VC	3	**2**
SD & VC	2	**1**
SD & LV	1	**1**
No. of lesions:		
1	106	**76**
2	22	**16**
⩾3	11	**8**
Total	139	**100**

Table 2

Distribution of number of lesions according to site of lesion and type of disease

Site of lesion	Type of disease			
	LV	vc	SD	Tub
Toe, Sole, Foot	6	41	0	0
Leg	5	3	0	0
Knee	14	4	3	0
Thigh	12	4	6	0
Gluteal region	12	2	0	0
Trunk	3	2	4	3
Fore-arm	5	2	0	2
Upper-arm (including axilla)	9	4	3	2
Palm, hand, finger	2	6	1	0
Neck	5	0	3	0
Face	6	0	0	18
Total lesions	79	68	20	25

Of 139 patients, 90 (65%) are less than 25 years of age on admission, 35 (25%) between 25-44 years and 14 (10%) are aged 45 years or more. Of the 139 patients, 117 (84%) have either lupus vulgaris or verrucose cutis type of disease. Single lesion was observed in 76% of the patients. Foot is the most frequently affected part in verrucose cutis amounting to 41 (60%) of 68 lesions. Knee, thigh and gluteal region are the most affected parts of the body in lupus vulgaris amounting to 38 (48%) of 79 lesions.

Table 3

Results of histopathology and culture examinations

Histopathology	Culture					Total
	M. tuberculosis		NTM*	Cont-amin-ation	Not done	
	Positive	Negative				
Positive	50	33	10	2	6	101
Negative	8	9	6	0	2	25
Probable	5	4	0	0	0	9
Not done	2	2	0	0	0	4
Total	65	48	16	2	8	139

* Non tuberculous mycobacteria.

The results of the bacteriological and histopathological examinations are shown in table 3.

The diagnosis of cutaneous tuberculosis was confirmed in 116 (83%) of 139 patients either histopathologically and/or bacteriologically.

Of the 139 patients admitted to the study, 82 patients have completed 9 months of chemotherapy; of these 27 patients were excluded from the present analysis for the following reasons; in 7, the disease was not confirmed by histopathology and there was no clinical response to anti-TB treatment and 20 received less than 75% of chemotherapy. A total of 55 patients who had received 75% or more of chemotherapy, are available for analysis (14% of patients received 100% chemotherapy; 37% received 91-99% and the remaining 49% of patients received 75-90% of prescribed chemotherapy).

The response of patients during the chemotherapy phase is shown in table 4.

Table 4

Resolution of skin lesion according to type of disease

Type of disease	No. of pts.	Resolved by (Month after starting Rx.)						Not resolved by 9 months
		1	2	3	4	5	6-9	
Lupus vulgaris	26	1	8	8	4	3	1	1
Verrucose cutis	23	1	2	9	5	3	1	2*
Scrofuloderma	3	0	1	1	1	0	0	0
Tuberculid	3	0	0	0	1	0	1	1

* Including 1 patient who had complete resolution at 12 months.

In 24 (92%) of 26 patients with lupus vulgaris, the lesions had resolved by the end of the 5th month. In 1 case, resolution was seen during the seventh month. One patient with lupus vulgaris (whose biopsy was culture negative and histopathology positive) showed no clinical improvement till the end of treatment period. However, this patient showed dramatic response at 2 months after the administration of an alternate regimen (Ofloxacin, Pyrazinamide, Ethambutol and Ethionamide).

In 23 patients with verrucose cutis, lesions were resolved in 20 (86%) by the end of the 5th month and in one by the 9th month. Two patients did not respond to the regimen prescribed. One patient had complete resolution by the end of the 12th month without additional treatment and the other was not available for follow-up beyond the 9th month. Their cultures were sensitive to streptomycin, isoniazid and rifampicin.

All the three patients with scrofuloderma showed complete resolution of the lesion by the end of the 4th month.

Of three patients with tuberculid, one patient showed resolution by the 4th month, another in the 7th month and the remaining one patient did not respond to treatment.

The intake to the study is continuing.

*****

Controlled clinical study of multi-drug therapy for multi-bacillary leprosy - 2 year report

(Ongoing study, 1988-97)

As mentioned in previous (1988-1991) annual reports, the Centre is undertaking a controlled clinical trial to assess the relative efficacies of pyrazinamide and rifampicin in combination with clofazimine and DDS in the treatment of multi-bacillary leprosy, at the Government Royapettah Hospital, Madras. The following 4 regimens are being investigated.

Regimens

I. NLEP: Rifampicin 12 mg/kg body-weight once a month in addition to a daily dose of 12 mg/kg body-weight in the first 14 days, clofazimine 300 mg once a month in addition to a daily dose of 100 mg for the first 14 days and 50 mg daily thereafter, and dapsone 100 mg daily, for a total period of 24 months (regimen in use in the National Leprosy Eradication Programme).

II. NLEP + Addition of PZA: Rifampicin, clofazimine and dapsone as in regimen I plus pyrazinamide 35 mg/kg body-weight daily for the first 3

months and 50 mg/kg body-weight twice-weekly for the next 9 months.

III. NLEP + Extension of Rif.: Clofazimine and dapsone as in regimen I, plus rifampicin 12 mg/kg body-weight, daily for the first 3 months, twice-weekly for the next 9 months and once a month for the next 12 months.

IV. NLEP + Extension of Rif. & Addition of PZA: Clofazimine, dapsone and pyrazinamide as in regimen II and rifampicin as in regimen III.

Chemotherapy was stopped at the end of 24 months from the start of treatment, irrespective of the BI value. However, if a patient had reactions and was getting steroids, the chemotherapy was extended for one more year and the case reviewed.

In all, 80 patients have been admitted so far to the study. Of these, 76 patients (19 NLEP, 19 NLEP + Add. of Z, 19 NLEP + Ext. of R and 19 NLEP + Ext. of R + Add. of Z) have information up to 24 months. Of these, 11 patients were excluded; three patients died due to reasons other than leprosy - carcinoma of the stomach, suicide, late complication (stricture oesophagus) of attempted suicide by swallowing sulphuric acid; one had treatment changed due to jaundice and one patient due to reaction; five were uncooperative; one patient migrated and the findings in the remaining 65 patients (18 reg. I; 15 reg. II; 18 reg. Ill and 14 reg. IV) are presented here.

Clinical progress: Clinical progress was assessed by an independent assessor, who was unaware of the regimen or bacteriological status of the patients, using scores based on semi-quantitative assessments (as described in previous annual reports); the classification of clinical progress is presented in table 1. Over 0-3 months, moderate or marked improvement was reported in 9 of 18 for reg. I, 6 of 15 for reg. II, 6 of 18 for reg. Ill and 2 of 14 for reg. IV. The corresponding figures were 10, 10, 4 and 6 at 0-6 months; 14, 10, 9 and 10 at 0-12 months and 17, 13, 16 and 11 at 0-24 months, respectively. Thus at the end of treatment there was an excellent clinical improvement in all the four groups.

Table 1

Clinical progress according to regimen

Regimen	Progress	Period (months)			
		0-3	0-6	0-12	0-24
I (n=18)	Improvement marked/moderate	9	10	14	17
	Slight	4	5	4	0
	No change	5	1	0	0
	Deterioration	0	0	0	0
	Absent for assessment	0	2	0	1
II (n=15)	Improvement marked/moderate	6	10	10	13
	Slight	4	2	4	1
	No change	1	3	1	1
	Deterioration	2	0	0	0
	Absent for assessment	2	0	0	0
III (n=18)	Improvement marked/moderate	6	4	9	16
	Slight	5	7	6	2
	No change	6	6	3	0
	Deterioration	0	0	0	0
	Absent for assessment	1	1	0	0
IV (n=14)	Improvement marked/moderate	2	6	10	11
	Slight	7	5	1	1
	No change	4	2	3	2
	Deterioration	0	0	0	0
	Absent for assessment	1	1	0	0

Bacterial indices: The mean bacterial indices (BI) for the 4 groups on admission and at 3, 6, 12 and 24 months are shown in table 2. The mean BI was 4.55 for reg.1, 4.51 for reg. II, 3.95 for reg. III and 4.20 for reg. IV on admission and 3.04, 3.16, 2.86 and 2.88 at the end of the treatment period. No significant difference was observed in the fall in BI by the end of the treatment period among the 4 regimens, the fall being 1.51, 1.35, 1.09 and 1.32, respectively, for the four groups.

Table 2

Bacterial Indices according to regimen

Regimen	BI	Month(s)				
		0	3	6	12	24
I	Mean	4.55	4.11	3.91	3.68	3.04
(n=18)	Range	3.67-5.00	2.33-5.00	1.50-4.83	0.83-4.50	1.00-4.33
II	Mean	4.51	4.06	3.83	3.61	3.16
(n=15)	Range	3.67-5.17	3.00-5.00	2.17-4.83	1.00-4.50	0.83-4.33
III	Mean	3.95	3.80	3.63	3.76	2.86
(n=18)	Range	2.67-5.00	1.83-4.83	1.50-5.17	1.83-5.00	1.00-4.17
IV	Mean	4.20	4.09	4.05	3.68	2.88
(n=14)	Range	3.67-4.67	2.00-5.00	2.17-4.83	1.50-4.67	0.00-4.00

In summary, the findings at 24 months have shown similar improvement clinically and bacteriologically in all the 4 groups. Any additional benefit derived from the supplement of rifampicin and pyrazinamide may perhaps be seen in the follow-up period.

Mouse foot-pad experiments: The mouse foot-pad technique of Shepard for cultivation of **M. Leprae** was undertaken in this trial to assess the viability of the organisms. Skin biopsy specimens were taken from the patients on admission, at 2 weeks, 3 weeks, 12 months and 24 months. **M. Leprae** recovered from the biopsy specimens were inoculated into mouse foot-pads (10^4 AFB/foot-pad) and harvesting was done at 6, 7, 8, 9 and 10 months after inoculation and multiplication if present, was noted. The results are given in table 3. It can be observed that on admission, out of 61 cases, only 19 showed multiplication i.e., growth of one or more log (at any time point of harvest) and 42 showed no growth. At 3 months out of 64 cases, none showed growth. At 12 months out of 63 cases only one showed growth. At 24 months out of 58 cases, none showed any growth. Similarly, scrotal biopsy/areolar biopsy for 56 cases (50 males and 6 females) was done at 24 months and none showed growth in the harvest.

Table 3

Mouse foot-pad harvest results at different periods

Harvest results	Biopsy						
	On admis-sion	at 2 weeks	at 3 months	at 12 months	at 24 months	at 24 months	
						Scrotal	Areola
Viable (Growth of one or more log at any harvest)	19	1	0	1	0	0	0
No Growth	42	60	64	62	58	50	6
Results available	61	61	64	63	58	50	6
Not done	4	4	1	2	4	4	1
Total patients	65	65	65	65	62 *	54**	7

Results awaited: * 3 patients: ** 4 patients

Table 4

Inoculum size of biopsies at different periods

Size of Inoculum	On admi-ssion	at 2 weeks	at 3 months	at 12 months	at 24 months	at 24 months	
						Scrotal	Areola
10^4	56	55	57	41	22	6	0
$<10^4$	5	6	3	14	26	27	1
Nil	0	1	4	8	14	21	5
Biopsies done	61	62	64	63	62	54	6
Not done	4	3	1	2	3	4	1
Total patients	65	65	65	65	65	58	7

The inoculum size (standard of 10^4 AFB/foot-pad) at different periods is presented in table 4. At 24 months, 22 (35%) of 62 cases had 10^4 AFB/foot-pad, 26 (42%) had less than 10^4 AFB/foot-pad and in 14 (23%) cases there was no bacilli. In all these cases for whom mouse foot-pad inoculation was done, there was no growth or multiplication seen in the harvest.

*****

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

(Ongoing study, 1993-94)

A pilot study was initiated during the year to evolve an objective criteria for assessing the diagnosis and progress in paucibacillary leprosy. Patients for the study are selected from those referred from Leprosy clinics in and around Madras City.

A patient was ineligible for the study if he/she was aged less than 5 years, was too ill, had evidence of major systemic illness including active pulmonary tuberculosis (if mantoux is ≥ 20 mm, to have an X-ray and rule out P.Tb.), was in reactionary state, was positive by skin smear, had had previous specific treatment (DDS for more than 1 month, or even a single dose of rifampicin in the last 6 months) and had 10 patches or more.

A patient was eligible for admission to the study, if the disease was classified as BT or TT clinically (and later confirmed by histopathology) and was willing to attend as and when required. It is proposed to admit 50 patients. All the patients are prescribed NLEP regimen (Rifampicin once a month plus DDS daily for 6 months). The dosage schedule is given in table 1.

All the patients are treated on an ambulatory basis. They attend the treatment centre once a month for 6 months except in the first month when they attend on the days specified for mantoux reading. After 6 months, patients attend at 9th and 12th month. Further follow-up will be decided at the end of 12 months.

Table 1

Dosage schedule

Weight(kg)	Rif. (mg)	DDS(mg)
<20	150	25
20-29	300	50
30-39	450	50
≥40	600	100

General examination and assessment on admission: 1)Complete clinical examination including a search for conditions that render the patient ineligible for the study, 2) urine examination for albumin, sugar and deposits, if indicated, 3) urine spot test, 4) estimation of haemoglobin, total and differential WBC counts, 5) stool examination for ova and cyst, 6) estimation of serum bilirubin, serum AST and ALT, blood urea, serum creatinine and serum alkaline phosphatase, 7) weight of the patient (kg) on the day of allocation and 8) 1-TU Mantoux test (PPD RT 23 with tween 80) on the left forearm to be read after 1,2,3, 7±1, 14±2 & 21 ± 2 days.

Examinations and assessments for leprosy condition on admission: 1) Clinical examination of the leprosy condition (marking of lesions on body out-line chart), 2) previous anti-leprosy chemotherapy, 3) skin smears from active sites and one ear lobe (3 to 6 sites), 4) lepromin skin test (Dharmendra antigen) on the right forearm read after 2 days and on the 21st day (Mitsuda), 5) independent assessment, 6) colour photographs of the lesions, 7) neurological examination to detect motor and sensory functions, 8) disability grading, 9) serum for PGL antibody and 10) skin biopsy: **Histopathology:** skin sections will be stained (a) with haemoloxylin eosin (b) for AFB by fite farraco method; **Immuno Histology:** Immuno enzymatic staining procedure for (a) presence of mycobacterial antigens, (b) complement C3d, B-cells and T-cells with appropriate poly and monoclonal antibodies.

For differentiating reversal reactions and relapse, the following will be done: 1) Granuloma index, apoptotic index and mitotic index, 2) the type, number and disposition of cells participating in the granuloma and 3) the presence, amount and disposition of mycobacterial antigen(s) and complement C3d.

Investigations and assessments which were done to assess the leprosy condition on admission will be repeated at periodical intervals

A total of 24 patients have been admitted (till December 1993); the intake to the study is continuing.

*****

Controlled clinical trial of dapsone as continuation chemotherapy beyond 7 years

(Ongoing study, 1977-97)

The 10 year findings were already presented in the 1992 annual report.

In more than 95% of the cases, at 120, 132, 144 and 156 months the BI was less than 0.50 and the patients were clinically inactive. Hence it was decided to stop anti-leprosy drugs for all the patients at 144 months and follow them once a year till 240 months.

Mouse-footpad experiments: In July 1987, it was decided to do scrotal and areolar biopsies for the patients who had completed 60 months and/or 84 months for any evidence of persisters. A total of 73 scrotal biopsies (12 at 60 months and 61 at 84 months) and 5 areolar biopsies (1 at 60 months and 4 at 84 months) were done; bacilli was seen in 13 of the 73 scrotal (10^4 in 2 and less than 10^4 in 11) tissues and no bacilli was seen in the 5 areolar tissues. There was no growth in the harvests at any time point (6, 7, 8, 9 and 10 months) for any of the 73 cases.

Follow-up of lost cases: Among the 210 patients who were admitted to the study, 43 were excluded for 120 months analysis. Of these, where-abouts were not known for 7, two were attending irregularly and one migrated (went abroad). Efforts were made to contact the remaining 33 patients for one time assessment. The action taken were, posting of letters and visits including out station by a team consisting of a Medical Officer, a Social Worker and a Laboratory Technician.

Twenty one patients have been retrieved. Clinical and bacteriological assessments were done. One patient was found to be active requiring retreatment; all the remaining patients were found to be inactive.

So far, we have come across 2 cases of relapse among the 167 patients, 51 of whom have completed 15 years since admission to the study.

*****

LABORATORY STUDIES - COMPLETED

Use of vancomycin in Selective Kirchner's liquid medium for culture of tubercle bacilli

(Completed study, 1993)

Selective Kirchner's liquid medium (SKLM) has been found useful in augmenting the yield of positives among paucibacillary extrapulmonary specimens. However, in the case of gastric lavage (GL), this medium very often gets heavily contaminated. In such cases, aerobic spore bearers have been found to be the chief contaminants, the four antibiotics present in the medium being apparently ineffective against these organisms. Vancomycin is known to be bactericidal for most gram-positive bacteria including the **Bacillus** species, without having any effect on **Mycobacterium tuberculosis.** Therefore this investigation was carried out to assess the usefulness of incorporating vancomycin in SKLM to reduce contamination and also to ascertain whether the drug has any adverse effect on the growth of **M. tuberculosis,** especially when it is present in small numbers.

The investigation was carried out using 221 GL samples collected from children during active case finding in the field. Since very few positives were expected among them, 26 scanty smear positive sputum samples were included as positive controls. Sputum and GL samples were processed by the modified Petroff's method. Portions of GL and sputum deposits obtained after decontamination were inoculated on LJ medium. The remaining portions were equally divided and inoculated into SKLM and SKLM with 10 mg/l vancomycin (SKLMV) in random order. The SKLM and SKLMV cultures were examined weekly, and when growth/contamination appeared or at the end of 6 weeks, these were subcultured on LJ medium directly and after decontamination.

The results showed that 135 of 221 GL (61%) and 13 of 26 (50%) sputum samples cultured in SKLM were contaminated as compared to 47 (21%) GL and 3 (12%) sputum samples in SKLMV medium (Table 1).

In all, there were 8 specimens positive by SKLM, of which 5 were positive by SKLMV also. On the other hand there were 15 specimens positive by SKLMV of which 5 were positive by SKLM also.

Table 1

Gastric lavage and sputum culture results on SKLM and SKLMV

Culture result on LJ*	Gastric lavage (221)				Sputum (26)			
	SKLM		SKLMV		SKLM		SKLMV	
	No.	%	No.	%	No.	%	No.	%
Positive	3	**1**	5	**2**	5	**19**	10	**38**
NTM	20	**9**	45	**20**	1	**4**	4	**15**
Negative	63	**29**	124	**56**	7	**27**	9	**35**
Cont.	135	**61**	47	**21**	13	**50**	3	**12**

* NTM = Non-Tuberculous Mycobacteria; Cont. = Contamination

Considering all the 247 samples (GL and sputum) together (Table 2) it can be seen that contamination was significantly lower in SKLMV ($P<0.00001$).

Table 2

Comparison of contamination in SKLM and SKLMV

SKLMV	SKLM		Total
	Contaminated	Not Contaminated*	
Contaminated	33	17	50
Not Contaminated*	115	82	197
Total	148	99	247

* Positive/NTM/Neg

In order to see whether vancomycin had any adverse effect on the growth of **M. tuberculosis** or NTM, the analysis was repeated with 70 GL and 12 sputum specimens which were not contaminated in either medium. Among these samples, 7 positives each were detected in SKLM and SKLMV, 5 being common (Table 3). There was no difference between SKLMV and SKLM in the rate of isolation of **M. tuberculosis** or NTM

suggesting that vancomycin had no adverse action on their growth. However the numbers are small to draw firm conclusions.

Table 3

Comparison of isolation of positives in SKLM and SKLMV from uncontaminated samples*

SKLMV	SKLM			Total
	Positive	NTM	Negative	
Positive	5	0	2	7
NTM	0	9	12	21
Negative	2	8	44	54
Total	7	17	58	82

*70 GL and 12 sputum samples

Thus vancomycin could to be a valuable adjunct for culture of tubercle bacilli from pathological specimens such as the gastric lavage,

*****

A direct test for rifampicin susceptibility of tubercle bacilli

(Completed study, 1993).

Resistance of **M. tuberculosis** isolates to rifampicin is nearly always associated with resistance to isoniazid and often to other drugs as well and indicates poor prognosis. Therefore early detection of rifampicin resistance is of vital importance. At present, drug susceptibility testing for **M. tuberculosis** isolates from patients is done by the indirect method on L-J medium which takes 2-3 months to yield results. The cost and sophistication of rapid methods such as the slide culture and Bactec methods make them beyond easy reach of laboratories in developing countries. Therefore a simple direct sensitivity test using L-J medium has been studied with the aim of obtaining early results.

Smear positive sputum deposits from pulmonary tuberculosis patients obtained before and during treatment, were inoculated on duplicate slopes

of L-J medium, without drugs and with 32 mg/l (R32) and 64 mg/l (R64) of rifampicin. The slopes were examined weekly for 8 weeks and the growth recorded. A further pair of L-J slopes inoculated from the same deposit provided the primary culture for setting up the conventional indirect test using rifampicin concentrations of 32, 64 and 128 mg/l. These slopes were read at 4 weeks and the cultures classified as sensitive or resistant.

The definitions of resistance used are as below:

	Growth on plain medium	**Growth on drug containing medium**
Indirect test	$\geq$ 2+	$\geq$ 20 colonies on R64
	< 2+	test repeated
Direct test	$\geq$ 2+	$\geq$ 20 colonies on R32 or R64
	1-99 colonies	$\geq$ 1 colony on R32 or R64 (i.e.>1% resistance)

Of 243 samples tested, 50 were excluded (41 no growth, 7 contaminated and 2 NTM) leaving 193 samples in the analysis. Classification of the susceptibility of the isolates on the basis of growth on LJ containing R 32 mg/l in the direct test, showed agreement with the classification based on the indirect test in 92.6% of 136 samples which could be read at 3 weeks after setting up, in 94.6% of 185 at 5 weeks and in 95.3% of 193 at 8 weeks (Table 1).

The extent of agreement based on R 64 mg/l in the direct test were, 89.7% at 3 weeks, 93.5% at 5 weeks and 95.9% at 8 weeks. Analysis showed that the grade of culture positivity did not affect the agreement between the indirect and the direct test results based on R32; however, the direct test based on R64 showed significantly better agreement when the cultures had confluent growth than when they had lesser growth (100% vs. 91-94%).

At the end of 8 weeks, 4 sensitive cultures were classified by the direct test as resistant on R32 (2 on R64 also) and 6 resistant cultures were classified as sensitive on R64 (4 on R32 also). Of the former, 3 were from patients with history of rifampicin resistance and 1 from a referred case which just missed being classified as resistant with 19 colonies on R64 in

the indirect test. Of the latter, 4 were from patients known to excrete resistant and sensitive organisms, probably because they harbour a mixed population. For one culture, the indirect test was based on a single colony

Table 1

Agreement between results of the indirect and the direct sensitivity tests read at different weeks

Rifampicin concent-ration	Sensitivity		3 weeks		5 weeks		8 weeks	
	Indirect test	Direct test	No.	%	No.	%	No.	%
32 mg/l	Sens.	Sens.	98	92.6	123	94.6	127	95.3
	Res.	Res.	28		52		57	
	Sens.	Res.	0	P<0.02	3	NS	4	NS
	Res.	Sens.	10		7		5	
64 mg/l	Sens.	Sens.	98	89.7	125	93.5	129	95.9
	Res.	Res.	24		48		56	
	Sens.	Res.	0	P<0.02	1	P<0.02	2	NS
	Res.	Sens.	14		11		6	
Total			136		185		193	

NS = Not Significant & P>0.05

on the plain medium with no growth on R32 and R 64 in the direct test and the remaining culture was wrongly classified as sensitive by the direct test.

The direct test detected 61 resistant cultures based on R32 and 58 based on R64. On R32,28(45%) were detected by the third week and 55 (90%) by the. fifth week. On R64, 24 (41%) were detected by the third week and 49 (84%) by the 5th week. Thus, resistant cultures were detected on R32 earlier than on R64.

The direct test described here is simple to perform and detected resistance mostly within 5 weeks with again of 4 weeks or more over the indirect test. The results are based on the primary culture and loss of information due to contamination or failure to grow on subculture were

minimised. For routine application, R32 which detects more of the resistant cases and yields results earlier may be recommended.

*****

Shortening the duration of susceptibility tests of M. tuberculosis strains by bioluminescence assay

(Completed study, 1990-93)

Drug susceptibility tests by bioluminescence assay were done with single colony growths of 60 cultures using nucleotide releasing agent for bacteria (NRB) for ATP extraction (see 1992 annual report). The crude agreement reported between ATP assay and conventional method with regard to S, H, R and E was 83%, 74%, 72% and 68% respectively.

Using a different ATP extraction method involving boiling TRIS-EDTA, the assay was repeated for 16 cultures. Better association with the conventional method was obtained with this method.

Table 1
Comparison between conventional method and ATP assay

		Conventional method											
		Streptomycin			Isoniazid			Rifampicin			Ethambutol		
		R	S	Both	R	S	Both	R	S	Both	R	S	Both
ATP assay	R	3	1	4	7	0	7	2	3	5	2	7	9
	S	4	32	36	12	21	33	4	31	35	4	27	31
	Both	7	33	40	19	21	40	6	34	40	6	34	40
Crude agreement (%)		**88**			**70**			**83**			**73**		

R=Resistant; S=Sensitive

Another 40 **M. tuberculosis** cultures were tested by ATP assay using boiling TRIS-EDTA method and compared with the conventional method using standard inoculum and also with 10^4 organisms/ml as that of ATP assay. The drug concentrations used were 0.1, 1 and 10 mg/l for all the

four drugs. The crude agreements between the ATP assay and the conventional method were 88% (35/40), 70% (28/40), 83% (33/40) and 73% (29/40), respectively, for S, H, R and E (Table 1).

Agreement for all drugs between the two tests was observed in 20 out of 40 cultures (50%) and for S, H and R the results of 25 cultures (63%) showed agreement. Based on these results of only a few resistant cultures, the ATP assay is not promising.

Recently a luciferase reporter phage system has been developed by Jacobs and colleagues (1993) which has been genetically engineered to express luciferase gene. This phage confers the ability to produce luciferase enzyme to the viable mycobacterium which it infects. Viable mycobacteria liberate photons in the presence of phage and luciferin which can be measured as relative light units (RLU) in a biocounter. Preliminary experiments have been carried out to standardise the technique. Drug sensitivity testing within 48 hours of the receipt of specimen is possible by this method.

* * * * *

Modulation of Delayed Type Hypersensitivity and protective response in guinea-pigs by M. avium-intracellulare (MAI) complex isolates from clinical specimens and environment of the BCG trial area

(Completed study, 1992-93)

As a part of the larger study on the characterisation of **M. avium-**intracellulare-scrofulaceum (MAIS) complex and **M. fortuitum** complex organisms isolated from clinical and environmental samples in the BCG trial area, an experiment was carried out to examine the modulation of delayed type hypersensitivity (DTH) and protective immune response by these organisms in the guinea-pig model.

A total of 75 guinea-pigs were taken and divided into 5 groups. Group 1 was the control group in which the guinea-pigs were not sensitised or immunised. At 6-weeks, guinea-pigs in group 2 were only immunised with BCG. Groups 3, 4 and 5 were sensitized with the standard strain, environmental isolate and clinical isolate of **Mycobacterium avium-intracellulare** (MAI), respectively, and were immunised with BCG six weeks later. In the fifth week after immunisation (i.e., at 11 weeks), all

the animals including the controls were skin-tested simultaneously with PPD-RT23 and PPD-B. In the sixth week after immunisation (i.e., at 12 weeks), all the animals were challenged with a South Indian low virulent strain of **M. tuberculosis** (SIV). From each group, 5 animals each were sacrificed at 2, 6 and 12 weeks after challenge. Spleen viable counts were set up at each time point.

Table 1
Delayed type hypersensitivity and protective response in guinea pigs exposed to different strains of MAI and BCG

Group	Mean diameter (mm) and range of reaction at 24 hours to		Mean $\log_{10}$ cfu of SIV in spleen after challenge		
	PPD-RT23	PPD-B	2-weeks	6-weeks	12-weeks
1. Control	0.20 (0-3)	0.40 (0-4)	5.321	0.480	0.000
2. BCG-SIV	8.60 (0-15)	4.28 (0-10)	2.022	0.000	0.000
3. MAI std.- BCG-SIV	9.50 (2-13)	6.60 (3-13)	2.140	0.000	0.000
4. MAI envt. - BCG-SIV	5.89 (0-12)	3.03 (0-8)	1.924	1.585	0.000
5. MAI clin.- BCG-SIV	9.62 (5-14)	6.04 (0-11)	2.527	0.000	0.000

The results (Table 1) showed that skin-test response to PPD-RT23 and PPD-B were negligible in the control group (group 1) animals. Compared to this group, significantly higher skin-test response was seen in animals immunised with BCG (group 2) and in animals exposed to different strains of MAI (groups 3, 4, and 5) prior to immunisation with BCG. The skin-test response to PPD-RT23 and PPD-B was significantly lower (P=0.02) in animals exposed to the environmental strain of MAI (group 4) prior to immunisation with BCG as compared to that in animals exposed to standard and clinical strains of MAI (groups 3 and 5) prior to immunisation with BCG.

The log colony forming units (cfu) of SIV in spleen was high (>5.0) in the control animals (group 1) at 2 weeks after challenge but these organisms could not be detected in the spleen at 6 and 12 weeks after challenge. The log cfu of SIV in spleen was much lower in animals immunised with BCG (group 2) as compared to the control group at 2 weeks after challenge indicating a good protective response due to BCG. At this time-point, similar protective response was also seen in the animals exposed to different strains of MAI (groups 3, 4 and 5) prior to immunisation with BCG. However, at 6 weeks after challenge, while the challenge organisms could not be detected in the spleens of the animals immunised with BCG (group 2) or exposed to standard and clinical strains of MAI (groups 3 and 5) prior to immunisation with BCG, detectable numbers were still present in the spleen of animals exposed to environmental strain of MAI (group 4) prior to immunisation with BCG. In none of the groups, organisms could be detected in the spleen of the animals at 12-weeks after challenge. These results indicate that, over time, prior exposure to environmental strain of MAI appears to have a modulatory effect on the protective response induced by subsequent BCG vaccination in the guinea pig-model studied.

*****

Pharmacokinetics of rifampicin, ethambutol, isoniazid and pyrazinamide following administration of the drugs individually or in different combinations to healthy subjects.

(Completed study, 1992-93)

The aims and objectives, the design and conduct of the study have been discussed in detail in 1992 annual report.

In brief, only limited information is available on the pharmacokinetic interactions between Rifampicin (R), Ethambutol (E), Isoniazid (H) and Pyrazinamide (Z) which are in current use in the treatment of tuberculosis. An investigation to obtain information on the plasma concentrations and the urinary excretion of these drugs together with their primary metabolites, when administered alone or in various combinations and also the bioavailability of rifampicin when given with isoniazid (because of the phenomenon of antagonism between rifampicin and isoniazid), was undertaken.

The investigations were undertaken in a total of 17 healthy volunteers and the drugs and their combinations were administered on 4 different occasions employing a cross-over design. There was an interval of at least one week between occasions thus ensuring that the induction due to R, if any, on the subsequent results will be minimal and fairly similar in the different groups.

The pharmacokinetic variables such as the mean plasma peak concentration, the time taken to attain the same (t-max), the area under the time concentration curve (AUC) and the half-life of the drugs were estimated. In addition, the proportions of the dose excreted as Rifampicin plus desacetyl Rifampicin(RMP+DRMP), total INA metabolites and pyrazinamide plus pyrazinoic acid were also estimated. The bioavailability index was calculated as the ratio of AUC when R was administered alone to that of AUC when other drugs were administered in combination(RE, RH, RZ or REHZ). The results are presented in tables 1-4.

Table 1

Pharmacokinetics of rifampicin in healthy subjects after administration of rifampicin alone (R) and rifampicin and ethambutol (RE) together

Pharmacokinetic variable	Mean ± Standard deviation	
	R	RE
Peak concentration (μg/ml)	11.9 ± 3.4	12.7 ± 1.8
t max (hours)	2.0 ± 0.6	1.8 ± 0.8
AUC(0-12; μg/ml. hours)	72.4 ± 20.0	64.6 ± 4.4
Half-life (hours)	4.8 ± 1.0	4.7 ± 1.6
% dosage excreted as RMP + DRMP in urine (0 - 12 hours)	17.5 ± 3.8	16.0 ± 2.2
Mean bioavailability index of RE based on AUC	0.96 ± 0.32	

Administration of E or Z do not appear to have any significant effect on the plasma levels or the urinary excretion of R (Tables 1 and 2). However, administration of R with H alone or in combination with E and Z, appears to significantly lower the AUC ($P < 0.01$) and urinary excretion of R ($P \leq 0.01$), the mean decrease based on the AUC being about 24% with RH and about 39% with REHZ(Tables 3 and 4). Since the peak concentrations are lower and the time taken to attain the peak

concentrations (t-max) are significantly higher with RH (P = 0.03) and REHZ (P = 0.04) than with the corresponding groups receiving R alone and since the mean values for, plasma half-lives are fairly similar, it is likely that H probably interferes with the gastrointestinal absorption of R and not its metabolism or elimination.

Table 2

Pharmacokinetics of rifampicin in healthy subjects after administration of rifampicin alone (R) and rifampicin and pyrazinamide (RZ) together

Pharmacokinetic variable	Mean ± Standard deviation	
	R	RZ
Peak concentration (µg/ml)	13.8 ± 3.0	14.5 ± 1.7
t max (hours)	2.0 ± 0.6	2.0 ± 0.6
AUC(0-12; µg/ml. hours)	79.9 ± 12.3	85.6 ± 15.3
Half-life (hours)	4.7 ± 1.1	4.1 ± 1.0
% dosage excreted as RMP + DRMP in urine (0 - 12 hours)	18.4 ± 3.6	17.0 ± 3.3
Mean bioavailability index of RZ based on AUC	1.08 ± 0.20	

Table 3

Pharmacokinetics of rifampicin in healthy subjects after administration of rifampicin alone (R) and rifampicin and isoniazid (RH) together

Pharmacokinetic variable	Mean ± Standard deviation	
	R	RH
Peak concentration (µg/ml)	13.5 ± 4.3	11.1 ± 3.8
t max (hours)	1.7 ± 0.5	2.3 ± 0.5
AUC(0-12; µg/ml. hours)	77.3 ± 22.1	58.6 ± 16.7
Half-life (hours)	5.0 ± 0.9	5.1 ± 1.4
% dosage excreted as RMP + DRMP in urine (0 - 12 hours)	19.3 ± 2.7	16.4 ± 2.5
Mean bioavailabil ity index of RH based on AUC	0.76 ± 0.11	

Table 4

Pharamacokinetics of rifampicin in healthy subjects after adminstration of rifampicin alone(R) and rifampicin, ethambutol isoniazid and pyrazinamide (REHZ) togethher

Pharmacokinetic variable	Mean ± Standard deviation	
	R	REHZ
Peak concentration (µg/ml)	13.6 ± 4.2	7.9 ± 2.4
t max (hours)	1.8 ± 0.8	2.8 ± 0.4
AUC(0-12; µg/ml. hours)	73.1 ± 20.4	44.6 ± 11.2
Half-life (hours)	4.3 ± 0.7	5.1 ± 1.9
% dosage excreted as RMP + DRMP in urine (0 - 12 hours)	16.8 ± 2.9	13.2 ± 2.9
Mean bioavailability index of REHZ based on AUC	0.65 ± 0.25	

Comparing the RZ series with the RH series, only the difference between the AUC of R was significant (P=0.02). However, the mean values in the RZ series were significantly higher than in the REHZ series ($P < 0.001$ for AUC and peak concentration and $P = 0.03$ for urinary excretion). The mean t-max value in the RZ group was, however, lower than that in the REHZ group (P=0.02). None of the differences between the RE and RH groups were significant. However, the mean values for AUC, peak concentration and t-max with RE were significantly different ($P \leqslant 0.02$) from those with REHZ. There was a suggestion that the mean % dose excreted as R plus desacetyl R in the RH group was higher (P=0.06) than that in the REHZ group.

The mean proportions of the dose of H excreted as total INA metabolites in urine collected over the 0-12 hour period were 60.7, 62.8, 58.9, 60.3 and 64.8% with H, HR, HE, HZ and HREZ respectively. Similarly, the AUCs of pyrazinamide were 382, 393, 374, 451 and 378 µg/ml hours with Z, ZR, ZE, ZH and ZREH respectively. The proportions (%) of the dose of Z excreted in urine (0-12 hours), as pyrazinamide plus pyrazinoic acid were 20.9, 19.9, 21.0, 20.9 and 23.1 respectively in the 5 groups. The mean values are based on the findings from 8 volunteers in each group and none of the differences, with respect to either H or Z was significant. Thus there was no evidence of any decrease in the bioavailability of H or Z.

In summary, the bioavailablity of R is decreased by isoniazid, as indicated by the bioavailablity indices 0.76 and 0.65 when RH and REHZ were administered, respectively. It is, therefore, apparent that if double drug combinations are to be employed on alternate days (as in the current clinical trial) it may be preferable to employ R in combination with E or Z than with H. These findings would however need to be confirmed in a larger group of subjects.

*****

LABORATORY STUDIES - IN PROGRESS

Follow-up bacteriological results of patients admitted to Short Course Chemotherapy under District Tuberculosis Programme in the Union territory of Pondicherry

(Ongoing study, 1984-94)

To assess the efficacy of short-course regimens under programme conditions, it is important to have reliable information on the bacteriology of sputum from the patients on admission, at the end of treatment and during follow-up. We have reported earlier the bacteriological profile and the prevalence of drug resistance among patients on admission and at the end of treatment in North Arcot District and Pondicherry (Paramasivan et al Tubercle and Lung Disease,l993, 74: 23-27). It was found that of the patients who had received 80% or more of the prescribed short course chemotherapy, as many as 80% from North Arcot and 92% from Pondicherry had become culture negative by the end of treatment. Since the overall efficacy of treatment depends upon the relapse rates in these patients, we report here the long-term follow-up bacteriological findings in patients who belong to Pondicherry. They were treated with a regimen ($2RHZ/4RH_2$) consisting of rifampicin 450mg plus isoniazid 300mg plus pyrazinamide 1.59 daily for 2 months, followed by rifampicin 600mg plus isoniazid 600mg twice a week for 4 months; in the first two months, the drugs were collected once in 15 days for self-administration, and in the next 4 months, all doses are administered under the supervision in the clinic. Those who were not willing to accept this regimen were given an alternate regimen (2RHZ/6TH) consisting of the same drugs in the first 2 months followed by thioactazone 150mg plus isoniazid 300mg daily for the next 6 months, the drugs being collected by the patients once in 15 days for self-administration.

Since June 1984, 2728 patients were examined. Of these, 634 (23%) were excluded for reasons such as death (53), "lost" during treatment (576) and migration (5). Of the remaining 2094 patients, sputum specimens could not be obtained from 991 (47%) at the end of treatment because the study was carried out under programme conditions. Of the 1103 patients from whom sputum specimens were collected and examined, 128 (12%) had a positive culture at the end of treatment, and 975 (88%) had sputum conversion at the end of treatment (Table 1).

Table 1

Pondicherry - Study population

		No.	%
No. of patients admitted to the study		2728	
Exclusions		634	**23**
	i) Died 53		
	ii) Lost 576		
	iii) Migrated 5		
No. of patients in the analysis		2094	
Patients who had not given specimens at the end of Rx.		991	**47**
Patients who had given specimens at the end of Rx.		1103	**53**
Patients with positive culture at the end of Rx		128	**12**
Patients who had sputum conversion		975	**88**

Among 975 patients who had sputum conversion, only 237(24%) gave sputum specimens between 10-14 months. Of these 29(12%) were culture positive. Four of these 29 cultures had a growth of 1-colony only, but three of them were also smear positive (1+). Fourteen patients (6%) were positive by smear examination only. Over all, there were 43 patients (18%) positive by either smear or culture or both.

These findings are limited by the fact that there were more than 50% exclusions and a very high proportion of patients have not given specimens after the completion of treatment. However, this is to be expected since the study was carried out under programme conditions and there was no regular annual collection of specimens which probably resulted in an appreciable proportion of patients not giving any specimen during the follow-up.

*****

Evaluation of bactericidal and sterilising action of ofloxacin and sulbactam/ampicillin, alone and in combination with other drugs, on M. tuberculosis In vitro and in the murine model

(Ongoing study, 1993-1995)

A study has been initiated to evaluate the bactericidal and sterilising action of ofloxacin (O) and sulbactam/ampicillin (S/A), alone and in combination with rifampicin (R) and isoniazid (H), starting with **In vitro** experiments in 7H9 liquid medium containing albumin and Tween 80.

Standardisation experiments were carried out first to estimate the residual concentrations of R, H, O and ampicillin (A) during incubation at 37°C in 7H9 liquid medium with albumin and Tween 80. To 10ml of 7H9 medium in McCartney bottles, R and H were added to give 1mg/l; O to give 1.25 and 5mg/l; A to give 8 and 15mgA; and S/A in 1:2 ratio to give 8 and 15mg/l of sulbactam/ampicillin (day 0), each drug individually in triplicate. After the addition of drugs, the bottles were incubated at 37°C. On days 0 (immediately after addition of drugs), 1, 3 and 5, the residual concentrations of the drugs in the medium were estimated using a standard biochemical method for H and microbiological assay using Staphylococcus aureus, E. coli and Micrococcus luteus, respectively, for R, O and A. It was seen that by day 3, there was a loss in the activity of the drugs by 4.7%, 33.9%, 36.4% and 37.1%, in the case of O, R, H and A, respectively.

In the main experiment, on day 0, the log phase and stationary phase cultures of **M. tuberculosis** H37Rv were distributed in 10 ml aliquots in 48 McCartney bottles. Viable counts (VC) were set up from all the bottles on 7H11 selective medium. O, R, H, RO, HO, HR and HRO with O at two concentrations, 1.25mg/l (O1) and 5mg/l (O2), and S/A, A, RS/A, HS/A and HRS/A at two final concentrations of ampicillin, 8mg/l (S/A1 and A1) and 15mg/l (S/A2 and A2), were added, each into two bottles. R and H were added to give final concentrations of 1mgA each. Cultures not treated with any drug over the experimental duration of 6 days were also included as controls. In the cultures with drugs, additional drugs were added on day 3 to make up for the loss in activity as estimated from the standardisation experiment. In addition to day 0 (before the addition of drugs), viable counts were set up from all the cultures on days 2, 4 and 6.

Results for the bactericidal activity on log phase cultures of **M. tuberculosis** H37Rv are presented in table 1.

Table 1

Bactericidal activity on log phase cultures

Drug	$Log_{10}((CFU/ml)+1)$		Reduction in log_{10} cfu/ml/day
	Day 0	Day 6	
Controls			
1 No drug	6.60	8.70	-0.35
2 A1	"	8.56	-0.33
3 A2	"	8.28	-0.28
Single drug			
1 O2	,,	1.80	0.80
2 H	,,	2.07	0.76
3 S/A2	,,	3.18	0.57
4 O1	,,	3.18	0.57
5 R	,,	5.27	0.22
6 S/A1	,,	5.96	0.11
2-drug combinations			
1 HO2	,,	1.25	0.89
2 HS/A1	,,	1.65	0.82
3 HS/A2	,,	1.77	0.80
4 HO1	,,	2.27	0.72
5 HR	,,	3.95	0.44
6 RO2	,,	5.14	0.24
7 RS/A2	,,	5.21	0.23
8 RO1	,,	5.28	0.22
9 RS/A1	,,	5.33	0.21
3-drug combinations			
1 HRO1	,,	3.35	0.54
2 HRS/A1	,,	3.81	0.46
3 HRS/A2	,,	3.95	0.44
4 HRO2	,,	3.99	0.44

Considering single drugs, the bactericidal activity on log phase cultures (Table 1) measured as the average reduction in log_{10} cfu/ml/day during the 6 days of exposure to the drug, was high for O2 and H, moderate for S/A2 and O1, and low for R and S/A1. Ampicillin alone (A1 and A2) without

sulbactam did not have any bactericidal activity.

Table 2

Bactericidal activity on stationary phase cultures

Drug		$Log_{10}((CFU/ml)+1)$		Reduction in log_{10} cfu/ml/day
		Day 0	Day 6	
Controls				
1	No drug	9.24	8.70	-0.04
2	A1	,,	>9.00	<0.04
3	A2	,,	>9.00	<0.04
Single drug				
1	O2	,,	8.49	0.12
2	H	,,	8.87	0.06
3	S/A2	,,	>9.00	<0.04
4	O1	,,	>9.00	<0.04
5	R	,,	>9.00	<0.04
6	S/A1	,,	>9.00	<0.04
2-drug combinations				
1	HO2	,,	8.16	0.18
2	HS/A1	,,	8.17	0.18
3	HS/A2	,,	8.19	0.17
4	HO1	,,	8.21	0.17
5	HR	,,	8.45	0.13
6	RO2	,,	8.51	0.12
7	RS/A2	,,	8.79	0.07
8	RO1	,,	9.02	0.04
9	RS/A1	,,	9.04	0.03
3-drug combinations				
1	HRO1	,,	7.90	0.22
2	HRS/A1	,,	8.05	0.20
3	HRS/A2	,,	8.12	0.19
4	HRO2	,,	8.18	0.18

Among the 2-drug combinations, HO1, HO2, HS/A1 and HS/A2 had high activity, HR had moderate activity and RO1, RO2, RS/A1 and RS/A2

had low activity. Combinations of H with O1, O2, S/A1 and S/A2 were either additive (the combined activity being higher than the activity of the single drugs in the combination) or indifferent (combined activity being similar to the activity of thesingle drugs). Combinations of R with H, O1, O2 or S/A2 were antagonistic (combined activity being lower than the activity of the single drug with the greater activity).

All the 3-drug combinations (HRO1, HRO2, HRS/A1 and HRS/A2) had only a moderate activity similar to that of HR. The combined effect was additive in the case of O1 but indifferent with O2, S/A1 or S/A2. R, as in the 2-drug combinations, had an antagonistic effect while H had an additive effect.

The bactericidal activity, measured on stationary phase cultures of **M. tuberculosis** H37Rv is given in Table 2. Considering single drugs, the bactericidal activity measured as the average reduction in $\log_{10}$ cfu/ml/day during the 6 days of exposure to the drugs, O, S/A and A at both concentrations had very low activity compared to the activity of R and H.

Among the 2-drug combinations, 0 or S/A at both concentrations in combination with R had very high activity compared to the high activity of O2 or R with H, moderate activity of S/A1 with H and low activity of O1 or S/A2 with H. In contrast to the activity on log phase cultures, R always had an additive effect in the activity on stationary phase cultures while H was additive with O2, indifferent with O1 or S/A1 and antagonistic with S/A2.

All the 3-drug combinations, again in contrast to the activity on log phase cultures, showed greater activity on stationary phase cultures. The combined effect of adding O or S/A to HR was synergistic, R was always additive or synergistic while H was additive or indifferent.

*****

In vitro activity of ampicillin/sulbactam on South Indian isolates of M. tuberculosis

(Ongoing study, 1993-94)

Combinations of Beta-lactam antibiotics with Beta-lactamase inhibitors have been suggested as possible chemotherapeutic agents for the treatment of patients with multiple-drug resistance to the commonly used

anti-TB drugs. Recently, Prabhakaran and others (Microbios, 72, 137, 1992) have reported that ampicillin plus sulbactam inhibited the multiplication of drug-resistant **M. leprae** in the mouse foot-pad. The same combination when tested against **M. tuberculosis** by the radiometric system was found to be more active than amoxycillin/clavulanic acid.

It has therefore been proposed to test the **In vitro** activity of ampicillin with sulbactam on 50 drug sensitive as well as on 50 drug resistant (SHR/HR) strains of **M. tuberculosis** on 7H11 agar plates.

*****

Comparison of culture results by cetylpyridium chloride (CPC) and NaOH methods under field conditions

(Ongoing study, 1993-94)

In an earlier controlled laboratory experiment (see 1991 annual report), using sputum specimens collected from patients attending the Centre's Clinic, it was found that aliquots of heavily culture-positive specimens, treated with CPC-NaCI reagent and stored at room temperature for 7 days, yielded more culture positives (88%) than the corresponding sputum aliquots stored without CPC-NaCI reagent (67%) and processed by the NaOH method. However, the aliquots of 12 scanty positive sputum specimens treated similarly, showed no difference in the rate of isolation (Selvakumar et al., 1993, IJT, 40, 95). The present study aims to find the isolation rates with the CPC and NaOH methods in sputum specimens collected, stored under field conditions in Thiruvallur area and transported to the Centre for culture examination.

The objectives of the present study were:

1. to compare the culture results from sputum aliquots, collected, stored and transported as in routine field conditions (cold chain maintained) and processed, the next day, for culture using NaOH decontamination (1-day NaOH) with that of aliquots of the same specimens, treated with CPC-NaCI reagent, stored and transported at ambient temperature, and processed, the next day, for culture without NaOH decontamination (1-day CPC),

2. to compare the culture results of aliquots of the same specimens stored and transported at ambient temperature with and without CPC-NaCI reagent and processed on the 8th day for culture, without (8-day CPC) and with (8-day NaOH) NaOH decontamination and

3. to compare the culture results obtained by using 1 in 10 diluted sputum deposits as inoculum in CPC and NaOH methods.

Design:

Sputum aliquot randomly allocated to (method)	Condition of storage / transportation			
	Addition of CPC	No. of days	Temperature	Decontamination with NaOH
1-day NaOH	No	1	4°C	Yes
1-day CPC	Yes	1	Ambient	No
8-day NaOH	No	8	Ambient	Yes
8-day CPC	Yes	8	Ambient	No

NaOH method: The sputum aliquot was processed by a modified Petroff's method. A loopful of the deposit was inoculated onto each of 2 LJ slopes. To the remaining deposit, 1.8 ml of sterile distilled water was added and again inoculated as above on 2LJ slopes

CPC Method: An aliquot, treated with CPC-NaCI, was centrifuged and the deposit was inoculated as mentioned above.

The culture results of 63 specimens are presented in Tables 1 and 2.

The 1-day NaOH method yielded 63% positive cultures compared to 43% by 1-day CPC method and the difference was statistically significant ($P<0.001$). The difference in culture positives between the 8-day CPC and 8-day NaOH methods was not statistically significant, although the 8-day NaOH method yielded more positive cultures.

Table 1

Culture results of, 1-day CPC and 1-day NaOH

		1-day CPC			
		Positive	Negative	Contami-nated	Total
1-day NaOH	Positive	27	6	7	40
	Negative	0	15	5	20
	Contaminated	0	2	1	3
	Total	27	23	13	63

Table 2

Culture results of 8-day CPC and 8-day NaOH

		8-day 2PC			
		Positive	Negative	Contami-nated	Total
8-day NaOH	Positive	14	2	9	25
	Negative	3	14	15	32
	Contaminated	1	2	3	6
	Total	18	18	27	63

The contamination rates were 21% and 43% for specimens processed by the 1-day CPC and 8-day CPC methods, respectively compared to 5% and 10% for 1-day and 8-day NaOH methods. The higher rates of contamination in CPC methods were due to breakege/spoilage of LJ slopes, perhaps as a result of interaction of CPC with the medium. Therefore, the deposit was washed with distilled water and used for inoculation (with neat and 1:10 dilution) for the remaining 57 aliquots. As more culture positives were obtained with the neat sputum deposit than in the 1:10 diluted deposit, the results obtained with the former were considered for the analysis. The results are given in Tables 3 and 4.

Table 3

Culture results of 1-day CPC and 1-day NaOH

		1-day CPC			
		Positive	Negative	Contami-nated	Total
1-day NaOH	Positive	16	7	0	23
	Negative	1	31	1	33
	Contaminated	0	1	0	1
	Total	17	39	1	57

Table 4

Culture results of 8-day CPC and 8-day NaOH

		8-day CPC			
		Positive	Negative	Contami-nated	Total
8-day NaOH	Positive	8	5	1	14
	Negative	4	32	0	36
	Contaminated	1	5	1	7
	Total	13	42	2	57

The results obtained by the two procedures were similar and the difference was not statistically significant.

The study is in progress.

*****

Betalactamase activity in mycobacteria and its possible role in Cefadroxil resistance in M. tuberculosis

(Ongoing study, 1991-95)

Cefadroxil (CEF) is a semisynthetic cephalosporin antibiotic with a broad antibacterial spectrum and a high chemotherapeutic potential when administered orally. About 55% of 29 clinical isolates of **M. tuberculosis** were found to be susceptible to this drug at a concentration of 10 mg/l in 7H11 agar, while the remaining isolates were not inhibited even at 40 mg/l which is much above the peak plasma level (28 mg/l) attained in human beings. One of the mechanisms attributed to betalactam resistance is betalactamases produced by the bacterial species. So it was proposed to study the role of betalactamase of **M. tuberculosis** in Cefadroxil resistance.

M. tuberculosis is known to produce betalactainase. So, hydrolytic activity of betalactamase of **M. tuberculosis** H37Rv was studied using CEF as the substrate replacing penicillin G in the betalactamase assay (Dufour et al., ARRD, 1966, 94, 965). It was found that CEF was not hydrolysed by betalactamase produced by **M. tuberculosis** H37Rv. Therefore, the mechanism of CEF resistance in **M. tuberculosis** may not be attributable solely to betalactamase.

An lodimetric method (Perret et al., Nature (London), 1954, 174, 1012) to quantify the betalactamase was standardised. Cefadroxil-resistant mutants are being selected by serial passage in drug-containing 7H9 broth and subcultures.

The study is in progress.

*****

Characterisation of MAIS isolates and M. fortuitum complex isolates in clinical and environmental specimens from the BCG trial area

(Ongoing study, 1992-94)

A study on the characterisation of **M. avium- intracellulare-**scrofulaceum(MAIS) complex and **M. fortuitum** complex organisms

isolated from clinical and environmental samples in the BCG trial area, based on drug and heavy metal susceptibility patterns, plasmid profiles, HPLC patterns of mycolic acids,and immune response induced in guinea-pig model is in progress, starting with the drug resistance patterns of the isolates of the **M. fortuitum** complex and the immune response in guinea-pigs.

Drug susceptibility patterns of **M. fortuitum** complex isolates: It has been shown earlier that drug susceptibility patterns can be helpful in differentiating the subgroups and subspecies of the **M. fortuitum** complex. Therefore a total of 70 **M. fortuitum** complex isolates from various sources including 16 from sputum, 40 from soil, 8 from water and 6 from dust were tested for their susceptibility patterns to 8 drugs by the broth microdilution procedure as described by Wallace et al (Antimicrob Agents Chemother 1982, 22: 186-192). The drugs tested included amikacin, clofazimine, doxycycline, erythromycin, gentamycin, kanamycin, ofloxacin and sulbacin. Of these drugs, amikacin and ofloxacin had the lowest MIC for these **M. fortuitum** complex isolates while sulbacin, doxycycline and erythromycin had the highest.

The MIC profiles are similar for **M. fortuitum** complex isolates from soil and sputum. Isolates from water had a lower MIC with respect to the aminoglycosides, amikacin and kanamycin as compared to the other groups. The MIC profiles for isolates from dust were also markedly different from the profiles for isolates from soil and sputum.

The analysis of the results based on the subgroups of **M. fortuitum** complex **(M. chelonae abscessus, M. chelonae chelonae, M. fortuitum fortuitum, M. fortuitum peregrinum)** among the isolates tested indicates that **M. fortuitum peregrinum** is more susceptible to the aminoglycosides (amikacin, gentamycin and kanamycin) than the other subgroups. This is in accordance with published reports. All the 4 subgroups were susceptible or moderately susceptible (MIC <2 or $\leqslant 4$, respectively) to ofloxacin. **M. chelonae chelonae and M. fortuitum fortuitum** had similar MICs for gentamycin, doxycycline and erythromycin.

Doxycycline and erythromycin had almost no inhibitory action on most of the isolates at the concentrations tested and were not helpful in differentiating among the different subgroups of this complex. Sulbacin, a combination of sulbactam (β-lactamase inhibitor) and ampicillin (β-lactam antibiotic) in the ratio of 1:2, also had very high MICs for the isolates tested.

Immune response induced by MAI complex isolates in guinea-pigs: An experiment has been started to study the delayed type hypersensitivity response and protective response induced by standard, environmental and clinical strains of **M. avium-intracellulare** (MAI) in guinea-pigs, as compared to that induced by BCG as a follow-up of the animal model experiments carried out earlier on the modulation of the immune response to BCG by NTM.

A total of 40 guinea-pigs were taken and divided into five groups. Group 1 is the control group. Groups 2, 3, 4 and 5 have been immunised with BCG, a standard strain, a clinical isolate, and an environmental isolate of MAI, respectively. These animals will be skin-tested simultaneously with PPD-RT23 and PPD-B, five weeks later. Six weeks after immunisation, all the animals will be challenged with a South Indian Low Virulent strain of **M. tuberculosis** (SIV). From each group, four animals each will be sacrificed at 2 and 6 weeks after challenge. Scores will be given for the extent of infection in the lungs, liver, spleen and lymph nodes, and spleen viable counts will be set up. On the completion of this experiment, the immune response induced by the MAI isolates from various sources will be compared on the basis of skin-test reactivity, scores and spleen viable counts. A similar experiment is planned using **M. fortuitum** complex isolates.

*****

Methods for the estimation of ofloxacin in body fluids

(Ongoing study, 1993-94)

Ofloxacin, belonging to the family of fluoroquinolones is now widely used in the treatment of lower respiratory tract infections. Mouse foot pad studies reveal. its bactericidal activity against leprosy. At our Centre, ofloxacin is currently given to patients having multi-drug resistance in pulmonary tuberculosis.

Very little information is available about the pharmacology of ofloxacin in tuberculous and leprosy patients. Prior to undertaking an investigation to study the biodisposition of ofloxacin, it was proposed to standardise the estimation of ofloxacin in plasma, saliva and urine by two methods viz.

microbiological and fluorimetric.

Preliminary experiments in our laboratory have shown that the organism, **E.coli** is sensitive to ofloxacin and that the zone of inhibition exhibited is proportional to the amount of ofloxacin present. Although the microbiological method is simple, requiring lower volume of the sample, it takes 24 hours for completion and to get the assay results. Another alternate method involving fluorimetry was also studied. This method is based on the principle that ofloxacin exhibits fluorescence, whose intensity can be quantified depending upon the level of the drug present. With this method, results will be available within 2-3 hours, but a larger sample volume is needed.

The estimation of ofloxacin by microbiological and fluorometric methods in plasma, saliva and urine was standardised. The following aspects are being studied:

i) Recovery of ofloxacin from plasma, saliva and urine on 5 different occasions.
ii) Interference of rifampicin, isoniazid, pyrazinamide, ethambutol, dapsone and clofazimine in the estimation of ofloxacin in plasma.
iii) Stability of ofloxacin in plasma, saliva and urine by storing the sample containing the drug for different periods.
iv) Protein-binding of ofloxacin based on its level in plasma and saliva.

Microbiological method: This is based on the degree of inhibition of **E.coli** by the diffusion of ofloxacin in nutrient agar medium. The concentration of the drug (in samples set up in quadruplicates), which is proportional to the zone of inhibition, is calculated from the regression line of the log concentration of the standards and the diameter of the zone of inhibition.

Fluorimetric method: Ofloxacin could be estimated fluorimetrically. Plasma, saliva and urine containing ofloxacin are deproteinised using perchloric acid. The resulting protein-free filtrate containing ofloxacin is read in a fluorimeter with an excitation wave-length of 310 nm and an emission wavelength of 510 nm. From the standards, the concentration of ofloxacin in the unknown sample can be calculated.

Recovery of ofloxacin from plasma, saliva and urine : On each of 5 occasions, ofloxacin was set up in duplicates, in concentrations of 0, 0.5, 1, 2, 4, 8 and 16 µg/ml in human plasma, saliva and urine. The samples

were randomised and the zones of inhibition in the case of microbiological method and the fluorescent units in the case of fluorimetric method were recorded.

Results of the microbiological assay showed that the diameter of zones of inhibition (mm) increased with increasing concentration of ofloxacin over the range tested. The coefficients of variation for replicate estimations for plasma, saliva and urine were 10.4, 11.2 and 13.3 % for a concentration of 0.5 µg/ml and 4.9, 5.7 and 4.3 % for a concentration of 16.0 µg/ml, respectively.

The fluorescent units obtained with fluorimetric method were proportional to the concentration of ofloxacin employed. The co-efficients of variation for replicate estimations for plasma, saliva and urine were 9.0, 11.7 and 7.5 % for a concentration of 0.5 µg/ml and 1.4 , 0.7 and 4.3 % for a concentration of 16.0 µg/ml, respectively.

The other aspects are being investigated and the study is in progress.

*****

Characterization and purification of antigenic components of M. tuberculosis

(Ongoing study, 1988-95)

It has been reported last year (see 1992 annual report) that **M. tuberculosis** culture filtrate (CF) and **M. tuberculosis** Pressate antigens are being prepared in large quantities for purification and identification of antigens using preparative Isoelectric Focussing (IEF).

Preparative IEF is a relatively gentle, non-denaturing technique. It affords very high resolution, since two proteins which differ by a single amino acid substitution can also be resolved as 2 different components. Since the method achieves 25-fold purification in a single run, the native conformation of the proteins and thereby the bioactivity is maintained.

Preparative IEF was carried out using BioRad Rotophor Electrofocussing Cell. Ampholytes of broad pH range (3-10), as well as narrow range (4-6) were used to obtain a high degree of resolution. A total volume of 55 ml of crude antigenic preparation was applied along with 4M

Urea, 0.2% CHAPS, 0.2% Digitonin, 5% Glycerol and 1-2% ampholytes. The conditions have been standardized for best separation and minimal precipitation during focussing. The run was carried out at a constant power of 12W for about 4 hrs, till the voltage reached a plateau. It was observed that pH 4-6 gradient offers a better separation of proteins (Fig.1).

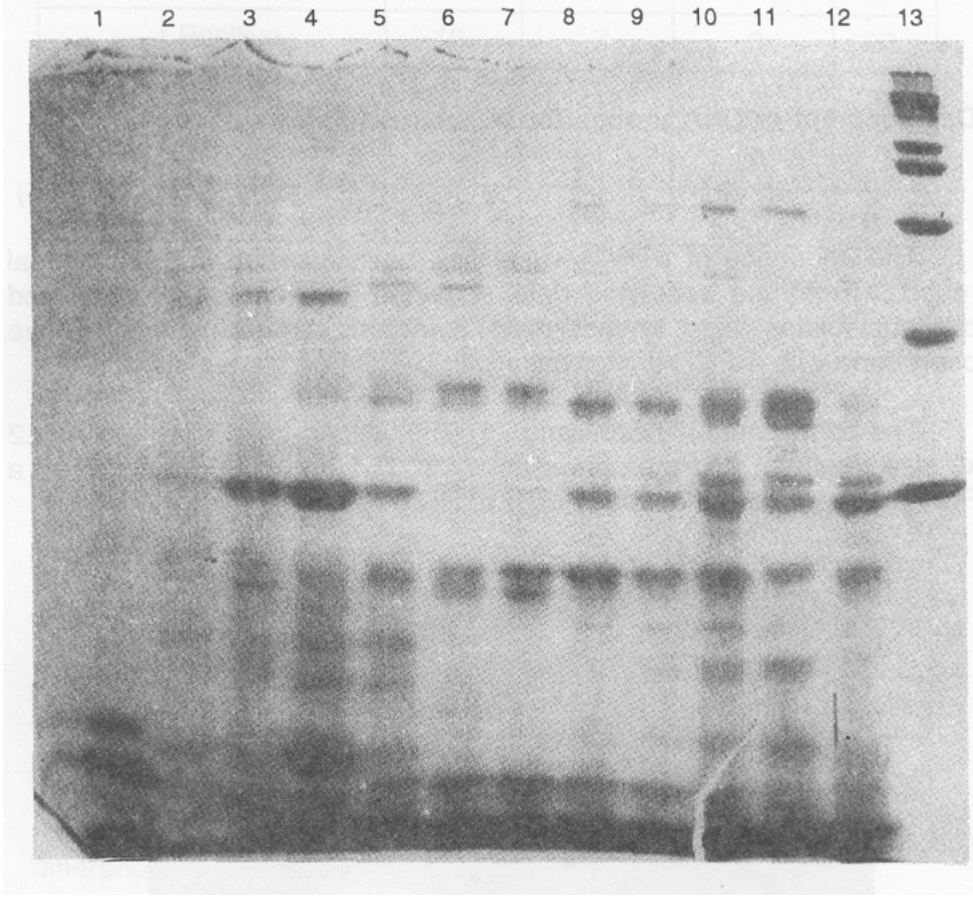


Fig.1 IEF pattern of **M. tuberculosis** $H_{37}Rv$ culture filtrate (pH 4-6)
Lanes 1, 13 - Molecular weight markers
Lane 2 - $H_{37}Rv$ culture filtrate
Lanes 3 - 12 - Separated fractions

Each of the fractions, obtained in IEF with 4-6 gradient, was run on SDS-Polyacrylamide Gel slabs and Westem blots were done. It was observed that there is specific recognition of 17 KDa (pl 3.4) and 38 KDa (pl 4.3) antigens from culture filtrates with pooled tuberculous sera (N=20)

and clear-cut absence of recognition with pooled normal sera (N=65). Individual sera from each category are yet to be tested.

Molecular sieving chromatography of the Rotophor purified fractions will be used for the "second dimensional" separation, to obtain individual antigens. This step will be carried out employing Preparative Electrophoresis on acrylamide gels and electro-elution.

*****

Development of DNA probes for M. tuberculosis

(Ongoing study, 1988-95)

The sequence of pTRC4 clone has been reported in 1992 annual report. From the sequence data, 4 primer pairs were designed and oligonucleotides were synthesised. Standardization experiments have been done with 4 PCR primer pairs with pTRC4 as the template.

The primer pairs 733/734 and 757/759 yielded a PCR product of 1.2 kb size fragment and the primer pairs 750/751 and 758/760 amplified a PCR product of 600 basepairs from pTRC (Fig.2).

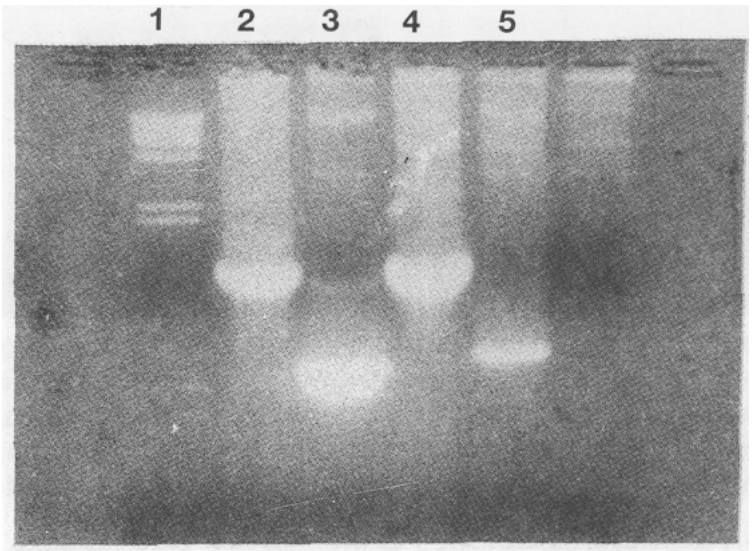


Fig.2 PCR amplification of **M. tuberculosis** - DNA using pTRC4 primers and Gel electrophoresis

Subsequently attempts are being made to standardise PCR protocol with clinical isolates.

Fig. 2 shows the photograph of PCR products amplified by the following PCR primer pairs run on 1% agarose gel with ethidium bromide.

Lane 1 - Hind Ill marker
2 - 733/734 amplified product (1.2 kb)
3 - 750/751 amplified product (600 bp)
4 - 757/759 amplified product (1.2 kb)
5 - 758/760 amplified product (600 bp)

These PCR primer pairs are being evaluated for their usefulness in clinical diagnosis.

*****

Human leucocyte antigen (HLA) studies in tuberculosis

(Ongoing study, 1990-97)

The main objective of this project is to use a combination of serological and DNA probes to analyse the phenotype ana the genotype of a number of individuals to find out whether there exists an association between any serological and/or DNA marker and the occurrence of tuberculosis.

HLA-phenotyping and genotyping will be carried out by HLA-antisera and HLA gene probes, respectively.

Further, the role of Class-ll genes/gene products on immunity to tuberculosis will also be studied in patients with active pulmonary tuberculosis and healthy volunteers.

The progress of the studies is reported under two different sections.

*****

I. HLA studies - HLA and Immune response: Role of HLA Class-II genes/gene products on immunity to tuberculosis

(Ongoing study, 1990-95)

It has been shown that Human leucocyte antigens influence immune functions, especially the Class-II antigens. The products of the HLA genes mediate the recognition and immune response to any pathogen by presenting antigens to the T-lymphocytes. To understand the role of HLA Class-II genes and their gene products (HLA-DR, -DQ and -DP) on antibody and cell-mediated immune responses against **M. tuberculosis** antigens, the present study was undertaken.

During the year, the humoral immune response to **M. tuberculosis** culture filtrate antigens was studied using plasma samples from 47 treated pulmonary tuberculosis patients who remained quiescent even after the completion of 5-years of follow-up.

The antibody response to **M. tuberculosis** culture filtrate antigen and the **M. tuberculosis** antigen recognition pattern of the plasma samples were carried out by ELISA and immunoblot methods. The antibody response to **M. tuberculosis** antigen is expressed as antibody titer.

Table 1
Influence of HLA-DR2 antigen on M.tuberculosis antigen (selected) recognition by the plasma samples of treated pulmonary tuberculosis patients

HLA-DR	Percentage of recognition by plasma samples	
	M. tuberculosis antigens* (Molecular weight in kDa)	
	32-34	36-38
DR2 Positive (n=25)	72	72
DR2 Negative (n=22)	50	59

* H37Rv culture filtrate antigens.

Since a higher phenotype frequency of HLA-DR2 is seen in the pulmonary tuberculosis patients than in the control subjects, the antibody

response was studied for HLA-DR2 positive (25) and HLA DR2 negative (22) patients. An increased mean antibody titer 653 was seen in the HLA-DR2 positive patients than the HLA-DR2 negative patients, for whom the mean antibody titer was 354. Further, the plasma samples of most of the HLA-DR2 positive patients recognize 32-34 and 36-38 kDa antigens of **M. tuberculosis** culture filtrate antigens unlike HLA-DR2 negative patients. (Table-1).

The study will also be carried out in 50 active pulmonary tuberculosis patients and 50 healthy volunteers. The influence of heterozygous combination of HLA-DR2 with other HLA-DR and -DQ antigens on immunity to tuberculosis will be studied.

*****

II. HLA Studies - Investigation in quiescent and relapse cases of pulmonary tuberculosis

(Ongoing study, 1992-95)

An exploratory study was undertaken to find out whether there is any association between HLA-antigen and/or haplotype and the occurrence of relapse of tuberculosis in successfully treated pulmonary tuberculosis patients. A total of 100 quiescent patients and 100 relapse patients will be investigated.

During the year, HLA-A, -B, -DR and -DQ serological typing were carried out in 19 quiescent and 19 relapse cases of pulmonary tuberculosis in addition to the 9 quiescent and 9 relapse cases mentioned in 1992 annual report. DNA were extracted from the peripheral blood white cells of these patients and stored at -70°C. The DNA will be later used for HLA-genotyping and tumor necrosis factor and T-cell receptor gene polymorphism.

The study is in progress.

*****

Cytokine profiles in tuberculosis by PCR detection of Cytokine mRNA

(Ongoing study, 1993-96)

Cytokines are the essential soluble transmitters of cell to cell communication. The important role of cytokines in mycobacterial infections is well established. The cytokines primarily associated with CD4+ cells namely IL1, IL2, TNF and IFN-r have been shown to be capable of influencing the course of mycobacterial infections.

As a pilot project, it is proposed to study the cytokine profiles in tuberculosis patients and compare it with that of normal subjects. The lymphokines IL2, IL4, IL6, IL10, IFN-r and GMCSF will be studied by PCR detection of mRNA as described by Ehler et al., (J. Exptl. Medicine, 1991, 173, 25)

Hepatinised peripheral blood (20 ml) was collected from 10 pulmonary tuberculosis patients in age group 20-51 years (smear and culture positive cases). Mononuclear cells were separated and stimulated with mitogen PHA (5 pμ/ml) and antigen PPD (5 μg/ml) for 24 hrs. Cells were washed, lysed and total RNA was extracted from lymphocytes. Reverse Transcription of mRNA was done in these samples to get more stable CDNA copies. These samples were stored at -20°C for further amplification of CDNA by PCR using specific cytokine primers.

Similarly 10 normal individuals in age group 13-25 years with Mantoux negative status were selected from the BCG trial area. Blood samples will be collected from these individuals and processed as described above.

PCR amplification of cytokines in tuberculosis patients' sample is in progress.

*****

RFLP analysis of M. tuberculosis isolates from South Indian patients

(Ongoing study, 1993-95)

Many genetic elements contribute to the polymorphism of **M. tuberculosis** because they occur as multiple copies in the genome of **M. tuberculosis.** This polymorphism has been used to study the strain

differentiation. IS6110 is one such genetic element which has been extensively studied. It is an excellent genetic marker to trace individual **M.tuberculosis** strains during micro epidemics, nosocomial infections and clonal dissemination of multiple drug resistant strains.

Although most of the **M. tuberculosis** complex strains carry multiple IS 6110 copies, occasionally isolates have been encountered which contain only one or a few copies. All **M. bovis** BCG strains contain a single IS copy. During a preliminary analysis of strains from pre and post treatment cultures from our Centre a large number of strains with none or only a single IS copy was encountered (see annual report 1992). Since these strains could not be differentiated by IS6110 DNA fingerprinting, it was proposed to see whether they could be differentiated with other genetic markers like DR and pTRC4 and also to study the frequency of exogenous tuberculous infection using DR probe and compare it with IS6110.

DR element is the 36 base pair Direct Repeat genetic element first identified in **M. bovis** BCG. Based on the published sequence of Direct Repeat of **M. bovis** BCG, oligonucleotides were synthesised. pTRC4 is a 2kb mycobacterial element cloned at the Centre(TRC).

DNA isolation purification and restriction analysis: DNA from the clinical isolates will be isolated and purified using standard procedure. A preliminary experiment was done in which the clinical isolates were subjected to restriction digestion with different restriction enzymes PVU II, Sal I, PST I, ECOR I, Hind Ill, Rsa I and Alu I. Out of these enzymes Rsa I and Alu I gave a better banding pattern when probed with DR.

Labelling of DNA: pTRC4 will be labelled by P32 dCTP. DR element has been labelled using ECL 3' oligolabelling and detection system. This utilises the enhanced chemiluminescence (ECL) associated with horse radish peroxidase catalysed oxidation of luminol to detect the presence of oligonucleotides tailed at the 3' end with fluorescein -d UTP (FI-dUTP) hybridised to target sequences on membranes. The labelling reaction is catalysed by terminal deoxy nucleotidyl transferase. The emitted light is detected in x-ray film.

Southern blot hybridisation: Digests of chromosomal DNAs will be electrophoretically separated on 1.0% agarose gel containing ethidium bromide (500 ng/ml). After denaturation and transfer to Gene Screen plus membranes (Dupont NEN products Boston Mass) by vacuum blotting (TC 80 Trans Vac TM Hoefer Scientific Instruments, San Francis Co.), the DNA

will be hybridised with P32 labelled probes. Hybridisation and washing will be done at 65°C. Membranes will be exposed to x-omat film (Eastman Kodak Co., Rochester N.Y.) at -70ºC for various lengths of time. The experimental procedures for the enhanced chemiluminescence gene detection system recommended by the manufacturer will be used.

From each of the fifty-two patients who relapsed, two clinical isolates (sputum cultures) obtained, one as pre-treatment and the other as relapse have been coded to conceal their identity. DNA from the coded clinical isolates have been extracted and purified using the above procedure. Alu I has been used in analysing the 52 pairs of pre-treatment and relapse cultures by RFLP studies with DR probe.

The study is in progress.

*****

Investigation of clones of M. tuberculosis isolated from primary cultures of patients for their phenotypic/genotypic differences: A pilot investigation

(Ongoing study, 1992-96)

As described in the 1992 Annual Report, 87 clones, generated from 9 pre-treatment cultures were grown in Middlebrook $7H_9$ medium for 4-6 weeks. After coding the cultures, the DNA was extracted, restricted and electrophoresed as per the protocol of Hermans et al., (Journal of clinical Microbiology, 1990, 28, 2051). The bands were visually read and their Restriction Fragment Length Polymorphism (RFLP) patterns are given in table 1.

Of the 87 clones, 5 were contaminated; the restricted fragments from the DNA of another 5 clones were unreadable. Therefore the results were available only for 77 clones. Totally 8 RFLP patterns were observed. The clones generated from the respective culture showed similar RFLP patterns. A different RFLP pattern was observed for one clone from each of 3 isolates (V, Vlll and IX), and this could be attributed to reading or experimental error.

Table 1

RFLP patterns of clones from pre-treatment cultures of M. tuberculosis

Pre-treatment cultures of M.tuberculosis	Number of Bands having same RFLP pattern								
	0	1	3	4	5(A)	5(B)	12	15	Total clones
I	7								7
II		8							8
III		10							10
IV			8						8
V				8		1			9
VI					9				9
VII						7			7
VIII	1						8		9
IX	1							9	10
Total clones	9	18	8	8	9	8	8	9	77

The study is in progress.

*****

Immunopathology of cutaneous tuberculosis

(Ongoing study, 1992-95)

The aims, objectives and methodology of this study were outlined in the 1992 Annual Report. Briefly, it is proposed to correlate the histopathological features with the various clinical forms of skin tuberculosis and lay down histological diagnostic criteria for this disease. Further, it is expected that this study would throw new light on the immunopathogenesis of tuberculosis.

So far, biopsies from 150 patients have been examined. Of these, 121 showed evidence of tuberculosis. It is interesting to note that while various types of necrosis were seen in nearly 60% of biopsy specimens from tuberculous lymphadenitis patients (see annual report 1992), necrosis was seen only in 8 (6.6%) cases of skin tuberculosis. Necrosis was observed

in seven of the eight cases of scrofuloderma and one of seventy cases of lupus vulgaris.

In addition to the routine staining with haematoxylin-eosin and Ziehl-Nielson (ZN) stains, mycobacterial antigen(s) and B-cells were demonstrated using polyclonal anti **M. tuberculosis** antibody and anti CD20 monoclonal antibody. Although acid fast bacilli could not be demonstrated in any of these tissues using ZN stain, the presence of mycobacterial antigen(s) was noted in 28 out of 35 (80%) biopsies stained so far. Further, the presence of B cells, mostly in clusters and rarely as isolated infiltrates, was found in 14 of the 22 (64%) biopsies stained with anti CD20 antibody.

*****

Evolution of experimental mycobacterial granuloma

(Ongoing study, 1993-94)

This study was initiated to follow the course of experimental mycobacterial granuloma vis a vis the presence of AFB and antigen in the skin of sensitized guinea pigs challenged with heat-killed **Mycobacterium tuberculosis** (HKMTB).

Twenty male guinea pigs were sensitized with HKMTB in Freund's incomplete adjuvant in the foot pad. After a fortnight, the guinea pigs were challenged with 0.1ml of 10^5 to 10^8/ml HKMTB intradermally in the flank. The development of granuloma was assessed by measurement of the injected skin using a pair of Vernier calipers. Two guinea pigs were sacrificed initially at days 4 and 7 and subsequently, at two weekly intervals. The skin at the injected site was taken out and was processed. Subsequently, the sections were stained with H & E, Ziehl Nielson stain and anti **M. tuberculosis** antibody.

So far, in the pilot experiments conducted, the dose of organisms to produce a granuloma has been standardised and the study is in progress.

*****

Development of an experimental model for fibrosis

(Ongoing study, 1993-95)

Morbidity in treated tuberculosis patients is usually due to fibrosis. This study was therefore initiated to understand **M. tuberculosis -** induced fibrogenic mechanisms that occur in an experimental animal model. It is hoped that a delineation of the process of fibrosis would pave the way for appropriate intervention strategies.

Twenty-four guinea pigs, which were sensitized with killed **M. tuberculosis,** were challenged with 1 mg(im/sc) live **M. tuberculosis** (harvested in mid-log phase). Four guinea-pigs were sacrificed at fortnightly intervals starting from 2 weeks. The site of infection, spleen, liver and lungs were collected and one half of each of the specimens was processed routinely and stained with haematoxylin-eosin and Ziehl-Nielsen(Z.N), anti **M. tuberculosis** and Van Gieson's stains. Collagen, elastin and hexosamine contents of the other half of the specimens are being measured. The methods of extraction of these from tissues have been standardized in the pilot experiment conducted so far.

*****

EPIDEMIOLOGICAL STUDIES - COMPLETED

Longitudinal Study of Bacteriological Quiescence and Relapse in Pulmonary Tuberculosis Under Programme Conditions

(Completed study, 1989-93)

This study was undertaken in order to have a better understanding of quiescence and relapse in patients with smear-positive pulmonary tuberculosis for whom anti-tuberculosis chemotherapy had been started under programme conditions, and relate it to the amount of chemotherapy, and the pre-treatment drug resistance. The methodology for this study has been described in detail in the 1989 annual report.

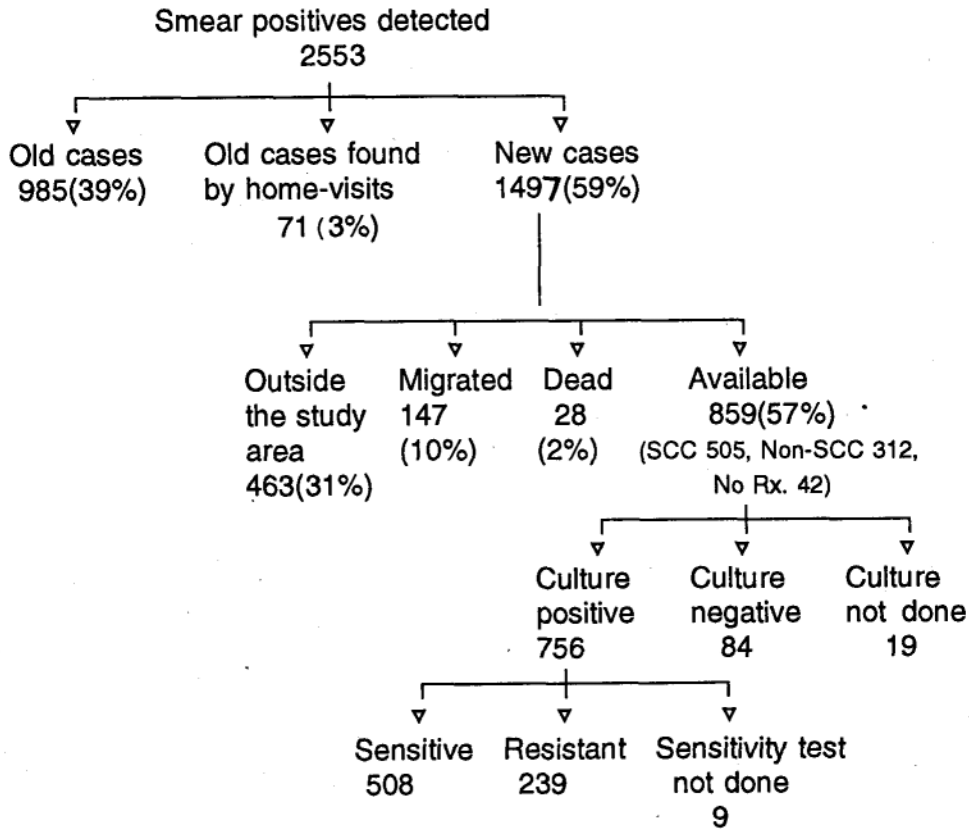


Fig. 1: Diagrammatic representation of cases

Previously untreated smear-positive patients detected at the four selected centres, viz, the District Tuberculosis Centre at Vellore, the Government Hospitals at Gudiyattam and Ami, and the Government Sanatorium at Adukkambarai, and living within 20Kms of these centres were eligible for inclusion in this study. The regimens in operation under the programme were: daily isoniazid with thioacetazone, ethambutol or PAS for one year (or) fully intermittent supervised twice-weekly (S)HRZ for 2 months followed by HR twice-weekly for 4 months (or) daily unsupervised RHZ for 2 months followed by TH for 6 months.

Figure 1 shows the status of patients found to have a positive smear under programme conditions. Forty-one percent of the patients have had treatment for tuberculosis previously. Thirty-one percent of new patients reporting to the clinics were from beyond 20kms. Ten percent had migrated and two percent had died within 10 days of diagnosis. This left 859(57%) patients for inclusion in the study. The age and sex distribution of the patients included in the study as compared to those who did not get included is shown in table 1. There were four times as many males as females.

Table 1

Age-Sex distribution of new cases

Age	Sex	Externals	Migrated	Dead	Available	Total
10-14	M	1	-	-	-	1
	F	1	-	-	3	4
15-24	M	38	10	1	74	123
	F	25	7	-	46	78
25-44	M	148	58	7	246	459
	F	54	15	2	72	143
45-64	M	152	37	12	315	516
	F	23	7	1	57	88
65 +	M	19	12	4	37	72
	F	2	1	1	9	13
Total	M	358	117	24	672	1171
	F	105	30	4	187	326
	Both	463	147	28	859	1497

Table 2

Initial smear vs culture

Smear	Culture					
	Neg	1-99 col.	1+	2+	3+	Total
Neg	65	14	12	0	0	91
1+	15	53	72	103	24	267
2+	4	12	37	178	178	409
3+	0	1	1	28	43	73
Total	84	80	122	309	245	840

The association between smear and culture results for 840 patients is shown in table 2. Sputum specimens could not be collected in 19 patients (SCC: 6, Non-SCC: 10 and No. Rx.: 3) at intake.

Table 3

Drug consumption according to initial smear status

Regimen	Drug consum-ption(%)*	Initial smear status				
		Neg	1+	2+	3+	Total
SCC	<50	23	62	139	22	246
	50-79	4	21	24	3	52
	≥80	18	68	93	22	201
	Total	45	151	256	47	499
Non-SCC	<50	17	61	68	12	158
	50-79	8	20	16	2	46
	≥80	19	28	47	4	98
	Total	44	109	131	18	302
Initial defaulters		2	7	22	8	39
Grand total		91	267	409	73	840

* Drug collection is considered as drug consumption

The pre-treatment culture results were not available for 19 patients and were negative for 84. Eighty had scanty growth. Of 91 patients with negative smears only 26 has produced positive culture and cultures were negative in 19 patients whose smears were positive. About two-fifth (358) of the patients had scanty or negative smears (Table 2). However, the smear status did not affect the proportion of patients completing chemotherapy in both SCC and standard regimens (Table 3).

Table 4

Drug consumption* in relation to duration of treatment

Regimen	Duration of Rx. (months)	Drug consumption (%)			
		<50	50-79	≥80	Total
SCC	<2	129	0	0	129
	2-3	55	14	0	69
	4-5	22	14	36	72
	≥6	45	25	165	235
	Total	251	53	201	505
Non-SCC	< 3	95	0	0	95
	3-8	49	33	0	82
	9-14	11	12	31	54
	≥15	10	2	69	81
	Total	165	47	100	312

*42 initial defaulters excluded

Table 4 shows the relationship of drug consumption to duration of treatment. It can be observed that 129 (51.4%) of 251 patients receiving SCC, discontinued treatment within 2 months and 95 (57.6%) of 165 patients receiving Non-SCC, discontinued treatment after the first 2 collections.

The pre-treatment sensitivity status of 747 patients for whom results were available is shown in table 5.

A total of 508 (68%) of 747 patients were sensitive to streptomycin, isoniazid and rifampicin (SHR), 19% to isoniazid (H) and 12% to rifampicin (R). Multiple drug resistance was seen in 90 (12%) cases. Isolated rifampicin resistance was also seen in 6% of cases.

Further analysis relates to those who were started on treatment and for whom pre-treatment drug sensitivity results are available (i.e., 710 patients).

Table 5
Initial drug sensitivity status

Treatment	No. eligible	No. examined	Drug sensitivity status							
			Sensitive to SHR	Resistant to						
				S	H	R	SH	SR	HR	SHR
SCC	460	455	319	30	29	31	27	6	5	8
Non-SCC	259	255	166	10	28	14	21	2	6	8
No Rx.	37	37	23	2	3	2	1	0	2	4
Grand Total	756	747	508	42	60	47	49	8	13	20

The status of the patients at the end of chemotherapy, according to the initial drug sensitivity status and the level of drug consumption in the SCC and Non-SCC regimens is shown in table 6.

There were more deaths and migrations among those patients who had less than 50% of chemotherapy (40% in SCC and 43% in Non-SCC). Among those who had completed 80% of chemotherapy with initially drug sensitive organisms, sputum conversion was similar in both types of regimens. However, in those who had initial drug resistance, sputum conversion among those taking SCC is about twice as high.

Among those who have had less than 50% of chemotherapy whose initial cultures showed sensitive organisms, 48% appear to have achieved conversion after SCC, and 53% after standard regimens. The sputum conversion rates, especially in those who have had less than fifty-percent of chemotherapy, appears to be higher among those with initial smear grades of negative or 1+ as compared to those with initial smear grades of 2+ or 3+ (Table 7).

Table 6

Status at the end of treatment period according to drug consumbtion and initial sensitivity status

Drug consum-ption(%)	Status at the end of treatment	SCC(9 months)			Non-SCC(18 months)		
		Sensi-tive	Resistant to		Sensi-tive	Resistant to	
			Single drug	Multiple drug		Single drug	Multiple drug
< 50	No. eligible	161	43	18	89	31	19
	Left	27	6	4	18	7	4
	Dead	42	6	4	18	7	6
	Available	92	31	10	53	17	9
	Examined	85	30	10	51	16	8
	Negative	41**[48]**	13**[43]**	3**[30]**	27**[53]**	7**[44]**	2**[NC]**
50-79	No. eligible	35	8	5	24	8	5
	Left	5	2	0	1	3	0
	Dead	3	0	1	2	0	0
	Available	27	6	4	21	5	5
	Examined	25	6	4	19	5	4
	Negative	16**[64]**	2**[NC]**	1**[NC]**	9**[47]**	2**[NC]**	3**[NC]**
≥ 80	No. eligible	123	39	23	53	13	13
	Left	4	2	0	1	0	0
	Dead	4	0	1	0	0	0
	Available	115	37	22	52	13	13
	Examined	110	36	20	51	13	12
	Negative	86**[78]**	28**[78]**	13**[65]**	45**[88]**	6**[46]**	2**[17]**
Total	No. eligible	319	90	46	166	52	37
	Left	36	10	4	20	10	4
	Dead @	49**(17)**	6**(8)**	6**(14)**	20**(14)**	7**(16)**	6**(18)**
	Available	234	74	36	126	35	27
	Examined	220	83	34	121	34	24
	Negative	143**[65]**	45**[54]**	17**[50]**	81**[67]**	15**[44]**	7**[29]**

Figures in square brackets show percentage converted; NC = Not calculated.
@ Figures in parentheses for this row show percentage dead

Table 7

Sputum conversion at the end of treatment according to initial smear status

Drug consu m-ption (%)	Sputum examination at the end of treatment	SCC			Non-SCC		
		Initial smear			Initial smear		
		Neg.	1+	2+ or 3+	Neg.	1+	2+ or 3+
<50	No. Examined	3	35	87	7	29	39
	Negative	2(**NC**)	22(**63**)	33(**38**)	2(**NC**)	16(**55**)	18(**46**)
50-79	No. Examined	1	17	17	2	11	15
	Negative	1(**NC**)	9(**53**)	9(**53**)	2(**NC**)	5(**45**)	7(**47**)
≥80	No. Examined	9	58	99	3	27	46
	Negative	9(N**C**)	44(**76**)	74(**75**)	3(**NC**)	22(**81**)	28(**61**)
Total	No. Examined	13	110	203	12	67	100
	Negative	12(**92**)	75(**68**)	116(**57**)	7(**58**)	43(**64**)	53(**53**)

Figures in parentheses show percentage converted; NC = Not calculated.

Of those patients with initial drug-sensitive organisms, relapse rates for SCC and Non-SCC regimens are similar, 12% and 11%, respectively (Table 8). Among patients with drug-resistant organisms, the relapse rates are lower in those who had SCC (7%), compared to those who had received Non-SCC regimen (16%).

In summary, it appears that in patients with drug sensitive organisms, the greater the amount of chemotherapy (SCC or Non-SCC) received, the higher is the proportion of sputum conversion. Among patients who had less than 50% chemotherapy (SCC), those with resistant organisms to single drug had 43% sputum conversion, compared to 30% in those with multiple drug resistance. Further, the proportion of patients with sputum conversion was lower among those with higher grades (2+ or 3+) of smear positivity at intake, compared with those who had negative or 1+ smear irrespective of whether they had SCC or Non-SCC.

Table 8

Relapse over 24 months among patients who had converted at the end of treatment according to drug consumption and initial drug sensitivity status

Drug consu m-ption	Status at the end of treatment	SCC			Non-SCC		
		Sensi-tive	Resistant to		Sensi-tive	Resistant to	
			Single drug	Multiple drugs		Single drug	Multiple drugs
<50	Eligible	41	13	3	27	7	2
	Left & dead	3	2	0	5	0	0
	Available	38	11	3	22	7	2
	Examined	35	11	3	22	6	2
	Positive	6	1	0	2	2	0
50-79	Eligible	16	2	1	9	2	3
	Left & dead	3	1	0	0	1	0
	Available	13	1	1	9	1	3
	Examined	12	1	1	9	1	3
	Positive	0	0	0	0	0	1
≥80	Eligible	86	28	13	45	6	2
	Left & dead	7	0	0	1	1	0
	Available	79	28	13	44	5	2
	Examined	77	28	12	43	5	2
	Positive	9	1	2	6	0	0
Total	Eligible	143	43	17	81	15	7
	Left & dead	13	3	0	6	2	0
	Available	130	40	17	75	13	7
	Examined	124	40	16	74	12	7
	Positive	15 **(12)**	2 **(5)**	2 **(12)**	8 **(11)**	2 **(17)**	1 **(NC)**

Figures in parentheses indicate percentage relapsed; NC = Not calculated

*****

Pilot study of case finding for tuberculosis in children at the community level

(Completed study, 1990-93)

This study was undertaken in Kadambathur Panchayat Union with the objective of developing a methodology for periodic screening of children in a community by health workers, to identify children likely to be suffering from tuberculosis as early as possible and to refer them for further investigations. Earlier studies by the Centre, have documented the methods for the development of an algorithm for screening of children by health workers (see 1990 annual report) and for training and assessment of health workers in the use of this algorithm (see 1989 annual report) as well as the feasibility of carrying out the investigations for tuberculosis at the community level (see 1988 annual report).

The population for this study comprised children aged less than 10 years in 15 randomly selected panchayats of Kadambathur. The intake for this study was started in December 1990 and completed in December 1991. There were 6-monthly follow-ups up to 18 months.

At intake, all children were screened by health workers for clinical signs and symptoms attributable to tuberculosis. In addition, they were subjected to tuberculin tests with PPD 1-TU RT 23 in tween 80 and full-plate chest radiographs for children aged 3 months and above. Those who had an abnormal X-ray had a repeat X-ray at 3 months. At 6 and 12 months, X-rays were taken only if indicated clinically. Tuberculin test was not repeated.

At 6, 12 and 18 months, complete house to house screening of all children by health workers was carried out. Clinical examination was carried out by a paediatrician and X-rays taken when clinically indicated. Criteria for X-ray referral and clinical examination were different for the 18th month follow-up.

Children with an X-ray abnormality at intake or with persistent screening abnormality or clinical abnormality in any one round were referred for X-ray in addition to those referred by the paediatrician after clinical examination.

In addition to those referred by the algorithm, children with the following were included for clinical examination.

1) All children referred for X-ray

2) Children with those abnormalities on screening that were not included in algorithm for referral on more than one occasion.

This was done, in order that cases that might have developed during the 18 months of follow-up, will be examined clinically at least once. At each point in time, a randomly selected proportion of the normals were also clinically examined as controls.

The procedures are summarised in table 1.

Table 1
Schedule for different examinations

	Tuber-culin test	X-rays	H.W. screening	Clinical exam. by paediatrician	Gastric Lavage/ sputum gland biopsy	Others
Intake	All	All	All	As indicated *	As indi-cated *	As indi-cated *
3 months	Not done	Abnormal at intake	Abnormal at intake	-do-	- do -	- do -
6 & 12 months	Not done	Clinical referrals	All	-do -	- do -	- do -
18 months	Not done	Abnormal at intake+persistent screening abnormality + had anti-TB Rx treatment +clinical cases + ARI	- do -	All X-ray referrals + those with screening with abnormality on any two previous rounds	- do -	- do -

* See text

There were a total of 6250 children in the villages included in the study area (defacto population). Of these, 6150 were permanent residents (dejure population).

Coverages for intake examinations, as well as for individual rounds (follow-up) are calculated for the defacto population (Table-2).

Table 2

Coverages obtained for different examinations

Investigations	Intake		3 months		6 months		12 months		18 months	
	No. eligible	%	No. eligible	%	No. eligible	%	No. eligible	%	No. eligible	%
Screening	6250	**97**	2336	**97**	6511	**95**	6589	**97**	6967	**97**
Tuberculin (test-read) (age≥1 month)	6182	**94**	-	-	-	-	-	-	-	-
X-rayed (age≥3 months)	6151	**94**	1465	**94**	-	-	-	-	2497	**91**
Clinical examination based on:										
X-ray	231	**89**	54	**91**	-	-	-	-	189	**78**
Screening	350	**94**	221	**81**	210	**91**	272	**81**	259	**72**
Both	19	**100**	14	**79**	-	-	-	-	11	**82**
Total	600	**92**	289	**83**	210	**91**	272	**81**	457	**75**
Control	897	**92**	202	**89**	127	**94**	331	**98**	1668	**95**
Investigations:										
Sputum	506	**98**	163	**78**	214	**100**	202	**98**	477	**97**
Gastric Lavage	472	**97**	168	**80**	252	**100**	224	**97**	396	**97**
Biopsy	21	(18)	7	(6)	11	(10)	18	(9)	27	(14)

Figures in parentheses are number biopsied

Consistently high coverages have been obtained for each round of follow-up. This has resulted in 93% of the cohort being retained at the end of 18 months among 6150 Children registered at intake, as shown in table 3. Of these 89% have been screened. This report gives information on 89% of the original cohort over an 18-month period.

Cohort coverages are shown for the dejure population (Table 3). The first six groups could not be covered for clinical examination at the 6-month follow-up as a medical officer was not available during that time.

Table 3

Cohort follow-up status

Follow-up (months)	Eligible	Dead	Left	Not Available	Available	Examined
6	6150	17	165	0	5968 **(97)**	5656 **(92)**
12	6133	13	287	10	5823 **(95)**	5564 **(90)**
18	6120	0	420	9	5691 **(93)**	5455 **(89)**

Figures in parentheses show percentages available among intake population

Based on the results of gastric lavage and gland biopsy, children were identified as having tuberculosis (confirmed by bacteriology or histopathology). Eighteen such cases were detected at intake, 3 at 3 months of selective follow-up, 2 at 6 months, 5 at 12 months and 12 at 18 months. Thus, there was a total of 40 cases confirmed by bacteriology or histopathology.

In addition to the above, 104 children had both clinical disease and radiological pictures interpreted as tuberculosis, 82 were suspected clinically but were normal radiologically and 152 had X-ray shadows suggestive of tuberculosis but were normal clinically.

The ability of the algorithm to identify correctly children who needed further investigations as well as the yield of cases from the algorithm referrals is shown in table 4. Seven of the forty cases had been picked up because of an abnormal X-ray. Twenty-one cases had been correctly identified and the others were missed.

It is also possible that there were many more cases of tuberculosis occurring in the community than those picked up by the algorithm. These cases would have sought treatment from agencies outside the facilities available with the study staff and they would have been missed by the pilot

study. In order to study this aspect, the following was done. At each round after intake, the parents of children were asked if they had sought emergency treatment for the ,child from outside agencies.

Table 4

Yield of cases according to method of referral confirmed by histo-pathology or bacteriology

Method of Referral	Intake		3 months		6 months		12 months		18 months	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Algorithm										
for sputum	2	322	1	65	1	140	1	154	1	105
for clinical only	7	27	0	8	0	16	0	13	0	33
for sputum & clinical	2	70	1	80	1	162	2	116	0	90
X-ray	2	213	0	32	0	0	0	0	2	35
Controls	5	315	1	75	0	151	2	129	9*	575
Total	18		3		2		5		12	

* 6 out of 9 cases had an abnormality on screening in more than one occasion which is not referable for further investigation

The distribution of cases seeking anti-TB treatment or attending medical emergency is given in table 5 which includes also children who do not

Table 5

Distribution of the cases seeking treatment or attending medical emergency

Round of follow-up (months)	Eligible	Died	Medical emergency	No. treated outside
6	6525	18	20	10
12	6604	15	24	27
18	6967	0	18	26

*defacto population

belong to that village (visitors or externals) but were found at the time of follow-up. These children were then visited by a senior field officer who ascertained the reasons for death or seeking medical emergency. The reasons were diarrhoea, measles, jaundice, breathing troubles, fever and other Non-TB symptoms.

In none of the children seeking emergency medical care, nor in those who died, was TB recorded as a diagnosis. None of these children have had a diagnosis of TB either clinically or by X-ray or bacteriology, by the trial procedures.

In summary, it was possible to train health workers to use an algorithm. This algorithm picked up 19 out of 40 cases occurring in the community. Four of the 21 that were missed, had only an X-ray abnormality and would not have been picked up by any screening that did not include X-ray. The findings in the other 17 cases are being examined to see if the sensitivity of the algorithm could be improved.

It was also shown that periodic screening would pick up most of the cases of TB in children.

*****

Feasibility of quantitative evaluation of DTP implementation by Peripheral Health Institutions

(Completed study, 1993)

The Objective of this study is to test the feasibility of a quantitative evaluation method using a scoring system, for the assessment of the efficiency of DTP implementation.

Methods: This quantitative evaluation is an active method, in contrast to the passive method based on the monthly reports on tuberculosis(MRTs) submitted by the PHls. There are 8 PHls in and around Thiruvalangadu and Kadambathur Panchayat Unions. All these PHls were visited once a month by a Medical Officer, a Technical Officer (Field), a Deputy Team Leader (Field) and a Laboratory Technician. The Medical Officer was incharge of the monitoring team and carried out the duties of the DTO. The Technical Officer checked all the relevant records namely, Outpatient Register, Sputum Register, X-ray Register, Book of treatment cards and

collected the relevant data. The Deputy Team Leader checked all the treatment cards and classified the patients as completed, regular, defaulter and lost to treatment. He also verified the completeness of entries in each treatment card. The Lab. Technician checked a sample of stained smears (10 to 30%). The scores used are described below:

I **(a) Case referral:** Scores ranging from 0 to 5 were allotted on the basis of percentage of new outpatients referred for sputum examination (Nil - 0; <0.50 - 1; 0.50 to 0.99 - 2; 1.00 to 1.49 - 3; 1.50 to 1.99-4; ⩾2-5)

(b) Smear examination: Scores ranging from 0 to 5 were allotted on the basis of percentage of smear positives identified from the total smear examinations done. (Nil - 0; <2 - 1; 2 to 3.9 - 2; 4 to 5.9 - 3; 6 to 7.9 - 4; ⩾ 8 - 5)

II **Caseholding:** Scores ranging from 0 to 10 were allotted on the basis of the regularity index. Regularity index was calculated by giving scores to each treatment card as follows:
Regular - 1; Defaulter - 0.5; and lost - 0.
Regularity Index (R.I.) = Total scores / Total cards
Case holding is scored on the basis of R.I. values.

(R.I.)	0.01 to 0.10 - 1	0.41 to 0.50 - 5	0.81 to 0.90 - 9
	0.11 to 0.20 - 2	0.51 to 0.60 - 6	0.91 to 1.00 - 10
	0.21 to 0.30 - 3	0.61 to 0.70 - 7	
	0.31 to 0.40 - 4	0.71 to 0.80 - 8	

III **Registers:** Sputum register, X-ray register and Book of treatment cards (BTC) were checked; if complete - 1; incomplete or Not Available (NA) - 0; For each register these scores were given making the maximum score for registers - 3.

IV **Treatment cards:** Each treatment card is checked for all relevant entries. The percentage of treatment cards with all relevant entries was used for assigning scores as follows:

(Nil - 0; ⩽25 - 1; 26 to 50 - 2; 51 to 75 - 3; 76 to 99 - 4; 100 - 5)

V **Reporting:** The scores for reporting are as follows: Monthly reports up to date - 2; If reports are due - 1; If not at all sent earlier - 0.

Table 1

Scores for the evaluation of DTP implementation by XCs

PHI Category (XC)	Components assessed	Scores for month						Maximum score
		1	2	3	4	5	6	
Trivellore General Hospital	Referred for sputum exam.	*	*	4	2	2	2	5
	Detection of pos. smear	-	-	2	4	3	5	5
	Regularity score	7	10	9	8	9	9	10
	Records and reporting	8	10	8	10	10	10	10
	Total score	15	20	23	24	24	26	30
	Efficiency (%)	**(75)**	**(100)**	**77**	**80**	**80**	**87**	**100**
Sriperumbudur General Hospital	Referred for sputum exam.	2	2	1	1	2	3	5
	Detection of pos. smear	5	2	0	0	0	2	5
	Regularity score	9	10	10	10	10	10	10
	Records and reporting	9	10	10	10	10	10	10
	Total score	25	24	21	21	22	25	30
	Efficiency (%)	**83**	**80**	**70**	**70**	**73**	**83**	**100**
Perambakkam PHC	Referred for sputum exam.	*	2	2	*	*	4	5
	Detection of pos. smear	*	0	0	*	*	0	5
	Regularity score	9	9	8	8	7	9	10
	Records and reporting	8	10	10	9	8	10	10
	Total score	17	21	20	17	15	23	30
	Efficiency (%)	**(85)**	**70**	**67**	**(85)**	**(75)**	**77**	**100**

* Information not available; - Not applicable
Figures in parentheses indicate efficiency (%) based on incomplete data

Efficiency was calculated as a percentage of the total scores of components assessed for which information was available, to the total of corresponding maximum scores.

Results: All the 8 PHIs visited - three X-ray centres (XCs), three Microscopic centres (MCs) and two Referral centres (RCs) - are considered for discussion of the findings. The findings of six monthly visits to these PHIs are presented.

Efficiency for XCs ranges from 67 % to 100 % (Table 1). Case referral is generally poor and needs improvement. Case detection is also not satisfactory. Regularity records and reporting are satisfactory.

Table 2

Scores for the evaluation of DTP implementation by MCs

PHI Category (MC)	Components assessed	Score for month						Maximum score
		1	2	3	4	5	6	
Poonimangadu PHC	Referred for sputum exam.	*	*	2	1	5	4	5
	Detection of positive smear	-	-	0	0	0	0	5
	Regularity score	6	10	7	10	8	8	10
	Records and reporting	5	5	8	8	8	9	9
	Total score	11	15	17	19	21	21	29
	Efficiency (%)	**(58)**	**(79)**	**59**	**66**	**72**	**72**	**100**
Tiruvalangadu PHC	Referred for sputum exam.	1	1	3	1	1	1	5
	Detection of positive smear	1	1	*	0	0	0	5
	Regularity score	8	8	7	9	9	9	10
	Records and reporting	5	5	8	8	7	9	9
	Total score	15	15	18	18	17	19	29
	Efficiency (%)	**52**	**52**	**75**	**62**	**62**	**66**	**100**
Kanakamma chatram, PHC	Referred for sputum exam.	1	1	1	1	1	1	5
	Detection of positive smear	0	0	0	0	0	0	5
	Regularity score	9	10	10	10	10	8	10
	Records and reporting	9	8	8	9	9	9	9
	Total score	19	19	19	20	20	18	29
	Efficiency (%)	**66**	**66**	**66**	**69**	**69**	**62**	**100**

* Information not available; - = Not applicable

Figures in parentheses indicate efficiency (%) based on incomplete data

Efficiency for MCs ranges from 46 % to 75 % (Table 2). The detection of positive smear was nil in all the three MCs at all the months except for first and second month in Thiruvalangadu PHC and first month in Kanagamma Chatram PHC. Recording and reporting needs improvement. Referrals for sputum examination is also poor in 2 out of 3 MCs.

Efficiency for RCs ranges from 50% to 75% (Table 3). All the components need to be improved, especially recording and reporting. Case referral also is not satisfactory except for two months in Ulundai PHC and for one month in Kadambathur PHC.

Table 3

Scores for the evaluation of DTP implementation by RCs

PHI Category (RC)	Components assessed	Score for month						Maximum score
		1	2	3	4	5	6	
Kadambathur PHC	Referred for sputum exam.	1	0	0	0	2	3	5
	Regularity score	9	8	8	8	7	8	10
	Records and reporting	5	5	5	5	6	6	9
	Total score	15	13	13	13	15	17	24
	Efficiency (%)	**62**	**54**	**54**	**54**	**62**	**71**	**100**
Ulundai PHC	Referred for sputum exam.	*	*	0	0	3	5	5
	Regularity score	4	*	6	6	4	6	10
	Records and reporting	6	*	6	6	6	7	9
	Total score	10	-	12	12	13	18	24
	Efficiency (%)	**(53)**	**-**	**50**	**50**	**54**	**75**	**100**

***** Information not available: - = Not appliable
Figures in parenthesis indicate efficiency (%) based on incomplete data

The availability of the Anti-TB drugs is necessary for efficient case holding. But as the availability at the PHI is dependant upon availability at

the DTC, this component is not included in the scoring system. The table 4 presents the available anti-TB drugs in all the 8 PHIs during these six months.

Table 4

Availability of Anti-TB drugs

Name of the PHI	Drugs available at the following (months)					
	1	2	3	4	5	6
Perambakkam	A	A	A	A	A	A
Tiruvallur	A	A	A	A	A	A
Sriperumbudur	A	A	A	A	A	A
Kanakammachatram	A	A	A	A	A	A
Thiruvalankadu	A	A	SHREZ	SHREZ	HREZ	SHR
Poonimangadu	*	*	A	A	A	A
Kadambathur	A	A	A	A	*	*
Ulundai	A	A	A	HREZ	HREZT	HRZT

A - All drugs available; * - Information not available; S - Streptomycin; H - Isoniazid; R - Rifampicin; E - Ethambutol; Z - Pyrazinamide; T - Thioacetazone

All the anti-TB drugs were available in 6 out of the 8 PHIs at all the assessments. In the Thiruvalangadu PHC, Thioacetazone was not available for 4 months; Streptomycin, Ethambutol and Pyrazinamide were not available for one month each. In the Ulundai PHC, streptomycin was not available for 3 months; Thioacetazone and Ethambutol were not available for one month each. Rifampicin and isoniazid were available in all the PHIs in all the months.

In summary, it is feasible to assess two PHCs or one GH (XC) on a single day. The scores have been assigned empirically to make the assessment simple, quick and acceptable to the DTC monitoring team. Important aspects of the DTP implementation are covered during each visit. It is possible for the DTO to identify and concentrate on the weaker components in order to improve them. This scoring system could be an efficient monitoring tool if it is found to work in the hands of the staff of the district tuberculosis centre.

*****

EPIDEMIOLOGICAL STUDIES - IN PROGRESS

Development of surveillance methodology for tuberculosis

(Ongoing study, 1990-94)

This study was started in response to the need to develop a simple tool for epidemiological surveillance for tuberculosis in a community. Although, it is widely accepted that the annual risk of infection is the epidemiological index to be used for the surveillance of tuberculosis in a community, the usefulness of this index in areas with a high level of non-specific sensitivity to tuberculin testing has not yet been studied. This long-term community-based epidemiological study has been undertaken in the BCG Trial area (where the non-specific sensitivity is high) to study the annual risk of infection in a community. In addition, the following will be studied.

1. Age-specific prevalence of infection and its trend.

2. Age-sex-specific rates of disease (prevalence and incidence) and their trend.

3. The proportion of chronic excretors among prevalence cases and their drug sensitivity at each round.

Table 1

Coverage for different examinations during follow-up

Screening method *	6 months		12 months		18 months		24 months	
	Eligible	Covered (%)	Eligible	Covered (%)	Eligible	Covered (%)	Eligible	Covered (%)
Sympto-matic	9097	**89**	9821	**89**	6337	**91**	5457	**92**
X - ray	9097	**88**	9821	**89**	6337	**90**	5457	**91**
Sputum	4845	**93**	5118	**92**	3258	**89**	2758	**89**

* For the follow-up groups completed up to December 1993

The methodology of the study has been described in detail in the annual reports for 1990 and 1991. Six-monthly selective follow-ups were carried out in all the 30 panchayats. The coverages for the selective follow-up rounds are given in table 1.

The consistently high proportions of coverage is encouraging, since this means that a high proportion of the cohort will be preserved.

In all, 211 sputum positive cases were identified from follow-up examinations up to September 1993. The distribution of positive cases by smear and culture according to month of follow-up (round) of examination is given in table-2. It can be seen that 133 (63%) of the 21 1 cases were positive only on culture and 31(15%) were smear positive but culture negative.

Table 2

Distribution of sputum positive cases by smear and culture

Selective Follow-up * (Round)	No. of Villages covered	Smear + Culture +	Smear - Culture +	Smear + Culture -	All
6 months	30	31	61	14	106
12 months	17	6	34	9	49
18 months	15	8	32	8	48
24 months	6	2	6	0	8
Total		47	133	31	211

* For the follow-up groups completed up to December 1993

The drug sensitivity results among culture positives, according to the history of previous anti-tuberculosis treatment is given in Table-3.

It can be seen that among 177 culture positive cases,12 (6.8%) were resistant to streptomycin,20 (11.3%) and 5 (2.8%) were resistant to isoniazid and rifampicin respectively. Nine patients (5.1 %) were resistant to two or more drugs (SH/RH/SHR).

Management of Cases: Sputum positive cases were referred for treatment (with SCC) under the DTP. They were also followed up every three months in their homes, at which time sputum was collected, and

information regarding the symptomatic status and drug regularity elicited.

Table 3

Drug sensitivity according to the history of previous anti-tuberculosis treatment

History of previous Rx *	No. of culture positives	Sensi-tive to SHR	Resistant to					
			S	H	R	SH	RH	SHR
No	154	133	6	8	1	4	2	0
Yes	23	17	0	3	0	1	1	1
Total	177 ** (100)	150 (84.7)	6 (3.4)	11 (6.2)	1 (0.6)	5 (2.8)	3 (1.7)	1 (0.6)

Figures in parentheses indicate percentages

* As elicited by the health worker by questioning.

** Of the 180 culture positives drug sensitivity result was not available for 3

Abacillary cases, (as defined by an X-ray read as a tuberculous abnormality by two readers and sputum negative on smear and culture) were called to the passive case finding centre(PCFC) at Thiruvallur after collecting one sputum at their residence. When they reported at the PCFC, one more sputum specimen was collected. The Medical Officer examined these cases at PCFC and took a decision to refer them for treatment based on clinical grounds. However, if the sputum became positive, they were referred for anti-tuberculosis treatment with SCC. All treatment was provided by the programme(DTP).

In all, 421 such abacillary cases were referred to the PCFC. The sputum collected (two months after intake) at PCFC yielded positive cultures in 7% (11 out of 155) of the patients.

In Kadambathur Panchayat Union, the population was followed up to 24 months and the first resurvey was started in December. In the other Panchayat Union viz., Thiruvalangadu, the follow-up is still in progress.

*****

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

(Ongoing study, 1989-99)

A longitudinal cohort study has been ongoing from July 1989 with the objective of monitoring individuals with Human Immuno-deficiency Virus (HIV) infection to detect the occurrence of tuberculosis.

Methodology: Addresses of individuals identified to be positive for HIV infection on routine ELISA testing are obtained from the various surveillance centres in the state of Tamilnadu. These individuals are contacted in their houses and asked to report to the Centre (TRC), where they are registered along with their contacts for the study. On registration and at 6-monthly intervals, they are examined clinically by a medical officer. Weight is recorded and a radiograph of the chest is taken. At least two samples of sputum are collected from those individuals having abnormal radiographic shadows or with symptoms referable to tuberculosis. Those found to be positive on smear or culture and who could attend the Centre are started on a nine-month daily regimen with 2 months of ethambutol, isoniazid, rifampicin and pyrazinamide followed by 7 months of isoniazid and rifampicin. Patients who are unable to come to the Centre for treatment are treated with a routine short course regimen under the program. Tuberculin testing (1-TU RT23) is being done at intake and at yearly intervals. Finger prints are taken at the time of registration and at every follow-up and compared to ensure correct identification. The family members (including the spouse) and other sexual partners are also being registered and followed up at 6-monthly intervals to study the pattern of transmission of HIV infection.

A centre has been established in Pondicherry from June 1990 in the STD Clinic of the Government Hospital and the same procedures are being carried out. Up to December 1993, the addresses of 550 patients had been received from the different centres of which 281 (51%) were traced and registered (Table 1). All these individuals had a repeat ELISA carried out at the time of registration.

Among 281 traced and registered, ELISA test done at TRC was negative for 59 (21%) patients. The study cohort contains the remaining 222 patients proved to be HIV positive either by Western blot or by a repeat ELISA or with an equivocal result on Western blot on two occasions. Eighty-eight of these cases came from Vigilance Home, 58 from STD,

Table 1

Source of individuals registered for the study

Source	Refe-rred	Not traced	Died before regn.	Traced & Regd.	At TRC				
					ELISA nega-tive	ELISA positive			
						WB+	WBE Q	Twice	Total
STD Clinic	341	251	7	83	25	57	0	1	58
Vigilance Home	101	1	0	100	12	83	1	4	88
TB patients screening	72	0	0	72	16	33	6	17	56
Others	36	9	1	26	6	9	2	9	20
Total	550	261	8	281	59	182	9	31	222

WB = Western Blot; EQ = Equivocal result

Clinic, 56 from TB Screening and 20 from other sources. The age and sex distribution of these patients is given in table 2. Seventy-three percent of the patients were less than 30 years of age.

Table 2

Age and Sex distribution

Age group (years)	No. HIV infected	TB patients	
		No.	%
≤ 20	37	5	**14**
21 - 30	124	31	**25**
31 - 40	38	27	**71**
41 - 50	15	12	**80**
> 50	8	5	**63**
Total	222	80	**36**

At intake, 113 of 222 (51%) patients were reactors (reaction size ⩾ 12 mm) to 1-TU RT23, 127 had an x-ray abnormality of which 71 were interpreted as tuberculosis Shadows.

The coverage and follow-up status is given in table 3.

Table 3
Coverage and census status during follow-up

Month of Follow-up	No. eligible	Examined		Refu-sed	Died	Not traceable	
		No.	%			No.	%
06	197	150	**76**	3	14	30	**15**
12	175	123	**70**	2	3	47	**27**
18	155	89	**57**	1	4	61	**39**
24	136	72	**53**	1	2	61	**45**
30	97	50	**52**	0	4	43	**44**
36	63	26	**41**	0	1	36	**57**

Coverages at 6 and 12 months have been 76% and 70% respectively, which is not as high as for other studies. This is probably due to the highly unstable nature of this position. At 24 and 36 months, we had only 53% and 41% respectively, there being a sharp increase in the proportion of those not traceable. Significantly the "refusals" are very few. Twenty eight individuals had died over 36 months.

Occurrence of Tuberculosis among HIV infected: Out of the 222 patients in the study, 71 (32%) had tuberculosis at the time of registration. Of the remaining 151 patients who were known to be free of tuberculosis, 9 (6%) had developed tuberculosis during 30 months of follow-up (Table 4). Thus, we had a total of 80 patients with HIV and TB registered in this study.

Among the 80 patients, 60 had produced positive smears or cultures on sputum examination. This includes 13 patients for whom treatment had been started based on x-ray, whose cultures have subsequently become positive. Four of these had multi drug resistant TB. Twenty patients were treated on the basis of a radiological diagnosis. Forty-eight of the 80

Table 4

Occurrence of tuberculosis during follow-up according to HIV status from sources other than TB screening

HIV status	Total Regd*.	Month of follow-up						
		6	12	18	24	30	36	Total
WB Positive	135	1	2	4	1	1	0	9
WB Equivocal	3	0	0	0	0	0	0	0
ELISA Twice Positive	13	0	0	0	0	0	0	0
Total	151	1	2	4	1	1	0	9

* Excluding those who had TB at intake

patients who could attend the Centre, were offered the 9 month daily regimen containing ethambutol, isoniazid, rifampicin and pyrazinamide for 2 months followed by rifampicin and isoniazid daily for the next 7 months. Those 32 patients who could not attend the Centre for regular treatment had the routine 8-month short course regimen at the nearest Peripheral Health Institution. Thirty-seven patients had completed treatment and 26 of these had become sputum negative. Sixteen of 80 patients had died. The others are being followed up.

Mortality: Twenty-eight (12.6%) patients out of 222 HIV infected individuals had died during follow-up (Table 5). Mortality among HIV-TB was 20% compared to 8% among HIV patients without TB. Among those with HIV and TB, the mortality was 143 per thousand person years of follow-up compared to 38 per thousand person years of follow-up among those with HIV but without TB.

The cause of death was ascertained by scrutiny of records where possible, and by a qualitative verbal autopsy in the others. Of the 28 individuals who had died,10 had signs of Tuberculosis and AIDS,3 had only Tuberculosis,7 had signs of AIDS without Tuberculosis, and 4 died of unrelated causes and in 4 cause could not be identified.

Table 5

Mortality in HIV infected

Referred from	Patients	Total	No. dead	Rate per 1000 (person years)
Madras	TB	42	7	**108**
	Non-TB	85	8	**43**
Pondy	TB	9	3	**167**
	Non-TB	57	4	**30**
Vellore	TB	29	6	**207**
	Non-TB	-	-	**-**
Total	TB	80	16	**143**
	Non-TB	142	12	**38**
Total		222	28	-

Intra-familial transmission: Of the 222 individuals, 63 were married and had families. All 63 spouses and the 79 child contacts were registered and followed up (Table 6). Among the spouses, 22 had HIV at the time of registration. Of the 41 spouses who were free of HIV infection at registration, five had developed HIV infection over 18 months. Of the 27 HIV infected spouses, 7 had TB compared to 4 of 36 non HIV infected spouses. Of the 79 children, 2 had HIV at intake and 1 developed HIV during follow-up. One adult sibling of a HIV/MDR-TB patient was also found to have HIV infection and TB at intake.

Table 6

Intra familial transmission

Contact status	Number Registered	Found positive at				Annual attack rate*(%)
		Intake	6m	12m	18m	
Spouse .	63	22	4	0	1	**8.1**
Children	79	2	1	0	0	**0.9**

$$*\frac{\text{(Total Cases found positive at 6m, 12m or 18m.)}}{\text{(No. Regd. - Cases at intake)}} \times 100 \quad \text{(corrected for 1- year)}$$

Surveillance of chest symptomatics and tuberculosis patients for Human Immuno-deficiency Virus infection

(Ongoing study, 1988)

This surveillance aims to determine the proportion with HIV infection among tuberculosis patients attending the District TB Centre (DTC) and Government TB Sanatorium (GTBS), Vellore and its trend over time. All tuberculosis patients who report to these centres are screened irrespective of their bacteriological status. Extra-pulmonary TB cases are also included. Three ml of intravenous blood obtained from each patient is tested by ELISA for HIV antibody. Specimens found to be positive by ELISA were initially tested by Western Blot (WB) for confirmation. Western Blot was discontinued during September 1992, in accordance with the National Policy. Since then a repeat ELISA on a second specimen of blood is used to confirm the result. ELISA tests are done at TRC and Westem Blot tests are done at CMC, Vellore.

Screening of TB patients for HIV at DTC and GTBS, Vellore: There were a total of 3037 patients screened, of whom 33 were positive by both ELISA amd Western Blot or positive by ELISA on two different specimens. Another 7 were equivocal by Western Blot. In 74 patients a second specimen could not be obtained as the decision to discontinue WB was taken late and the mechanism for recalling the patients for a second specimen was not yet implemented. These 74 cases have not been included in the subsequent analysis. The number screened and the proportion of HIV positives are shown in table 1.

Table 1
HIV infection in TB patients

Centre	Number screened	HIV positive		
		ELISA + WB +	ELISA + WB EQ	Repeat ELISA +
DTC, Vellore	204	2 (1.0)	2 (1.0)	0 (-)
GTBS, Vellore	2763	24 (0.9)	5 (0.2)	7 (0.3)
Total	2963	26 (0.9)	7 (0.2)	7 (0.2)

Figures in parentheses indicates percentages; WB = Western Blot; EQ = Equivocal

Table 2

HIV infection in TB patients by case category

Case Category	Number screened	HIV positive	
		E+ WB+/ Rpt. E+	E+ WB EQ
New smear positive	1000	16	1
New X-ray positive, sputum negative	1117	12	3
Persistent sputum positive	161	0	2
Extra-pulmonary tuberculosis	20	0	0
Others	665	5	1
Total	2963	33	7

The distribution of the patients screened up to December 1993 is presented, according to case category, in table 2. The age, sex distribution for these patients is given in table 3. The highest proportion of positive (2.3.%) was among males aged 20-29 years. Most of the positives were clustered in the 20-49 years age group. Of 40 positives detected, 28 were from those with pulmonary tuberculosis.

Although the number of HIV infected individuals detected in this study is small, it appears that males between 20 and 50 years with tuberculosis would be a sentinel group for screening for HIV infection.

It is too early to observe a significant trend. The study methodology is now being revised to include more centres, such that each centre will have screening only during specified time points.

Table 3

HIV Infection in TB Patients by age and sex

Age group (years)	Sex	Number screened	HIV positive		
			E+ WB+/ repeat ELISA +	%	E+ WB EQ
<10	M	3	0	-	0
	F	8	0	-	0
	B	11	0	-	0
10-19	M	103	1	**1.0**	2
	F	75	0	-	0
	B	177	1	**0.6**	2
20-29	M	352	8	**2.3**	1
	F	224	2	**0.9**	0
	B	576	10	**1.7**	1
30-39	M	423	7	**1.7**	1
	F	195	1	**0.5**	0
	B	618	8	**1.3**	1
40-49	M	517	7	**1.4**	1
	F	154	2	**1.3**	0
	B	671	9	**1.3**	1
50-59	M	469	2	**0.4**	1
	F	86	1	**1.2**	0
	B	555	3	**0.5**	1
≥60	M	309	2	**0.6**	1
	F	46	0	-	0
	B	355	2	**0.6**	1
Total	M	2176	27	**1.2**	7
	F	787	6	**0.8**	0
	B	2963	33	**1.1**	7

Screening of chest symptomatics and TB patients for HIV at the Centre (TRC): Since occurrence of TB should be viewed with a high index of suspicion for the development of HIV, chest symptomatics referred to the Centre and patients with pulmonary and extra-pulmonary forms of tuberculosis registered at the Centre (Madras and Madurai) and on treatment or follow-up, were screened to identify their HIV status. However, screening is limited to the estimation of point prevalence at yearly intervals as per the recommendations of National Aids Control Organisation (NACO).

A total of 6114 patients were screened. Patients were classified based on the diagnosis made after the initial assessment. Thus a patient diagnosed as a bacillary case initially continues to be classified as bacillary even during follow-up phase for the purpose of this analysis.

A patient was considered as HIV positive if either 2 ELISA tests on two different samples of blood or 2 ELISA tests on a single sample were positive. Based on this definition, 239 (6.0%) of the 3954 TB cases, 40 (4.9%) of 823 Extra-pulmonary TB cases and 13 (3.1%) of 420 non-tuberculosis cases were positive for HIV. Among 825 classified as normal based on X-ray and sputum examination 38 (4.6%) were found to be HIV positive. Thus of the total 6022 chest symptomatics tested, 330 (5.5%) were detected to be HIV positive.

*****

ELECTRONIC DATA PROCESSING

During the year, further progress was made in developing computer programs for (a) Surveillance Study in Tuberculosis and (b) cohort analysis of Leprosy data. Routine database maintenance for pay-roll is being continued. Data entry for 2,03,594 records was carried out and 2,06,922 records were subjected to independent data verification on the computer during the year.

Development of Surveillance methodology for Tuberculosis: For this study, the data for 24 months follow-up round of examination was integrated with the main database file. New computer programs were developed for tabulation of various types of coverages.

Alphabetical and Household order listings were provided for each village along with the pre-printed forms that were supplied for 6-month follow-up examination.

Cohort analysis of Leprosy Data: Tabulation was done on cohort population after scrutiny of the database by computer programs. Age and sex distributions were obtained separately for BCG Vaccine groups (by strain and strength), by smear results and by size of reaction to PPD-S and PPD-9.

Other studies: For Childhood Tuberculosis study, print outputs on pre-printed screening forms and print output of clinical forms were provided. Pre-printed cards are supplied periodically for Socio-Economic Research (SER) study on Filariasis.

*****

LIBRARY & INFORMATION SERVICES

The year witnessed a change in the Library & Documentation services, namely, a shift from the conventional document oriented service to the Information Oriented service system.

'Tuberculosis Alert', a computerised Selective Dissemination of Information (SDI), is being brought out fortnightly. The bibliographic database "TUBERCULOSIS" developed for the purpose, now consists of 2000 records. The Resource Sharing programme with the Vector Control Research Centre (VCRC), Pondicherry has been continued. "Current Contents" on disks and forty-two journals are being shared.

Library Computerisation: During the year an IBM PC-AT/386 computer (8Mb RAM, 330Mb Hard Disk and Colour Monitor) with Multi-Tech Modem, a CD-ROM drive and a 300 cps dot-matrix printer, have been installed in the Library. Computerisation of the library systems and services and the holdings has also begun. The book collection of the Centre has been classified according to Dewey Classification Scheme (DDC) and computerised. The data bases such as LIBCAT (Library Book Catalogue), TUBERCULOSIS, TRCPUB (Publications by the Centre), Serials Control, (subscriptions to periodicals), and Mail List (for routine mailing), are updated periodically.

The CD-ROM monthly updates of the Medline Standards file beginning from 1991 have been provided in the Centre for search and retrieval of medical literature, using the computer.

Considering the impact and scope of Computer Communication Networks, the Centre is connected to SIRNET, the Computer Network of CSIR and the Government of India. The system facilitates Electronic Mail (E-Mail) services, for faster communication between scientists nationally and internationally, access to select databases, news groups (Bionet, Surfnet) and bulletin board services on various international networks.

During the year, a total of 210 (103 Indian and 107 foreign) issues of periodicals and 156 new books were added to the library collection.

*****

APPENDICES

TRAINING PROGRAMMES

WHO Fellows

Dr. (Mrs.) Vikarunnessa and Dr.Md. Sirajul Islam, Bangladesh, from 12.4.93 to 16.4.93.

Trainees

The following underwent training in different departments as follows:

Bacteriology

Thirteen M.D. (Microbiology) and M.Sc. (Microbiology) students from Dr. A.L.Mudaliar Post-graduate Institute of Basic Medical Sciences, Taramani, Madras, from 1.2.93 to 5.2.93.

Dr. S. Parvathy, Senior Tutor in Microbiology, P.S.G. Institute of Medical Sciences and Research, Coimbatore, from 2.3.93 to 11.3.93.

Dr. Ramanamurthy and Dr. Javed Nain, Institute of Microbial Technology, Chandigarh, from 26.4.93 to 30.4.93.

Eighteen students of Diploma Course in Medical Laboratory Technology, Voluntary Health Services, Adyar, Madras, from 3.5.93 to 15.5.93.

Ms. S.S. Geetha and Ms. M.G. Manimegalai, Research Assistants, Perundurai Medical College and Research Centre, Perundurai, for 1 week from 23.8.93.

Lt. Col. J. Jena, Microbiologist, Military Hospital (CTC), Pune, from 9.9.93 to 17.9.93.

Cardio-Pulmonary Medicine

Mr.Ravi Kumar and Mr. Balamurugan, Technologists, Apollo Hospitals, Madras, from 8.2.93 to 13.2.93.

Dr.D.J. Christopher, Lecturer, Christian Medical College, Vellore, from 2.4.93 to 30.4.93.

Dr.K.P. Sooraj, Dr.S.Venkatasubramanian and Dr.C.M. Shyam, Calicut Medical College, Calicut, from 16.4.93 to 26.4.93

Dr.Sanjoy Gupta, Deputy Consultant, Calcutta Medical Research Institute, Calcutta, from 1.7.93 to 10.7.93.

Dr.K.Shanmugam, Physician, Sir Ivan Stedford Hospital, Ambattur, Madras, from 8.11.93 to 20.11.93.

Immunology

Dr. Michael, P.S.G. College of Arts & Sciences, Coimbatore, for 3 weeks from 10.5.93.

Epidemiology

Mrs. Prabha Datta, Public Health Nurse, R.M.R.C., Andamans, from 13.9.93 to 25.9.93.

Statistics

Mr. Alok Ranjan, Statistical Assistant, Rajendra Memorial Research Institute of Medical Sciences, Patna, from 2.8.93 to 12.8.93.

General

Dr. Gopi Krishna and Dr. S. Vinod Kumar, M.D. (TB & CD) students, Andhra Medical .College, Visakhapatnam, for 14 days from 9.8.93.

Three D.T.R.D. students, Institute of Thoracic Medicine, Madras, from 23.8.93 to 30.8.93.

Dr. Dipankan Chakraborti, Institute of Thoracic Medicine, Madras, from 2.11.93 to 15.11.93.

Dr. R. Porkodi, Dr. R. Panchapakesan and Dr. Rajendran, Department of Rheumatology, Madras Medical College, Madras, from 1.12.93 to 31.12.93.

Compulsory Rotating Resident Internee posting students, Ramachandra Medical College, Porur, Madras, for 2-3 weeks during 1993 - 6 batches.

Others

One- or two-day training programmes were arranged at the Centre for batches of medical students, post-graduates, nursing students and para-medical personnel, as given below:

Medical students

Kilpauk Medical College, Madras - 1 batch.

Post-graduate students

Three M.D. (Community Medicine) students from Madras Medical College, Madras.

Eleven M.Sc. (Microbiology) students from Gulbarga University, Gulbarga.

Nursing and para-medical students

B.Sc. (Nursing) students from Christian Medical College, Vellore - 2 batches.

B.Sc. (Nursing) students from Fr. Muller's College of Nursing, Mangalore - 1 batch.

B.Sc. (Nursing) students from Madras Medical College, Madras - 3 batches.

B.Sc. (Zoology) students from Loyola College, Madras - 1 batch.

General Nursing Students, Apolla Hospitals, Madras - 3 batches.

Students of S.M.L.T. Durgabai Deshmukh Polyclinic, Andhra Mahila Sabha, Madras - 1 batch.

Students of Diploma Course in Medical Laboratory Technology, Loyola College, Madras - 1 batch.

STAFF DEVELOPMENT PROGRAMMES

1. Dr.P.Selvaraj underwent training in "Heteroduplex analysis of DQ alpha and DQ beta HLA genotyping" at the Centre, by Dr.Peter Zimmerman of the National Institutes of Health, Bethesda, Maryland, USA, in February, 1993.

2. Mr. A.S. Kripasankar underwent training in "Biostatistics and hospital statistics, Epidemiological methods and analysis" at the Christian Medical College, Vellore, from 14.6.93 to 2.7.93.

3. Mr. V. Chandrasekaran underwent training in "Applied multivariate techniques and PC based statistical software in health care" at the Christian Medical College, Vellore, from 14.6.93 to 2.7.93.

4. Mr. S.I. Eusuff underwent training in "PC based statistical software in health care" at the Christian Medical College, Vellore, from 14.6.93 to 2.7.93.

5. Mr. J. Devan underwent training in "Epidemiological methods and analysis" at the Christian Medical College, Vellore, from 14.6.93 to 2.7.93.

6. Dr. M.S. Jawahar was awarded 1-year WHO Fellowship for training in Epidemiology at the London School of Hygiene and Tropical Medicine, London, from September, 1993.

7. Mrs. P.S.Jayavani attended a workshop on "Preventive Education and Counselling for HIV and AIDS in Physio-Social aspects of AIDS" at the Resource and Reference Centre, Christian Medical College, Vellore, from 26.10.93 to 30.10.93.

8. Mr. K. Sampathkumar, Mr. G.K. Loganathan and Miss R.Geetha underwent part-time computer training in "Word Perfect" at Micro Computer Centre, Nungambakkam, Madras, from 15.12.93 to 21.12.93.

9. Mr. M.G. Sreekumar was awarded M.L.I.S. degree by the University of Madras, Madras, during 1993.

PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

Name of conference, venue and date	Title of paper	Name of staff member
19th Annual Conference of Indian Immunology Society, Bangalore, 8-10 January, 1993	1) Protective response in guinea-pigs exposed to **M.avium intracellulare/M.scrofulaceum,** BCG and South Indian isolates of **M.tuberculosis**	Dr.Daniel Herbert
	2) IgG antibody levels to various mycobacterial antigens in tuberculin negative and positive children from the South Indian BCG trial area and in pre- and post- BCG/tuberculin conversion in young individuals from the South Indian trial area and Britain	
48th Annual Conference of Association of Physicians of India, New Delhi, 20-24 January, 1993	Drug-induced respiratory diseases (update on drugs and organs injury)	Dr.V.K.Vijayan.
XVII World Congress on Diseases of the. Chest, Amsterdam, 13-18 June, 1993	Tropical eosinophilia: Relationship between lower respiratory tract inflammation and changes in lung function following treatment at one year	Dr.V.K.Vijayan

Name of conference, venue and date	Title of paper	Name of staff member
20th Annual Andhra Pradesh State Conference on TB and Chest Diseases, Kurnool, 10-11 July, 1993	Newer antimycobacterial drugs (Dr.P.V.Benjamin Oration)	Dr.C.N.Paramasivan
VIII South Zone Conference of Indian Academy of Paediatrics, Symposium on Childhood TB, Madras, 13-15 August, 1993	Tuberculous meningitis in children (Guest lecture)	Dr. Padma Ramachandran
- do -	Indirect tests in the diagnosis of TBM in children	Dr. N.Selvakumar
- do -	-	Dr. Soumya Swaminathan
XIV Annual Conference of Indian Association of Biomedical Scientists, Madras, 11-12 September, 1993	Mycobacteriuria in pulmonary tuberculosis	Dr.N.Selvakumar
- do -	Effect of DEC on expiratory flow rates in tropical eosinophilia	Dr.K.V.Kuppu Rao

Name of conference, venue and date	Title of paper	Name of staff member
XI Annual Conference of the Indian Society for Medical Statistics and National Seminar on Growing Priority for Epidemiological Approaches in Environmental Studies, Visakhapatnam, 18-20 October, 1993	1) Modelling the hazards in environmental health 2) Ranking of prognostic factors in Cox's regression 3) An application of Aicken's measure of agreement	Dr.P.Venkatesan
2nd Conference of Tamil Nadu Science Congress, Madras, 6-8 November, 1993	A fast procedure for model selection	Dr.P.Venkatesan
5th National Conference of the Respiratory Chapter of the Indian Academy of Paediatrics, Coimbatore, 27-28 November, 1993	Control of respiration (Guest lecture)	Dr. Soumya Swaminathan
48th National Conference on TB and Chest Diseases, Bhopal, 9-13 December, 1993	Follow-up of patients discharged against medical advice in TB meningitis studies in children	Dr. Padma Ramachandran
- do -	Feasibility study of involving tribal literate youths for case finding in a tribal area in Tamil Nadu	Dr. Rani Balasubramanian

Name of conference, venue and date	Title of paper	Name of staff member
48th National Conference on TB and Chest Diseases, Bhopal, 9-13 December, 1993	1) Mycobacteriuria in pulmonary tuberculosis patients in Madras, South India 2) Isolation of **M.tuberculosis** from CSF by centrifugation and filtration methods a pilot study	Dr.N.Selvakumar
42nd Annual Conference of Neurological Society of India,Madras, 16-19 December, 1993	Adverse reactions to short course chemotherapy in tuberculosis with special reference to neurotuberculosis	Dr.Rajeswari Ramachandran
45th Annual Conference of the Cardiological Society of India, Madras,20-22 December, 1993	Relationship between aerobic threshold measured non-invasively and invasively in athletes	Dr.V.K.Vijayan
39th Annual National Conference of the Association of Physiologists and Pharmacologists of India, Thiruvananthapuram, 27-29 December, 1993	Ventilatory response to exercise in normal South Indian children	Dr.K.V.Kuppu Rao

PARTICIPATION BY THE CENTRE'S SCIENTISTS IN SYMPOSIA, WORKSHOPS, MEETINGS AND TRAINING COURSES HELD AT OTHER INSTITUTIONS

Name of the event, venue and date	Title of paper	Name of staff member
Seminar on Respiratory Physiology, Association of Physiologists and Pharmacologists of India (Vellore Branch), Christian Medical College, Vellore, 9 January, 1993	Flow-volume loops - their usefulness in clinical medicine	Dr.V.K.Vijayan
Workshop on Recent Trends in Teaching Physiology and Pharmacology, Tamil Nadu Dr.MGR Medical University and Association of Physiologists and Pharmacologists in India (Vellore Branch), Christian Medical College, Vellore, 9 January, 1993	-	Dr.V.K.Vijayan
National Seminar on the Management of Infection after Implant Surgery, Madras Institute of Orthopaedics and Traumatology, Madras, 17 January, 1993	Immunological approaches in the understanding of infection	Dr.Rajiswamy
Symposium on Asthma: Asthma and Bronchitis Association of India (Southern Chapter) and Association of Physicians of India (Madras Branch), Madras, 31 January, 1993	Inhalation therapy	Dr.V.K.Vijayan

Name of the event, venue and date	Title of paper	Name of staff member
Expert Committee Meeting of Pulmonary Medicine Centre (Bhopal), Madras, 23-26 February, 1993	-	Dr. V.K.Vijayan (Chairman)
CME Programme, Tamilnad Hospitals, Madras, 5 March, 1993	Transportation of sputum specimens for culture of tubercle bacilli	Dr.N. Selvakumar
Expert Committee Meeting of Pulmonary Medicine Centre (Bhopal), Bhopal, 15-16 March, 1993	-	Dr. V.K.Vijayan (Chairman)
Steering Committee Meetings of Filariasis, TDR/WHO, Geneva, 15-19 March and 14-17 September, 1993	-	Dr.V.Kumaraswami
Workshop for Librarians, British Deputy High Commission, British Council Division, Madras, 22-23 March, 1993		Mr. M.G. Sreekumar
32nd Annual Meeting of National Academy of Medical Sciences (India), New Delhi, 2-3 April, 1993		Dr.V.K.Vijayan (Awarded membership)
Seminar on Biomedical Databases on CD-ROM, Informatics India Ltd., NIMHANS, Bangalore, 15 April, 1993	-	Mr.M.G.Sreekumar

Name of the event, venue and date	Title of paper	Name of staff member
Third meeting of the World Public Health Forum, London, 18-21 April, 1993	Tuberculosis back to the future - Immunological aspects of BCG and epidemiological aspects of vaccine trials	Dr.Manjula Datta (Chair-person)
Indian Medical Association, Ambattur, Madras, 29 April, 1993	Recent advances in the management of bronchial asthma (Guest lecture)	Dr.V.K.Vijayan
Steering Committee Meeting, Therapy for Myco-bacterial Diseases, WHO, Geneva, 1-2 May, 1993	Shortening short course chemo-therapy - lessons from the past	Dr.T.Santha Devi
Indian Chest Society and SMS Medical College, Jaipur, 2 May, 1993	Basic pulmonary function tests in clinical practice - update in pulmonary medicine (Guest lecture)	Dr.V.K.Vijayan
Administrative Meeting of the International Academy of Chest Physicians and Surgeons of the American College of Chest Physicians, Amsterdam, 13 June, 1993	-	Dr.V.K.Vijayan (Governor)
XVll World Congress on Diseases of Chest, Symposium on Tropical Lung Diseases, Amsterdam, 16 June, 1993	Broncho-alveolar lavage studies in, tropical eosinophilia	Dr.V.K.Vijayan (Faculty member)

Name of the event, venue and date	Title of paper	Name of staff member
CME Programme on Recent Advances in Respiratory Medicine, Medical College, Trichur, 27 June, 1993	Chronic obstructive pulmonary disease - Recent trends	Dr.V.K.Vijayan (Faculty member)
Summer Institute on Advances in Veterinary Clinical Medicine, Tamil Nadu Veterinary and Animal University, Madras, 6 July, 1993	Bronchoalveolar lavage (Guest lecture)	Dr.V.K.Vijayan
Inter Country Workshop on Tuberculosis, WHO/SEARO, New Delhi, 12-16 July, 1993	Management of tuberculosis at district level	Dr.V.K.Vijayan
National Tuberculosis Training Course, National Tuberculosis Institute, Bangalore, 26-30 July, 1993	Revised strategy for controlling tuberculosis	Dr.V.K.Vijayan (Facilitator)
Workshop on Research Methodology and Critical appraisal, Clinical Epidemiology Unit, Madras Medical College and ACCERT (ICMR), Coimbatore Medical College, Coimbatore, 31 July - 1 August, 1993	-	Dr. Manjula Datta (Resource person)
- do -	-	Mr.P.V.Krishnamurthy (Resource person)

Name of the event, venue and date	Title of paper	Name of staff member
ICMR Workshop on Immunopathology and Lymphatic Filariasis, Tuberculosis Research Centre and Vector Control Research Centre (Pondicherry), Madras, 7-11 August, 1993	The role of secondary infections in pathology of lymphatic filariasis	Dr.C.N.Paramasivan
- do -	Techniques for processing of specimens for Immunopathology	Dr.V.D.Ramanathan
Seminar on Library Networks in India, Documentation Research and Training Centre (Bangalore) and Indian National Scientific Documentation Centre (New Delhi), Bangalore, 12-13 August, 1993	-	Mr.M.G.Sreekumar
Symposium on Chronic Obstructive Airways Disorder, Asthma and Bronchitis Association of India (Southern India Chapter), Madras, 22 August, 1993	Airways hyper-reactivity-mechanisms and measurements	Dr.V.K.Vijayan
- do -	-	Dr.A.M.Reetha

Name of the event, venue and date	Title of paper	Name of staff member
Workshop on Clinical Epidemiology, Research Methodology and Critical appraisal, Clinical Epidemiology Unit, Madras Medical College and ACCERT (ICMR), Thanjavur Medical College, Thanjavur, 30-31 August, 1993	-	Dr. Manjula Datta (Resource person)
- do -		Mr.P.V.Krishnamurthy (Resource person)
CME Programme in General Medicine, Govt. Stanley Medical College, Madras,12 September, 1993	Current trends in the management of bronchial asthma (Guest lecture)	Dr.V.K.Vijayan
CME Programme, Association of Graduate Technicians, Apollo Hospitals, Madras, 19 September, 1993	Recent advances in histopathological techniques	Dr.V.D.Ramanathan
Symposium on Filariasis, Joint TDR/International Society of Lymphology, (ISL), co-sponsored with 14th International Congress of Lymphology, Washington D.C., USA, 20-30 September, 1993	Clinical aspects of lymphatic filariasis	Dr.V.Kumaraswami
Seminar on Resource Sharing among Medical Libraries, TamiInad Hospitals, Madras, 25 September, 1993	Concept and role of computer communication networks in library resource sharing	Mr.M.G.Sreekumar

Name **of the event, venue and date**	**Title of paper**	**Name of staff member**
Two-week Training Programme on Research Methodology and Critical Appraisal, for the faculty of Medical Colleges of Tamil Nadu, Clinical Epidemiology Unit, Madras Medical College and ACCERT (ICMR), Madras Medical College, Madras, 4-16 October, 1993	-	Dr. Manjula Datta (Resource person)
- do -	-	Mr.P.V. Krishnamurthy (Resource person)
Symposium on Deep Picture of Blood Gas Analysis - A Useful Clinical Concept, conducted by Radiometer International A/S (Denmark), Madras, 12 October, 1993	Blood gas analysis (Guest lecture)	Dr.V.K.Vijayan
- do -	-	Dr.A.M. Reetha
Joint ICMR/TDR/WHO 2nd Workshop on Social and Economic Impact of Lymphatic Filariasis, Tuberculosis Research Centre, Madras, 25-31 October, 1993		Dr. V. Kumaraswami
- do -	-	Dr. Manjula Datta
- do -	-	Mrs. M.P.Radhamani
- do -	-	Mr.R. Selvaraj
CME Programme, Devaki Hospital, Madras, 6 November, 1993	Diagnosis and management of tuberculosis	Dr.Rajiswamy

Name of the event, venue and date	Title of paper	Name of staff member
CME Programme, Devaki Hospital, Madras, 6 November, 1993	Chemotherapy of tuberculosis	Dr.Rani Balasubramanian
Symosium on Respiratory Infections, Apollo Hospitals, Madras, 25 November, 1993	-	Dr.V.K.Vijayan (Chairman)
University of Madras, Tamil Nadu TB Association Endowment Lecture, Madras Medical College, Madras, 2 December, 1993	Antigenic relatedness in Mycobacteria and its relevance to immune response	Dr.C.N.Paramasivan
International Symposium on HLA in Health and Disease: Current State of the Art, All India Institute of Medical Sciences, New Delhi, 6-8 December, 1993	Association of HLA with pulmonary tuberculosis	Dr.P.Selvaraj
Symposium on an update on the Management of Tuberculosis - American College of Chest Physicians, Madras,15 December, 1993	-	Dr.V.K.Vijayan (Chairman)
- do -		Dr.A.M.Reetha
CME Programme, Neurological Society of India, Madras, 16 December,1993	Short Course Chemotherapy in neurotuberculosis	Dr.Rajeswari Ramachandran
Workshop on Transoesophageal Echocardiography - biplane and multiplane, Cardiological Society of India, Madras, 18 December, 1993		Dr.V.K.Vijayan

LIST OF PUBLICATIONS

Papers published

1. Mohan, V., Kumaraswami, V. and Viswanathan, M. Immunology of diabetes. **Journal of Association Physicians of India,** 1992, **40,** 461-463.

2. Ramamurthy, B., Manjula Datta and Kanthimathi David. Anticonvulsant drugs and women with seizurs. **Journal of Obstetrics and Gynaecology India,** 1992, **42,** 438-447.

3. Swaminathan, S., Venkatesan, P. and Mukunthan, R. Peak expiratory flow rate in South Indian children. **Indian Paediatrics,** 1993, **30,** 207-211.

4. Paton, J.Y., Swaminathan, S., Sargent, C.W., Hawksworth, A. and Keens, T.G. Ventilatory response to exercise in children with congenital central hypoventilation syndrome. **American Review of Respiratory Diseases,** 1993, **147,** 1185-1191.

5. Cheng, S.H., Walker, K.B., Lowrie, D.B., Mitchison, D.A., Rajiswamy, Datta, M. and Prabhakar, R. Monocyte antimycobacterial activity before and after **M.bovis** BCG vaccination in Chengelput, India and London, U.K. **Infection and Immunity,** 1993, **61**, 4501-4503.

6. Vijayan, V.K., Sankaran, K., Venkatesan, P. and Kuppurao, K.V. Prediction equations for maximal voluntary ventilation in non-smoking normal subjects in Madras. **Indian Journal of Physiology and Pharmacology,** 1993, **37,** 138-140.

7. Vijayan, V.K., Kuppurao, K.V., Venkatesan, P. and Sankaran, K. Reference values and prediction equations for maximal expiratory flow rates in non-smoking normal subjects in Madras. **Indian Journal of Physiology and Pharmacology,** 1993, **37,** 291-297.

8. Vijayan, V.K. Drug-induced respiratory diseases. **In : Medicine Update, Ed: Dr.S.Chandrasekharan, Association of Physicians of India,** 1993, **8,** 177-186.

9. Vijayan, V.K. and Kuppurao, K.V, Early clinical, pulmonary function and blood gas studies in victims of Bhopal tragedy. **Biomedicine,** 1993, **13,** 36-42.

10. Vijayan, V.K. Tropical eosinophilia. Indian scene. **Indian Journal of Clinical Practice,** 1993, **3,** 39-43.

11. Vijayan, V.K. Current status of bronchoalveolar lavage. **Indian Journal of Clinical Practice,** 1993, **3,** 51-54.

12. Kuppurao, K.V., Vijayan, V.K., Venkatesan, P. and Sankaran, K. Effect of treatment on maximal expiratory flow rates in tropical eosinophilia. **Ceylon Medical Journal,** 1993, **38,** 78-80.

13. Vijayan, V.K. Chronic obstructive pulmonary diseases - Recent trends. **In : Recent advances in respiratory medicine. Publ. Dept. of Physiology, Chest Diseases and Pharmacology,** Medical College, Trichur, 1993, 20-39.

14. Vijayan, V.K. Tropical eosinophilia: Relationships between lower respiratory tract inflammation and changes in lung function following treatment at one year. **Chest,** 1993, **101** , 249s.

15. Padma Ramachandran. TB meningitis in children. **In: Medical Times, Ed: Sandoz publication,** 1993, **23,** 1-5.

16. Venkataraman, P., Paramasivan, C.N. and Prabhakar, R. **In vitro** activity of rifampicin, rifapentine and rifabutin against South Indian isolates of **M.tuberculosis. Indian Journal of Tuberculosis,** 1993, **40,** 17-20.

17. Venkataraman, P., Paramasivan, C.N. and Prabhakar, R. **In vitro** activity of Capreomycin and Ciprofloxacin against South Indian isolates of **M.tuberculosis. Indian Journal of Tuberculosis,** 1993, **40,** 21-25.

18. Paramasivan, C.N.; Chandrasekaran, .V. , Sudarsanam, N.M., Santha, T. and Prabhakar, R. Bacteriological investigations for short course chemotherapy under District Tuberculosis Programme in two districts in India. **Tubercle and Lung Diseases,** 1993, **74,** 23-27.

19. Paramasivan, C.N., Daniel Herbert and Prabhakar, R. Bactericidal action of pulsed exposure to rifampicin, ethambutol , isoniazid and pyrazinamide on **M.tuberculosis In vitro. Indian Journal of Medical Research,** 1993, **98,** 145-150.

20. Selvakumar, N., Vanaja Kumar, Narayana, A.S.L., Suryanarayanan, D., Umapathy, K.C., Paramasivan, C.N. and Prabhakar, R. Use of cetylpyridinium chloride for the storage of sputum samples and isolation of **M.tuberculosis. Indian Journal of Tuberculosis,** 1993, **40,** 95-97.

21. Venkatesan, P. Mathematical models in biomedicine. **Biomedicine,** 1993, **12,** 15-18.

22. Venkatesan, P. A fast procedure for model selection. **In: Mathematics, Statistics and Computer Sciences, Ed: K.N. Ponnusamy, University of Madras, Madras,** 1993, 121-124.

23. Venkatesan, P., Viswanathan, K. and Prabhakar, R. Co-variate analysis of tuberculosis data using Cox's regression model. **Biomedicine,** 1993, 12, 43-46.

24. Raghu, G., Sarma, G.R. and Venkatesan, P. Effect of iron on the growth and siderophore production of Mycobacteria. **Biochemistry and Molecular Biology International,** 1993, **31**, 341-348.

25. Raghu, G., Sarma, G.R. and Venkatesan, P. Effect of iron-binding proteins on the growth of mycobacteria and production of siderophores. **Medical Sciences Research,** 1993, **21**, 657-658.

26. Raghu, G., Sarma, G.R. and Venkatesan, P. Siderophore-mediated iron uptake in mycobacteria. **Medical Sciences Research,** 1993, **21**, 773-774.

27. Raghu, G., Sarma, G.R. and Venkatesan, P. Effect of haemoglobin on the growth of mycobacteria and production of siderophores. **Indian Journal of Pathology and Microbiology,** 1993, **36,** 376-382.

28. Shantanu Kumar Kar, Sumitra Patnaik, Jayanti Mania and Kumaraswami, V. Ivermectin in the the treatment of Bancroftian filariasis infection in Orissa, India. **South East Asian Journal of Tropical Medicine and Public Health,** 1993, **24,** 80-86.

29. Shantanu Kumar Kar, Sumitra Patnaik, Kumaraswami, V. and Murty, R.S.N. Side reactions following ivermectin therapy in high density Bancroftian microfilaraemics. **Acta Tropica,** 1993, **55,** 21-31.

30. Shenoy, R.K, Kumaraswami, V., Rajan, K., Thankom, S. and Jalajakumari. A comparative study of the efficacy and tolerability of single and split doses of ivermectin and diethylcarabmazine in periodic Brugian filariasis. **Annals of Tropical Medicine and Parasitology,** 1993, **87,** 459-467.

31. Ajit P. Limaye, Eric A. Ottesen, Kumaraswami, V., John S. Abrams, Jaya Regunathan, Vijayasekaran, V., Kunthala Jayaraman and Thomas B. Nutman. Kinetics of serum and cellular interleukin-5 in post-treatment eosinophilia of patients with lymphatic filariasis. **Journal of Infectious Diseases,** 1993, **167,** 1396-1400

32. Mahanty, S., King, C.L., Kumaraswami, V., Regunathan, J., Maya, A., Jayaraman, K., Abrams, J.S., Ottesen, E.A. and Nutman, T.B. IL-4 and IL-5 - Secreting lymphocyte populations are preferentially stimulated by parasite-derived antigens in human tissue invasive nematode infection. **Journal of Immunology,** 1993, **151,** 3704-3711.

33. Christopher L. King, Siddhartha Mahanty, Kumaraswami, V., John S. Abrams, Jaya Regunathan, Kunthala Jayaraman , Eric A. Ottesen, and Thomas B. Nutman. Cytokine control of parasite-specific energy in human lymphatic filariasis: preferential induction of a regulatory T helper type 2 lymphocyte subset. **Journal of Clinical Investigations,** 1993, **92,** 1667-1673.

34. Manjula Datta. You cannot exclude the explanation you have not considered. **The Lancet,** 1993, **342,** 345-347

35. Manjula Datta, Radhamani, M.P., Selvaraj, R., Paramasivan, C.N., Gopalan, B.N., Sudeendra, C.R. and Prabhakar, R. Critical assessment of smear-positive pulmonary tuberculosis patients after chemotherapay under District Tuberculosis Programme. **Tubercle and Lung Diseases,** 1993, **74,** 180-186.

36. Soumya Swaminathan and Manjula Datta. Primary complex. **Indian Academy of Paediatrics Journal of Practical Paedatrics,** 1993, **1,** 21-23.

37. Thilakavathy, S., Jemima Shiela Fredricks, Fredricks, K.G., Parthasarathy, R., Santha, T., Somasundaram, P.R., and Prabhakar, R. High coverage for long-term follow-up of patients with spinal tuberculosis. **Indian Journal of Tuberculosis,** 1993, **40,** 90-94.

38, Thomas, A., Joseph, P. and Prabhakar, R. 'Flu' syndrome associated with other systemic manifestations with once a month rifampicin in the treatment of multi-bacillary leprosy. **Indian Journal of Leprosy,** 1993, **65,** 219-224.

39. Paramasivan, C.N. HIV and tuberculosis - Editorial - **Indian Journal of Medical Microbiology,** 1993, 10(4), 176-192.

Papers accepted for publication

1. Rani Balasubramanian, Sivasubramanian, S., Parthasarathy, R., Santha, T., Somasundaram, P.R., Shanmugasundaram, T.K. and Prabhakar, R. Prevalence, incidence and resolution of abscesses and sinuses in patients with tuberculosis of spine: 5-year results of patients treated with Short Course Chemotherapy with or without surgery in Madras. **Indian Journal of Tuberculosis.**

2. Thomas, D.A., Swaminathan, S., Beardsmore, C.S., McArdle, K.A., Macfadyen, V.M., Goodenough, P.C., Carpenter, R. and Simpson, H. A comparison of peripheral chemotherapy for twin infants. **American Review of Respiratory Diseases.**

3. Geetharamani Shanmugam, Chandrasekaran, V., Santha, T., Somasundaram, P.R., Sudarsanam, N.M. and Sudha Ganapathy. Feasibility of utilising address card system for obtaining accurate address of patients under programme conditions. **Indian Journal of Tuberculosis.**

A21

4. Vijayan, V.K. Interstitial lung diseases : Mechanism of lung injury, granuloma formation and fibrosis, cryptogenic fibrosing alveolitis, hypersensitivity pneumonitis, Farmer's lung, histocytosis X, idiopathic pulmonary hemosiderosis and radiation-induced lung injury. **In : Respiratory Medicine in Tropics, Ed : Pandey, J.N., Oxford University Press, New Delhi.**

5. Vijayan, V.K. Tropical eosinophilia. Aetiology, Pathology and Pathogenesis. **In : Respiratory Medicine in Tropics. Ed: Pandey, J.N., Oxford University Press, New Delhi.**

6. Selvaraj, P., Venkataprasad, N., Vijayan, V.K. and Narayanan, P.R. Altered bactericidal activity against staphylococcus aureus of tuberculous bronchoalveolar lavage fluids. **European Respiratory Journal.**

7. Selvaraj, P., Venkataprasad, N., Vijayan, V.K., Prabhakar, R. and Narayanan, P.R. Procoagulant activity in bronchoalveolar lavage fluids from the site of tuberculous lesion. **European Respiratory Journal.**

8. Vijayan, V.K. Pulmonary embolism - Diagnosis and management. **In : Recent Advances in Clinical Medicine, Ed: Rabbani U, Aligarh Muslim University, Aligarh.**

9. Vijayan, V.K. Hypersensitivity pneumonitis. **In : Clinical Immunology, Ed: Pravash C. Sen Gupta, Calcutta.**

10. Alamelu Raja, Narayanan, P.R., Jawahar, M.S. and Prabhakar, R. Evaluation of mycobacterium tuberculosis antigen 6 by Enzyme Linked Immuno Sorbent Assay (ELISA). **Tubercle and Lung Diseases.**

11. Padma Ramachandran. Follow-up of patients discharged against medical advice in TB meningitis in children. In**dian Journal of Tuberculosis.**

12. Venkataraman, P., Paramasivan, C.N. and Prabhakar, R. **In vitro** activity of Oflaxacin and Ciprofloxacin against South Indian isolates of **M.tuberculosis. Indian Journal of Tuberculosis.**

13. Kamala, T., Paramasivan, C.N., Daniel Herbert, Venkatesan, P. and Prabhakar, R. Evaluation of procedures for isolation of non-tuberculous mycobacteria from soil and water. **Applied and Environmental Microbiology.**

14. Kamala, T., Paramasivan, C.N., Daniel Herbert, Venkatesan, P. and Prabhakar, R. Isolation and identification of environmental mycobacteria in the **Mycobacterium bovis** samples in BCG trial area of South India. **Applied and Environmental Microbiology.**

15. Paramasivan, C.N., Daniel Herbert, Umapathy, K.C., Fathima Rahman, Krishnamurthy, P.V. and Prabhakar, R. Early bactericidal action of pulsed exposure to 4 drugs together thrice weekly (REHZ 3) and 2 double-drug combinations on alternate days (RE3HZ3 - alt) in pulmonary tuberculosis. **Indian Journal of Medical Research.**

16. Daniel Herbert, Paramasivan, C.N., Prabhakar, R. and Swaminathan, G. **In vitro** experiment with compound centella asiatica - investigation to elucidate the effect of compound on the acid fastness and viability of **M.tuberculosis. Indian Journal of Leprosy.**

17. Daniel Herbert, Paramasivan, C.N. and Prabhakar, R. Protective response in guinea pigs exposed to **M.avium intracellulare M.scrofulaceum,** BCG and South Indian isolates of **M.tuberculosis. Indian Journal of Medical Research.**

18. Paramasivan, C.N. Newer antimycobacterial drugs and their role in the treatment of tuberculosis patients. **Indian Journal of Tuberculosis.**

19. Selvakumar, N., Reetha, A.M., Vanaja Kumar, Tripathy, S.P., Narayana, A.S.L., Sivasubramanian, S., Paramasivan, C.N. and Prabhakar, R. Mycobacteriuria in pulmonary tuberculosis patients in Madras, South India. **Indian Journal of Tuberculosis.**

20. Raghu, G., Sarma, G.R. and Venkatesan, P. Effect of haptoglobin on haemoglobin supported growth and siderophore production in Mycobacteria. **Medical Sciences Research.**

21. Raghu, G., Sarma, G.R. and Venkatesan, P. Effect of anti-tuberculosis drugs on the iron sequestion mechanism. **Indian Journal of Pathology and Microbiology.**

22. Vijayan, V.K., Sankaran, K., Venkatesan, P. and Prabhakar, R. Characterisation of lower respiratory tract inflammation in patients with smear negative pulmonary tuberculosis. **Lung India.**

23. Manjula Datta and Krishnamurthy, P.V. Association between obesity and hypertension in South Indian patients. **Indian Heart Journal.**

24. Paramasivan, C.N. and Daniel Herbert. Mycobacteria. **In: Respiratory Medicine in Tropics, Ed: J.N. Pande, Oxford University Press, New Delhi.**

25. Manjula Dutta and Mohan Alladi. Epidemiology of tuberculosis. **In: Respiratory Medicine in Tropics, Ed:J.N.Pande, Oxford University Press, New Delhi.**

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, 33 such meetings were conducted during the year.

In addition, a video show of a New York TV programme on Tuberculosis and a quiz programme on tuberculosis and related diseases were conducted.

*****

LECTURES BY VISITING SCIENTISTS

Subject	Speaker
Immuno-efficient scid mice as models for human lymphatic filariasis	Prof.T.V.Rajan, Professor of Pathology, Connecticut, USA.
Research strategies for economic evaluation of the TB Control Programe	Dr.Henry Glick, Health Economist, University of Pennsylvania, Philadelphia.
Health Services in Andaman & Nicobar Islands	Surgeon Commander G.S.Nagra, Armed Forces Medical College, Pune.
HIV and Associated Diseases: Update on the treatment and vaccine development	Prof.J.C. Chermann, Director, Research Unit on Retrovirus and Associated Diseases, National Institutes of Health and Medical Research, Marseille, France.

DISTINGUISHED VISITORS

1. Dr.E.A.Ottesen, Chief, Section of Clinical Parasitology, Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.

2. Dr.P.Zimmerman, National Institutes of Health, Bethesda, Maryland, USA.

3. Dr. Astrid Brundin, University Hospital, Lincoping, Sweden.

4. Dr. Oiszewski, Polish Academy of Sciences, Medical Research Centre, Warsaw, Poland.

5. Dr. Mohlia Palat, Institute Malardie, Papeete, Tahiti, French Polynesia.

6. Prof. Navaratnam, Centre for Drug Research Universitiy Sains Malasia, Penang, Malasia.

7. Dr. M.J. George, National Programme Officer (WHO/India), New Delhi.

8. Dr.C.P.Ramachandran, Secretary, Steering Committee, Filariasis, TDR, WHO, Geneva

9. Mrs. Lina Chakrabarthi, Health Secretary, Govtemment of West Bengal, Calcutta.

10. Dr. S.P. Tripathy, Director-General, Indian Council of Medical Research, New Delhi.

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

12. Dr. Prahlad Kumar, Assistant Director-General (TB), DGHS, Ministry of Health and Family Welfare, New Delhi.

13. Mr. Pawar Chopra, Joint Secretary, Ministry of Health and Family Welfare, Government of India, New Delhi.

14. Dr.P.D.Nigam, Former Professor of Cardiology, RML Hospital, New Delhi.

STAFF MEMBERS ON ADVISORY COMMIITEES OF OTHER INSTITUTIONS

Staff member	Name of committee
Dr.R.Prabhakar	Fellow, International Academy of Chest Physicians and Surgeons of the American College of Chest Physicians, Illinois, USA.
- do -	Editorial Board, **Ceylon Medical Journal,** Colombo, Sri Lanka.
- do -	Project Review Committee, indo-US Science and Technology Initiative, Department of Science and Technology, Government of India, New Delhi.
- do -	Standing Technical Committee, Tuberculosis Association of India, New Delhi.
- do -	Governing Body, ICMR, New Delhi.
- do -	Executive committee, ICMR, New Delhi.
- do -	Project Review Committee for Tuberculosis, ICMR, New Delhi.
- do -	Editorial Board, **Indian Journal of Tuberculosis,** New Delhi.
- do -	Scientific Advisory Committee, Regional Medical Research Centre, ICMR, Port Blair, Andamans.

Staff member	Name of committee
Dr.R.Prabhakar (Contd.)	Research Advisory Panel, Schieffelin Leprosy Research and Training Centre, Karigiri.
- do -	Planning Board, The Tamilnadu Dr. M.G.R.University of Medical Sciences, Madras.
- do -	Senate, The Tamilnadu Dr.M.G.R. University of Medical Sciences, Madras
- do -	Board of Management, Vision Research Foundation, Shankar Netralaya, Madras.
- do -	Research Sub-Committee, Vision Research Foundation, Shankar Netralaya, Madras.
- do -	Steering Committee, Advanced Centre for Clinical Epidemiological Research and Training, Madras.
Dr.C.N.Paramasivan	Editorial Board, **Indian Journal of Tuberculosis,** New Delhi.
- do -	Editorial Board, **Indian Journal of Medical Microbiology,** Madras.
- do -	Board of Studies in Microbiology, University of Madras, Madras.

Staff member	Name of committee
Dr.T.Santha Devi	Steering Committee of Therapy for Mycobacterial Diseases, WHO, Geneva.
Dr.V.K.Vijayan	Expert Member, Central Crisis Group (CCG) for Chemical Disasters, Ministry of Environment and Forests, Government of India, New Delhi.
- do -	Consultant, Government of Madhya Pradesh for establishing a super speciality hospital for pulmonary medicine at Bhopal.
- do -	Respiratory Medicine Panel, Institute of Integral Health Studies, Madras.
- do -	Expert Member, Crisis Management group for Chemical Road Transportation Emergency, Tamil Nadu Pollution Control Board, Government of Tamil Nadu.
- do -	Editorial Board, Indian . **Journal of Chest Diseases and Allied Sciences,** V.P.Chest Institute, New Delhi.
- do -	Assistant Editor, **Lung India,** Madras.
- do -	Advisory Board, Lung Sounds, Asthma and Bronchitis Association of India (South India Chapter), Madras.

Staff member	Name of committee
Dr.V.K.Vijayan (Contd.)	International Governor, International Academy of Chest Physicians and Surgeons, USA.
- do -	Convener, Position Paper on "Spirometric Norms in India". Indian Chest Society, Bombay.
- do -	Panel of Judges to select the best paper, Indian Association of Biomedical Scientists, Madras, 1993.
- do -	Scientific Committee, XIII National Congress on Respiratory Diseases, Indian Chest Society, Madras.
Dr. Padma Ramachandran	State Resource Facility Continuing Medical Foundation In Paediatric update, Indian Academy of Paediatrics, Tamil Nadu State Branch, Madras.
Dr. Manjula Datta	Scientific Advisory Committee, Regional Medical Research Centre for Tribals, Jabalpur.
- do -	Curriculum Development Committee for Clinical Epidemiology, The Tamilnadu Dr.M.G.R University of Medical Sciences, Madras.

Staff member	Name of committee
Dr.V.Kumaraswami	Expert Committee, Parasitic Diseases (Filariasis), WHO, Geneva.
- do -	Steering Committee (Filariasis), TDR/WHO, Geneva.
Dr.Soumya Swaminathan	Journal Committee,IAP, **Journal of Practical Paediatrics,** Madras.
Dr.K.V.Kuppurao	Executive Council, Indian Association of Biomedical Scientists, Madras.
Dr.Manjula Datta, Mr.P.V.Krishnamurthy	Steering Committee, Advanced Centre for Clinical Epidemiological Research and Training, Madras.

PRIZES AND AWARDS RECEIVED BY STAFF MEMBERS

1. Dr. R. Prabhakar was awarded the "Dr. Subratnaniam Suresh Memorial Oration Award" for contribution to AIDS and Tuberculosis at Madras Medical College, Madras, during November, 1993.

2. Dr. R. Prabhakar was awarded the "Dr. Raman Viswanathan Oration Award" by the National College of Chest Physicians, during August, 1993.

3. Dr. R. Prabhakar was awarded the "Dr.Rathnavel Subramaniam Endowment Oration" on visceral Leishmaniasis at Madras, during 1992.

4. Dr. C. N. Paramasivan was awarded the "Dr.P. V. Benjamin Oration Award" by the Andhra Pradesh TB & Chest Diseases Society, Kurnool, for the year 1993.

5. Dr. V. K. Vijayan received the "Prof.B.K.Aikat Oration Award" for research in Tropical Diseases for the year 1991, in Award Function, ICMR Headquarters, New Delhi on 9th July 1993.

6. Dr. V. K. Vijayan was awarded an International Governorship for India by the International Academy of Chest Physicians and Surgeons of the American College of Chest Physicians, USA for the term 1993-96.

7. Dr. V. Kumaraswami was awarded the "Dr.M.O.T.lyengar Memorial Award" for contributions to chemotherapy and immunology of lymphatic filariasis for the year 1992.

8. Dr. Rema Mathew was awarded the "Dr.R.Krishna Memorial Award" for the best paper presented at the 47th National Conference on Tuberculosis and Chest Diseases held in Bombay in November 1992, entitled "Response of patients with initially drug-resistant organisms to treatment with short course chemotherapy".

ACKNOWLEDGEMENT

The Director acknowledges the effort of Mr.P.V.Krishnamurthy and Mr.S. Sivasubramanian in editing and organising the publication of this report. The enthusiastic and untiring effort of Mr. R. Segaran and Mr. V. Sundaram in compiling, processing and preparing this report using computer is greatly appreciated.