TUBERCULOSIS RESEARCH CENTRE

CHETPUT MADRAS-600 031

REPORT ON RESEARCH ACTIVITIES DURING 1992

INDIAN COUNCIL OF MEDICAL RESEARCH

NEW DELHI

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PREFACE

The Centre has undertaken operational research studies to evolve methodologies suitable for application in the National Tuberculosis Programme for improving its efficiency. The areas covered under this research programme are case finding and case-holding, two important components for improving the Programme. Towards this objective, different strategies to suit the local conditions are being tried. The operational research study in an urban set up (Madurai) utilising the NSS volunteers indicates that the case-finding efficiency could be substantially improved; however, with regard to case-holding, more inputs are needed to ensure better drug compliance. Hence, necessary steps are being taken to modify the procedures to ensure better drug compliance by patients. The study in a tribal area with inadequate medical facilities in the district of North Arcot Ambedkar, utilises literate youths for case finding and health education activities. If this strategy is found successful, it may be adopted in other tribal areas in the country and could be extended to other health programmes also.

Short Course Chemotherapy regimens of 6- month duration with split- dose double-drug combination administered on alternate days during an initial intensive phase of 2 or 3 months appear to be promising and could be as efficacious as when the four drugs are administered together on thrice- weekly basis. The interim findings of the study have shown similar sputum conversion rates at the end of 2 months of treatment in sputum positive pulmonary tuberculosis patients. However, long-term follow-up is necessary to confirm their efficacies. The success of the splid-dose double-drug combination therapy could lead to dispensation of these combinations in blister-packs and enable better supervision, ensure better acceptability of treatment and greater chances of reducing adverse reactions, all of which are aimed at successful completion of treatment without compromising on the efficacy of treatment. Long-term follow-up results of a trial with a fully oral 8-month daily regimen containing ethambutol have revealed the benefit of the drug in overcoming initial drug resistance to isoniazid. Encouraged by the results of our short course chemotherapy studies in various forms of extrapulmonary tuberculosis, a pilot study on cutaneous tuberculosis with an SCC regimen was started to standardise diagnostic criteria and to study the feasibility.

Fiberoptic bronchoscopic studies are being explored to evolve a bacteriologically confirmative test of diagnosis for pulmonary tuberculosis in adults and in children. Pulmonary physiology studies to assess the lung functions in pulmonary tuberculosis patients, which could eventually help to explain the respiratory crippling in relation to the gradation and duration of illness will be undertaken.

A bacteriological study in pulmonary tuberculosis patients to elucidate the principles of chemotherapy with the split double-drug combination of anti-tuberculous drugs has been completed. *In vitro* studies were conducted and reported, while *in vivo* studies in experimental tuberculosis in mice are in progress. A study on the occurrence

of mycobacteria in the environment in the Tiruvallur BCG Trial area indicated the predominance of two species of mycobacteria, namely, *M.Fortuitum* and MAIS complex. This study will lead to a better understanding of the hypothesis of immuno modulation brought about by the non-tuberculous mycobacteria in individuals vaccinated with BCG. Studies of Beta lactamase activity in *M.tuberculosis* are in progress. Search is being continued for newer drugs for the treatment of patients with drug resistant bacilli and for possible reduction of duration of chemotherapy. In this context, some derivatives of fluoro quinolones have been screened for their antimycobacterial activity *in vitro* and the results have been reported. Bioluminescence assays have been standardised for rapid screening of drug susceptibility in mycobacteria, especially in non-cultivable *M.leprae*. This will be a useful tool in the assessment of progress during treatment of multi-bacillary forms of leprosy. Further, this method of assay avoids using radio-labelled substances which are expensive and hazardous.

Pharmacological investigations relating to drug interaction with the commonly used anti-tuberculosis drugs in short course chemotherapy are being carried out. This is an off-shoot of the ongoing controlled clinical study with split double-drug combination of anti-tuberculosis drugs. Furthermore, the study will elucidate the effectiveness of combined formulations of the drugs.

Development of immuno-diagnostics is one of the frontier areas of research of the Centre. Accordingly, search is continuing for highly specific antigens and antibodies for early detection of pulmonary and extra pulmonary forms of tuberculosis. Efforts are also being made to develop DNA probes for diagnosis of mycobacterial infections. The technique of RFLP has been applied in epidemiological studies of South Indian isolates of *M.tuberculosis* and also in the study of isolates of *M.tuberculosis* from patients who had a relapse after favourable response to treatment. The latter study may help to distinguish between relapses and reinfection among patients treated successfully with bactericidal and sterilising regimens of short course chemotherapy. T-cell cloning technology has been established and will enable study of cytokines derived by the clones in response to specific antigenic stimulation in addition to the understanding of the role of T-cell subsets in the immunology of tuberculosis.

Studies of seroprevalence of HIV infection among tuberculosis patients and tuberculosis among HIV infected individuals are being continued to enable understanding of the dual infection. These studies are very important since there is a global awareness

of tuberculosis emerging as a major health problem in the years to come in the wake of an upsurge in HIV infection.

Valuable contributions are being made in the epidemiology of tuberculosis by the conduct of studies to evolve surveillance methodology and diagnosis of childhood tubercuolsis at field level on a community basis. The interim findings of the prospective long-term follow-up study being carried out to find out the fate of sputum positive patients treated under DTP may have far reaching implications.

During the year, significant improvements have been introduced in the library and information services at our Centre. A fortnightly publication 'Tuberculosis Alert' has been started. Facilities have been made for literature search and library resource sharing. Several library activities have been computerised.

The long felt need for a good animal house facility is going to be fulfilled as the construction of the building is taking shape rapidly. The building will house rabbits, guinea pigs and mice. This facility will be very useful in experiments for developing reliable immuno diagnostics, detailed pharmacological investigations with anti-TB drugs, screening of newer anti TB drugs and for development of vaccinee in addition to basic research with mycobacteria

The basic, applied and operatianal research studies conducted at this Centre are aimed at improving the efficiency of the National Tuberculosis Programme. The environment conducive for research, provided by the adminstartive and other infrastructure facilities being utilised by the Scientists of this Centre to turn out high quality research. The fact that a number of young and bright scholars are attracted every year by the Centre to pursue doctoral programmes bears testimony to the quality of guidance. Often, these programmes form part of the research activities of the Centre.

The Scientific Advisory Committee, which met on the 26th October 1992 under the Chairmanship of Dr.S.P.Tripathy, Director General, ICMR gave valuable guidance and helpful suggestions regarding the research activities of the Centre.

Finally, I wish to place on record my deep appreciation and grateful acknowledgement for the unstinted support received by me from my colleagues and for their untiring efforts which has enabled the Centre to make useful and significant research contribution in tuberculosis.

R. Prabhakar

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OPERATIONAL RESEARCH STUDIES - COMPLETED

Short course chemotherapy under District Tuberculosis Programme

(Completed study, 1983-92)

Short course chemotherapy was introduced in 18 districts spread over 10 states in India during the period March 1983 to March 1985. The Centre had been given the responsibility of implementation and monitoring of the programme. Periodic analysis is undertaken based on the returns received from these districts, and the data presented in each year's annual report (1983 onwards).

The Scientific Advisory Committee decided, in 1991, that the monitoring of the programme in far away districts should be transferred to National Tuberculosis Institute and that the Centre should confine its activities to four nearby districts to carry out its operational research studies in addition to monitoring. Accordingly, the Centre stopped its monitoring activity in 14 of the 18 districts in 1992. This report given a brief review of the main findings in the 18 districts.

The regimens prescribed were:

1. $2RHZ_2/4RH_2$: Rifampicin 600 mg plus isoniazid 600 mg plus pyrazinamide 2.0g given twice a week for 2 months, followed by rifampicin 600 mg plus isoniazid 600 mg twice a week for the next 4 months, all doses being administered under supervision in the clinic.

2. 2RHZ/6TH: Rifampicin 450 mg plus isoniazid 300 mg plus pyrazinamide 1.5g daily for 2 months, followed by thioacetazone 150 mg plus isoniazid 300 mg daily for the next 6 months, the drugs being collected by the patients once in 15 days for self administration.

3. $2RHZ/4RH^2$: Rifampicin 450 mg plus isoniazid 300 mg plus pyrazinamide 1.5g daily for 2 months, followed by rifampicin 600 mg plus isoniazid 600 mg twice a week for 4 months; in the first two month, the drugs are collected once in 15 days for self- administration, and in the next 4 months, all doses are administered under supervision in the clinic

Three policies of treatment, one each for 6 districts, are followed:

Policy A: Regimen 1, with regimen 2 as an alternative.
Policy B: Regimen 2.
Policy C: Regimen 3, with regimen 2 as an alternative.

Sputum positive pulmonary tuberculosis patients aged 15 years or more are eligible to be treated with short course chemotherapy, provided they have not received more than 2 months of previous specific chemotherapy for tuberculosis.

The programme of short course chemotherapy is integrated with the District Tuberculosis Programme; hence, implementation and running of the programme is the responsibility of the staff of the District Tuberculosis Centres and the PHIs (Peripheral Health Institutions). The Centre's staff made periodic monitoring visits to the districts.

Sputum examination and intake to SCC: The average sputum examination per month from the inception of SCC ranged from 740 to 3616 in the Policy A districts, 622 to 2289 in the Policy B districts and 589 to 1612 in the Policy C districts; the percentage of positivity ranged from 4.2% to 13.6%, 7.2% to 9.6% and 4.5% to 9.1% respectively. The percentage of eligible patients started on SCC ranged from 46% - 82% in the Policy A districts, 31% - 74% in the Policy B districts and 42% - 80% in the Policy C districts.

Detailed analysis on smear positivity in the districts over the years have shown that there was variation in some districts, but there was no clear-cut trend. Since the study was terminated in June 1992, this report covers the period January to June 1992 only. Considering all the 18 districts, the smear positivity in 1992 (January to June) was less than 5% in one district, 5 %- 9% in 10 districts and 10% - 15% in the remaining 7 districts.

As for the intake to SCC, in 1992, 80% or more of eligible patients were put on SCC in 4 districts, 70% - 79% in 2, 60% - 69%,in 3, 50% - 59% in 2 and less than 50% in 7.

Contribution of PHIs to case finding: The total number of sputum examinations at the DTC during 1992 (see Table 1) ranged from 1018 to 4267 in the Policy A districts (median 1793), 424 to 3355 in the Policy B districts (median 1189) and 940 to 3815 in the Policy C districts (median 1894). The corresponding figures for the PHIs are 660 to 19624 (median 6111), 1253 to 14253 (median 4240), and 2009 to 10508 (median 5694), respectively. The sputum positive cases diagnosed at the DTC ranged from 142 to 726 (median 301) in the Policy A districts, 128 to 352 (median 212) in the Policy B districts and 165 to 402 (median 314) in the Policy C districts. The corresponding figures for the PHIs are 65 to 1646 (median 540), 148 to 1316 (median 218) and 60 to 790 (median 186) respectively. The over-all positivity rate at the DTCs is 14.6% and at the PHIs, 6.3%. The PHIs examined 16 sputum specimens for every

positive obtained whereas at the DTCs, it was 7 specimens for every positive. Of the total 12846 sputum positive cases diagnosed during the period January to June, 1992, 59% were diagnosed at the PHIs and 41% at the DTCs.

Table 1
Percentage of sputum positives at DTC and PHIs
(Jan - Jun 1992)

	DTC			PHI		
	New sputum examined	Positive		New sputum examined	Positive	
		No.	%		No.	%
Policy A						
Total	13829	2053	*15*	49575	3627	*7*
Median	1793	301	*17*	6111	540	*10*
Range	1018-4267	142-726	*8-25*	660-19624	65-1646	*3-15*
Policy B						
Total	9341	1380	*15*	32985	2323	*7*
Median	1189	212	*18*	4240	218	*6*
Range	424-3355	128-352	*10-39*	1253- 14253	148-1316	*3-12*
Policy C						
Total	12841	1812	*14*	37351	1651	*4*
Median	1894	314	*15*	5694	186	*4*
Range	940-3815	165-402	*8-23*	2009- 10508	60-790	*1-8*

Data on contribution of PHIs to case finding from 1985 to 1991 are given in Table 2. Intensive monitoring of DTP was initiated by TRC in 1988. The PHI contribution to case finding has increased from an average of 50% during 1985- 88 to an average of 57% during 1989-91.

Treatment completion rate: From the inception of SCC, treatment completion rates for 8 different cohort periods (ending June, 1992) are available. In the current cohart(Ju1y 91 to June 92), the SCC treatment completion rate is 50% and ranges from 41 to 61% in the Policy A districts, 69% and 33 to 78% in the Policy B districts and 61% and 42 to 72% in the Policy C districts. Considering the treatment completion rate during the entire 8 cohort periods, 53% (median 56%) of patients in Policy A districts, 58% (median 54%) in Policy B districts and 56% (median 56%) in Policy C districts had received 80% or more of chemotherapy.

Considering the treatment completion rate according to the regimen during the 8th cohort period (July 1991 to June 1992), 44% of 1038 patients on the fully supervised regimen had received 80% or more of chemotherapy, compared with 64% of 8858 patients on the unsupervised regimen and 56% of 983 patients on the partially supervised regimen. The treatment completion rates for the previous 7 cohort periods were 49% of 4129, 53% of 1719, 47% of 1716, 46% of 1351, 51% of 1446, 50% of 1478 and 50% of 1186 patients for regimen 1, 48% of 2834, 50% of 4598, 57% of 6509, 54% of 5601, 55% of 6985, 54% of 10062 and 60% of 10026 patients for regimen 2 and 76% of 631,

55% of 1258, 63% of 1043, 60% of 1051, 61% of 1325, 55% of 1215 and 64% of 907 patients for regimen 3. Thus the completion rate remained more or less the same over the years with regimens 1 and 2; there are wide variations in the rates with regimen 3 but there is no clear-cut trend. The treatment completion rates for the entire 8 cohort periods were 49% for regimen 1, 56% for regimen 2 and 60% for regimen 3.

Treatment completion rates for SCC and standard regimens are available for 5 concurrent cohort periods. Results of the last two concurrent cohorts for the period July 89 to June 91 are given in Table 3 and the results of previous three cohorts were provided in the 1991 annual report. The treatment completion rate with SCC for the 5 cohort periods has been consistent for Policy A and B districts, the medians being 51%, 54%, 55%,53% and 50% in Policy A districts and 52%, 48%, 50%, 52% and 56% for Policy B districts. In Policy C districts, the median treatment completion rate has gone up from 52% and 51% in the 1st and 2nd cohort periods to 60%, 56% and 65% in the 3rd, 4th and 5th cohort periods respectively. Considering the standard chemotherapy data for all the 18 districts, the median treatment completion rate had improved from 27% in the 1st cohort period to 36% in the 2nd, 39% in the 3rd, 34% in the 4th and 38% in the 5th cohort period.

Table 2
PHI contribution[1]
Percentages of initial sputum examinations and sputum positives

Year	Sputum examination %		Sputum positive %	
	Mean	Range	Mean	Range
1985	70	47 - 94	49	18 - 80
1986	73	50 - 94	52	12 - 81
1987	72	49 - 88	51	14 - 83
1988	71	47 - 88	48	21 - 81
1989	73	38 - 84	55	20 - 87
1990	76	28 - 88	58	13 - 81
1991	75	35 - 96	59	15 - 91

1. Based on 16 districts only, for North Arcot and Anrangabad, separate figures for DTC and PHI were not reported from 1985 to 1988.

Table 3
Concurrent cohorts - SCC vs standard (STD)
(period 7/89 to 6/91)

District	SCC				STD			
	7/89-6/90 [1]		7/90-6/91		7/89-6/90		7/90-6/91	
	No. of Pts.	≥ 80 % Rx. %	No. of Pts.	≥ 80 % Rx. %	No. of Pts.	≥ 80 % Rx. %	No. of Pts.	≥ 80 % Rx. %
Policy A								
Total	4719	53	3322	50	2320	30	1976	48
Median	708	53	423	50	331	28	416	36
Range	368-1398	40-71	379-1193	45-66	209-936	23-47	210-934	35-62
Policy B								
Total	3453	54	4476	64	2338	51	2690	58
Median	549	52	548	56	395	49	521	51
Range	264-1049	33-67	390-1808	34-79	327-680	24-87	221-880	13-90
Policy C								
Total	4583	54	4321	62	2057	44	1855	45
Median	655	56	571	65	316	40	404	38
Range	247-1352	30-74	247-1530	39-81	173-533	21-64	194-546	23-76
Median	574	53	537	56	364	34	418	38

1.Month/Year

This study, therefore, demonstrates that short course chemotherapy can be introduced in DTP provided adequate infrastructure is available in districts and the mean treatment completion rates of five cohorts (1986 to 1991) were 55% and 41% with SCC and standard regimens respectively. Similarly, case finding can also be improved by intensive monitoring at PHI level.

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Utilisation of NSS volunteers to augment the components of a City TB Programme

(Completed study, 1990-92)

Details regarding rationale, aims, area of study, personnel involved in this pilot study along with some findings were given in our two previous annual reports. This report gives a brief review of all the activities of the National Service Scheme (NSS) volunteers.

Sensitisation methods adopted and cases detected : During the period December 1990-July 1992, four camps were held in Madurai and different strategies were adopted for educating the public to get their cooperation. In addition to student volunteers, other agencies also were utilized for sensitising the population. In the first two camps, students did a door to door enumeration, detected the chest symptomatics and motivated them to attend the camps. In the 3rd and 4th camps, the students enacted street plays only. Main sensitisation methods by other agencies consisted of announcements through All India Radio (AIR), public announcements with the use of microphone and wall posters in the area. The wall posters were descriptive of the features of chest symptomatics and gave details about date and time of camps, etc. These wall posters were seen or read by the local community. Table 1 gives the population covered and the symptomatics who attended these camps and sputum positives detected.

Table 1
Sensitization methods adopted and the cases detected in the four camps

	Camp			
	I (Dec 90)	II (Feb 91)	III (Sep 91)	
Area covered	Aruldaspuram	Sellur	Aruldaspuram and Sellur	Aruldaspuram and Sellur
Population	25,000	18,000	43,000	43,000
sensitization methods				
(a) by students	Enumeration	Enumeration & street plays	Street plays	Street plays
(b)other agencies	Film shows and hand bills	Wall posters and public announcements	Wall posters and AIR anno uncements	Wall posters
No.of persons registered	380	359	237	263
Symptomatics	288	330	233	209
Sputum specimens collected[1]	412	466	316	327
Smear positives	21	15	17	6

1.More than one sputum specimen was collected from each symptomatic, if necessary.

The chest symptomatics attending the camps were questioned regarding the factors which made them to attend the camp. This was done from the second camp onwards. The replies to the questions were analysed and are shown in Table 2. In the second camp, among all factors that were used to sensitise the community, enumeratian contributed 40%, wall posters 29% and on their own 25%. In the 3rd camp, enumeration was not repeated and other strategies were tried. Among them, 42% of

symptomatics replied that the wall poster was the main motivating factor for them to attend. All India Radio announcements contributed 16% and neighbours, who came to know through these sources motivated 37% of the symptomatics. In the fourth camp, wall posters accounted 54%, street plays 10% and neighbours 35% for the attendance of chest symptomatics. Over all, sensitisation through wall posters, in the absence of door to door enumeration, is found to be the best among the strategies tried.

Table 2
Source of information leading the symptomatics to attend the camp

	Symptomatics who attended					
	Camp II		Camp III		Camp IV	
Source of information	No.	%	No.	%	No.	%
Enumeration	143	*40*	-	-	-	-
Wall posters	103	*29*	99	*42*	143	*54*
Street plays	21	*6*	12	*5*	27	*10*
Mike announcements	3	*1*	-	-	-	-
AIR announcements	-	-	39	*16*	21	
On their own	89	*25*	-	-	-	-
Neighbour	-	-	87	*37*	91	*35*
Total	359	*101*	237	*100*	263	*100*

Case-finding activity and regimens used: Case finding activity was carried out routinely in the two health facilities during the periods between the camps, in addition to cases detected during the camps. As a result, 93 sputum positives and 38 x-ray positives were started on treatment during the period. Smear positives were started on SCC regimen ($2EHRZ_7/6EH_7$). The drug collection was fortnightly in first phase and monthly in the second phase. X-ray positives were given a 12 month regimen (TH or EH) with monthly collections.

Case-holding : Once a patient is started on treatment, student volunteers were asked to visit them twice a month, irrespective of the regularity of drug collection. Students during their visits motivated the patients to take drugs regularly and did pill counting and recorded visit details in a form. Table 3 gives the visit pattern of students during 1991. Of the total 410 visits made, 25% and 22% of the visits were done in August and September. Less than 5% of the visits were done in March, April, June, November and December. The months with low percentage of visits correspond to their examination and vacation periods. Students could visit only during evening and were able to meet the patients only on 165 (40%) of the occasions.

Table 3
Home visits by NSS volunteers in 1991 and the number of occasions patients were met

Month	Visits made No. (A)	%	Occasions patients were met No.	% of (A)
January	53	*13*	20	
February	11	*3*	2	*34*
March	2		0	
April	1	*1*	1	
May	39	*10*	12	
June	5	*1*	4	*36*
July	39	*10*	15	
August	101	*25*	39	*39*
September	90	*22*	48	
October	38	*9*	14	*48*
November	18	*4*	6	
December	13	*3*	4	*32*
Total	410[1]	*101*	165	*40*

1. includes visits made to smear-negative but x-ray positive patients.

Table 4 gives the frequency of visits (in 1991) to patients home for 57 patients. A total of 14% of the patients were not visited at all due to reasons like out of area, lack of proper address, early migration, death etc. A minimum of 1 to 3 visits were done in 18%, 4 to 6 in 26%, 7 to 9 in 23% and 10 or more in 19% of the patients. On an average, each patient was visited 5.8 times.

Table 5 describes the results in the cohort of patients admitted till the end of December 1991. Of 83 patients admitted, 43% collected 75% or more of prescribed drugs, 63% collected 50% or more of drugs and 28% of patients collected less than 25% of drugs prescribed. It is observed that the student force could not contribute to any improvement in case-holding in spite of several visits they made to contact each patient. It is therefore concluded that NSS volunteers may not be useful for case-holding which happens to be the most important component in the programme, but they can play a significant role in sensitising the community, particularly when intensive campaigns can be carried out for short periods.

Table 4
Frequency distribution of home visits by volunteers.

Frequency of visits	Patients No.	%
None	8	*14*
1 - 3	10	*18*
4 - 6	15	*26*
7 - 9	13	*23*
10 or more	11	*19*
Total patients	57	*100*
Total visits	331	
Mean visits	5.8	

Table 5
Percentage of doses of drugs collected by patients

Doses of drugs collected %	Patients [1] No.	%	Camp I to Camp II No.	%	Camp II to Camp III No.	%	Camp III to Dec. 1991 No.	%
Less than 25	23	*28*	5	*25*	8	*22*	10	*38*
25-	7	*8*	2	*10*	4	*11*	1	*4*
50-	17	*20*	5	*25*	8	*22*	4	*15*
75-	5	*6*	2	*10*	1	*3*	2	*8*
≥ 80	31	*37*	6	*30*	16	*43*	9	*35*
Total	83	*99*	20	*100*	37	*101*	26	*100*

1. Patients admitted upto the end of December, 1991.

However, since only the girl students were involved in this investigation, it is intended to undertake another investigation in which only the male students would be involved.

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OPERATIONAL RESEARCH STUDIES - IN PROGRESS

Pilot study in Jawadhu hills for augmentation of District Tuberculosis Programme among tribals

(Ongoing study, 1990-96)

As mentioned in 1991 annual report, a pilot study was undertaken in 19 roadside hamlets in a tribal area (Jawadhu hills). The aim was to find out the feasibility of utilising the services of literate youths and the ooran (leader) from each hamlet for identification of chest symptomatics in the community, proper collection of sputum from them, drug distribution to sputum positive patients and documentation of drug supply. The results have shown that literate youths were able to identify the chest symptomatics in the community but were not useful in supplying antituberculous drugs to the patients.

It was decided to extend this study to 3 sub-centres of Jamnamarudur PHC in stages. These 3 sub-centres cover both interior and roadside hamlets. Study was started in September 1992.

The Design of the Study is briefly described below :

1. The Para Medical Workers (PMW) of the Centre will contact and identify one or more literate youths in each hamlet, who would be willing to participate in the District Tuberculosis Programme. The services of the literate youths will be utilised for census enumeration of the households of their villages, identification of chest symptomatics in the community, collection of sputum specimens from them and transportation of sputum specimens to the PHC. There is a proposal to pay honorarium to the literate youths.

2. Required training and health education will be imparted to oorans and the literate youths by Centre's staff. Health education includes basic aspects of diagnosis, treatment and methods for prevention of tuberculosis.

3. Census books with necessary work instructions (in Tamil) filed in them will be given to the literate youths.

4. Each literate youth will be supplied with a pocket book for writing the names of chest symptomatics identified by him.

5. The Centre's study team will visit PHC once a month and stay there for about a week. The literate youths will be in advance informed about the dates of the visit. The literate youths collect the sputum specimens from symptomatica one day earlier to the team's visit and bring the specimens to PHC on the next day to hand them over to the study team.

   The sputum smears will be examined by the laboratory technician at the PHC and also at Centre's laboratory for confirmation. Culture examinations will be done only at our Centre.

6. Chest symptomatics identified by literate youths will be interviewed by PMW/ Medical Officer(MO)/SociaI Worker(SW) to check whether they are true symptomatics or not.

7. PMW/SW will visit, once in 3 or 4 months, a few hamlets and select some households, at random, and identify the symptomatics in those households to ensure that chest symptomatics have not been missed by the literate youths. If new symptomatics are found, PMW/SW will collect one sputum specimen from each new symptomatic.

8. All sputum positive patients will be started on $2HRZ^{7}/6TH$ and treatment will be initiated by MO/Village Health Nurse(VHN). The VHN will issue drugs once in 15 days to the patients and instruct them to take the drugs every day at night after food. She will also explain to the patient about the proper way of disposal of sputum.

9. PMW/MO/SW will visit once a month, at random, each patient under treatment and do pill count to know whether the patient is taking the drugs regularly or not. This visit is also made use of to find out whether any side effects are encountered by the patient.

Work carried out in the field from September 92 to December 92: Sixteen villages in one sub-centre (Athipattu) were covered. A total of 2426 persons were enumerated in 819 households and screened by the literate youths. Of 2426 persons, 1485 were aged 15 years and above.

To ascertain whether literate youths had identified all the chest symptomatics in the community, a 10% random sample of the eligible population and all the chest symptomatics identified by literate youths were interviewed by PMW/MO. The results are shown in Table 1.

The literate youths had identified 116 (8%) chest symptomatica in 1485 persons (eligible population). The sputum specimens were collected from each of the 115 persons and examined by smear and culture. Five specimens were positive by smear and two by culture only.

Table 1
Evaluation of identification of symptomatics by literate youths

		Chest symptoms assessed by literate youths		
		Present	Absent	Total
Cheat symptoms assessed by PMW/MO	Present	115	10	125
	Absent	1[1]	141	142
	Total	116	151	267

1. This person was a chest symptomatic but symptoms disappeared a the time of interview by PMW

Chemotherapy : Six of 7 positive patients were prescribed Rifampicin 450 mg plus isoniazid 300 mg plus pyrazinamide 1.5g daily for 2 months followed by thioacetazone 150 mg plus isoniazid 300 mg for a further period of 6 months. One patient in one village was started on $2HRZ_2/4RH_2$ as he was willing for supervised chemotherapy.

The study is in progress.

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

(Ongoing study, 1989-94)

'Dais' are traditional birth attendants, conducting deliveries at home in the villages. Primary Health Centres in Tamil Nadu are conducting annual training courses for the village Dais, to teach them to conduct deliveries in a proper way under aseptic conditions. A pilot study was undertaken in Sriperumbudur taluk to explore the feasibility of utilising the services of Dais tor the improvement of case finding and case holding in the District Tuberculosis Programme.

There are 44 villages with a population of 26413, divided into 12 clusters in Sriperumbudur taluk, Chingleput district, Tamil nadu. A voluntary health organisation ('Prepare') functioning in Sriperumbudur taluk, trains Dais to provide primary health care to the village community. The Dais supply drugs for minor ailments; their work is closely supervised by Community Health Assistants (CHAs) employed by 'Prepare', who work like Government-employed multi-purpose workers.

In order to study the operational aspects of primary health care provided to the community through Dais, a health visitor and a clinic nurse from the Centre visited 13 villages in Sriperumbudur area. The Dais in these villages were given practical training

in identifying chest symptomatics and in collecting sputum specimens from them, for transportation to the Centre. Five such training programmes were organised in all.

Case finding by village Dais : A total of 463 sputum specimens were collected from symptomatics identified so far and examined by smear for AFB and by culture for *M.tuberculosis* (see Table 1). Of these specimens, 57 (12%) were found to be positive by smear for AFB and 12 (3%) were negative by smear but positive by culture for *M.tuberculosis.*

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 at 'night after food.

Table 1
Case finding activity by village Dais

	No.	%
Population catered by Dais (1991 census)	26413	
Population aged > 15 years	16740	*63*
Total symptomatics identified by Dais and sputum examined	463	*3*
Sputum positives		
by smear alone	21	*12*
by smear and culture	36 } 57	
by culture alone	12	3
over-all positivity	69	16

Out of 69 patients who were positive by smear or culture, 3 died before starting treatment. One patient 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, and 1 patient could not be identified. Treatment was initiated for the remaining 63 patients. During treatment, 6 patients died and 2 patients migrated. Two patients complained of giddiness after taking treatment in the first month of treatment but with reassurarnce felt better and the other refused to bake treatment inspite of repeated motivation.

So far, 42 patients have completed one year of treatment. At the end of treatment, sputum was collected from 30 patients by the Dais.

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 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 smear was negative.

Attempts are being made to study how far Dais living in villages outside 'Prepare' area can be utilised for the improvement of DTP.

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

(Ongoing study, 199143)

In 1990, 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. 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 are admitted into this investigation. All the patients are treated with a 6-month fully supervised regimen with twice-weekly attendance. Half the patients are allocated, randomly, to routine motivation and the other half to patient-to-patient motivation.

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

Patient-to-patient motivation (PM) : Motivation by treated patients, in addition to clinic staff motivation. Patient motivation is done 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 allocation procedures have been adopted. The Medical Officers, Clinic Nurses and Health Visitors are not informed about the group to which the patient is allocated.

Defaulter action is similar for both groups, as prescribed in 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 for a month are considered "lost" .

So far, 210 patients have been admitted in the study and 142 have completed 6 months period of treatment. Sufficiently large number of patients would be completing 6 months period of treatment in 1993 and the effect of the patient-to-patient motivation will be presented next year.

The study is in progress.

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CLINICAL STUDIES - COMPLETED

Five-year follow-up of children treated for tuberculous meningitis with short course chemotherapy

(Completed study, 1982-92)

The five-year follow-up of patients admitted to short course chemotherapy study on tuberculous meningitis in children was completed this year. The detailed findings on the response to SCC were presented in 1988 annual report. In brief, patients aged between 1 and 12 years were randomly allocated, after stratification according to clinical severity, in equal proportion to the following 2 regimens.

Regimen I : $2S_7H_7E_7R_3Z_3/7R_2H_2$: Streptomycin, isoniazid and ethambutol daily with rifampicin and pyrazinamide thrice a week for 2 months, followed by rifampicin and isoniazid twice a week for 7 months.

Regimen II: $2S_7H_7E_7R_2Z_2/7R_2H_2$: Streptomycin, isoniazid and ethambutol daily with rifampicin and pyrazinamide twice a week for 2 months, followed by rifampicin and isoniazid twice a week for 7 months.

In addition, the patients received non-specific therapy in the form of I.V. fluids, anti-oedema measures, anti-convulsants, and vitamins; as a policy, all received steroids for a period of 6-12 weeks.

In all, 215 patients were admitted to the 2 regimens (107 to regimen I, 108 to regimen II). Of these, 1 died of a non-tuberculous cause and 29 patients were discharged against medical advice before completing therapy. The response to the allocated regimen could not be assessed in 35 patients, as their treatment had been modified because of the development of hepatitis or ocular changes. The analysis of response to treatment was therefore based on 150 patients. Of these, 40 (27%) patients died of tuberculous meningitis, 52 (35%) had neurological sequelae and 58 (39%) had complete recovery. The response was similar in the two regimens.

The survivors at the end of treatment were followed up to find out the relapse rates and the course of the lesions. They were seen once a month up to 24 months, once in 3 months up to 36 months and once in 6 months up to 60 months. The

follow-up investigations included (a) a complete physical examination with special reference to the central nervous system, (b) a chest radiograph at 3-monthly intervals for patients who had persistent abnormality at the end of treatment, till they became normal and (c) cerebro-spinal fluid (CSF) examination for cell count, biochemical characteristics and bacteriological examination for *M.tubcrculosis* every three month for patients with abnormal CSF findings at the end of treatment. In addition, between 48 and 60 months, the following investigations are done: (d) electro-encephalogram; (e) psychometric evaluation; (f) hearing assessment and (g) radiograph of skull for evidence of calcification.

Of the 110 survivors at the end of chemotherapy, 16 patients had a persistant abnormality in the chest radiograph. In 13 patients, it became normal (in 6 patients between 10-12 months, in 4 between 13-24 months, in 3 between 25-36 months). In 2 patients, there were calcified lesions at 18 and 28 months, respectively. In 1 patient, there were bronchiectatic changes (as a sequelae) up to 60 month the patient was seen by the surgeons and no surgical intervention was suggested. None of the 16 patients had any clinical deterioration or CSF abnormality necessitating retreatment.

Five patients had abnormal CSF fluid findings (high protein) at the end of chemotherapy. In 4 patients, the values became normal at 10,14,30 and 54 months, respectively. For the 5th patient, the CSF examination at 48th month was still abnormal and the examination could not be repeated there after as the parents were not willing for a lumbar puncture. None of the patients had clinical deterioration and the CSF culture was negative for *M.tuberculosis* in all.

Of the 110 survivors at the end of 9 months, 11 died subsequently and the remaining 99 patients (28 with moderate sequelae, 14 with mild sequelae urd 57 with complete recovery) have completed the 60th monthly examination, i.e., 51 months of follow-up after stopping treatment. Table 1 shows the status at 60 months compared to the status at 9 months.

Of the 28 patients with moderate sequelae, in 26, the status remained me, 1 patient improved to mild sequelae while 1 patient recovered completely. Of the 14 patients with mild sequelae, in 2 patients, the status changed to moderate,sequelae (1 patient developed weakness of lower and upper limbs and the other developed secondary epilepsy as a late sequelae), in 10 there was no change, while the remaining 2 patients recovered completely.

Of the 57 patients with complete recovery, in 4, the statua changed to moderate sequelae (all developed secondary epilepsy) and, in 3, to mild requelae, while in the remaining 50 the recovery was maintained.

Only one patient, who had mild sequelae at the end of treatment, had a relapse with in 3 months with reappearance of clinical symptoms and signs.The CSF biochemistry was abnormal and *M.tuberculosis* (sensitive to all drugs) was grown in culture. Intensive therapy was restarted but the patient died in 31st month.

Table 1
Status at 60 month compared to status at 9 month

Status at 9m	No.of pts.	Death after 9m due to		No. eligible	Status at 60 month Sequelae			
		TBM sequelae	Non TB causes		Severe	Moderate	Mild	Complete recovery
Severe sequelae	5	5	0	-	-	-	-	-
Moderate sequelae	30	1	1	28	0	26	1	1
Mild sequelae	17	2[1]	1	14	0	2	10	2
Complete recovery	58	0	1	57	0	4	3	50
	110	8[2]	3	99	0	32	14	53

1. Including one patient who had a relapse.
2. 5 patients died between 10 and 24 months, 2 between 25 and 36 months and 1 patient between 37 and 48 months.

In summary, there were no drop outs during the five year follow-up; only one patient had a relapse and all the five patients who had severe sequelae at the end of chemotherapy died during the follow-up period.

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Pulmonary function studies in patients who had been treated for spinal tuberculosis

(Completed study, 1982-92)

Pulmonary function studies were carried out in patients with tuberculosis of the spine who had been treated with short-course regimens (see 1985-86 annual report). The treatment had consisted of rifampicin plus isoniazid daily, for either 6 months with radical resection of the spinal lesion with bone grafting or 6 or 9 months chemotherapy alone. Since these tests had not been undertaken on admission to treatment, comparative pretreatment values for the different groups are not available. However, since allocation of the patients to the three treatment groups were at random, it is highly likely that the three groups were similar in respect of pulmonary function on admission.

The aims of the pulmonary function study were to find out (a) whether the correction of deformity by radical surgery makes a significant contribution to improved respiratory function, and (b) whether the presence of a lesion in the thoracic or thoracolumbar region of the spine compromises the respiratory function to a greater extent than a lesion in the lumbar region. The following pulmonary function tests were ecarried out at yearly intervals, using P.K.Morgan Transfer Test Model C:

1. Forced Vital Capacity (FVC)
2. Forced Expiratory Volume in 1 sec (FEV_1)
3. (FEV_1 x 100)/FVC
4. Maximum Voluntary Ventilation (MVV)

In addition, electrocardiograms were recorded in each patient every year and the patients were followed up for 10 years. Of the 269 patients who had first year assessments, 221 were assessed in the 5th year and 153 in the 10th year. The mean valuer of the four pulmonary function parameters for 1st and 10th year in ambulatory (AMB 6/9HR) and surgical (RAD 6HR) series are given in Table 1. It indicates that there is no improved pulmonary function in patients in surgical series compared with patients in ambulatory series.

Table 1
Pulmonary function parameters at 1st and 10th year according to regimen

Test	Year	AMB 6/9HR			RAD 6HR		
		n	Mean	SD	n	Mean	SD
FVC (L)	1	102	2.26	0.71	51	2.23	0.74
	10	102	2.32	0.65	51	2.39	0.72
FEV_1 (L)	1	102	1.87	0.63	51	1.83	0.63
	10	102	1.90	0.58	51	1.96	0.62
(FEV_1 x100)/FVC (%)	1	102	82.6	8.8	51	82.3	8.9
	10	102	81.9	9.4	51	81.8	7.0
MVV (L/min)	1	73	74.7	26.1	35	76.8	34.7
	10	73	79.1	30.1	35	81.3	28.7

Mean changes in pulmonary function parameters from 1st to loth year are shown in Table 2. Similarly, lesions in thoracic or thoraco-lumbar region have not compromised pulmonary function compared to lesions in lumbar region. Further detailed analysis is being carried out.

Table 3
Mean changes in pulmonary function parameters from 1st to 10th year according to region of lesion

	Thoracic or thoraco-lumbar Mean ± SD	Lumbar Mean ± SD
FVC (L)	0.12 ± 0.77	0.06 ± 0.71
FEV_1 (L)	0.11 ± 0.70	0.02 ± 0.64
$(FEV_1 \times 100)/FVC$ (%)	0.14 ± 10.60	1.53 ± 7.56
Total Patients	78	75

* * * * *

Controlled clinical trial of dapsone as continuation chemotherapy beyond 7 years

(Completed study,1977-92)

As mentioned in previous (1986-87; 90 & 91) annual reports, the Centre undertook a controlled clinical trial of a rifampicin and a non-rifampicin regimen in the treatment of leprosy at the Government Royapettah Hospital, Madras. The findings up to five years have already been published (International Journal of Leprosy, 1990; 58, 273). The patients are being followed up for a further period of 10 years. Interim findings up to ten years are presented here.

Patients who have completed 60 months of treatment were stratified according to the average Bacterial Index(BI) value at 57,58,59 and 60 months as 0.5 or more and less than 0.5, and were randomly allocated to one of two regimens, namely, clofazimine 50mg with dapsone(DDS) 100mg daily (CD group) or dapsone 100mg daily (D group) for a further period of 2 years. At the end of 84 months, the patients were randomly allocated to either dapsone or placebo if their BI value at 84 months was 1.0 or less; if the 31 value was more than 1.0; they continued to get the earlier treatment (CD or D).

In all 210 patients (104 rif., 106 non-rif) were admitted to the trial, of whom 159 had completed 7 years of treatment and were allocated to dapsone (81) or to Placebo (78). The remaining 51 patients were not allocated at 84 months (8 had died, 13 had migrated and 29 had failed to attend for long periods and 1 patient developed toxicity to dapsone). Of the 159 patients, 10 patients (8 DDS, 2 placebo) have been excluded (1 died, 2 developed) tuberculosis and were prescribed anti-tuberculosis chemotherapy, and 7 failed to attend for more than 6 months). The findings of the remaining 149 patients (73 DDS, 76 placebo) are presented here.

Clinical progress: Clinical progress was assessed by an independent assessor who was unaware of the regimen or bacteriological results of the patients, using scores based on semi-quantitative assessments (as described in the previous annual reports). The independent assessor's classification of clinical progress is presented m Table 1. Over 0-84 months, moderate or marked improvement was reported in 66 (97%) in the DDS group and 74 (97%) in the placebo group. The corresponding figures were 67 (100%) and 62 (98%) over 0.96 months, 60 (100%) and 65 (98%) over 0-108 months, and 62 (100%) and 63 (100%) over 0-120 months, respectively. Thus, there was excellent clinical improvement in both series.

Table 1
Clinical progress as assessed by the independent assessor

Regimen (85-120 months)	Progress	Period (months)							
		0-84		0-96		0-108		0-120 [1]	
		No.	%	No.	%	No.	%	No.	%
Dapsone (n=73)	Improvement								
	Marked	65	96	65	97	58	97	62	100
	Moderate	1	1	2	3	2	3	0	0
	Slight	2	3	0	0	0	0	0	0
	No change	0	0	0	0	0	0	0	0
	Deterioration	0	0	0	0	0	0	0	0
	Absent for assessment [2]	5	-	6	-	13	-	11	-
Placebo (n=76)	Improvement								
	Marked	68	89	60	95	63	95	61	97
	Moderate	6	8	2	3	2	3	2	3
	Slight	0	0	0	0	0	0	0	0
	No change	1	1	1	2	0	0	0	0
	Deterioration	1	1	0	0	1	2	0	0
	Absent for assessment [2]	0	-	13	-	10	-	12	-

1. Excluding one patient in the placebo group who was assessed as deteriorated at 107 months and retreated.
2. Excluded for percentage calculation.

Bacterial Indices: The mean bacterial indices (BI) for the 2 groups at 84, 96, 108 and 120 months are shown in Table 2. The mean BI was 0.53 for the DDS group and 0.49 for the placebo group at 84 months, 0.29 and 0.27 at 96 months, 0.18 and 0.19 at 108 months and 0.13 and 0.06 at 120 months respectively.

Table 2

The mean Bacterial Indices observed in the two groups

Regimen (85-120m)	BI	Months			
		84	96	108	120 [1]
Dapsone (n=73)	Mean Range	0.53 (0.00-1.50)	0.29 (0.00-2.00)	0.18 (0.00-1.17)	0.13 (0.00-1.00)
Placebo (n=76)	Mean Range	0.49 (0.00-1.67)	0.27 (0.00-1.33)	0.19 (0.00-1.83)	0.06 (0.00 0.67)

1.Excluding one patient in the placebo group who deteriorated at 107 months and retreated.

In summary, the interim findings show that patients in the two regimens have shown similar improvement clinically and bacteriologically. No additional benefit was observed in the group treated with dapsone for 3 years after 84 months.

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 methods that might help to overcome these difficulties is to split the four oral drugs into two 2-drug combinations, giving each combination on alternate days, thus making each two-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 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 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 tuberculosis 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 previous chemotherapy, to one of the following regimens:

1. **$2RE_3HZ_3$(alt.)/$4RH_2$(2 alt .):** 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.)/$3RH_2$(3 alt.):** This is similar to regimen 1, but the initial phase is for 3 months, followed by 3 months in the second phase. (For regimens 1 and 2, Sunday is a drug-free day.)

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

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

Table 1

Number of patients allocated to different regimens

Centre	2 alt.	3 alt.	Thrice	Total
Madras	104	104	106	314
Madurai	95	95	94	284
Both	199	199	200	598

A total of 598 patients (314 at Madras and 284 at Madurai) have been admitted up to December 1992 (Table 1).

So far, 458 patients have completed chemotherapy (246 at Madras and 212 at Madurai). Sixteen patients were excluded from analysis, early death (3), pre-treatment culture negativity (6), infection with non-tuberculous mycobacteria on admission (2), non-tuberculous death (2), chemotherapy modified for adverse reaction (1) and Western Blot positive (2). Of the remaining 442 patients, 329 (74%) patients received 90% or more of chemotherapy during phase I, i.e., 115 (77%) patients in '2 alt', 103 (70%) patients in '3 alt' and 111 (77%) 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	42		29		57	
95 – 99	39	77%	51	70%	33	77%
90 – 94	34		23		21	
80 – 89	17		23		16	
70 – 79	11		11		7	
60 – 69	3		7		4	
50 – 59	2		1		3	
< 50	1		1		3	
Continuous FTA > 1m	0		2		1	
Total	149		148		145	

Regarding chemotherapy during phases I and II, out of the 442 patients, 304 (69%) patients received 90% or more of chemotherapy, i.e. 101 (68%) patients in '2 alt', 99(67%) patients in '3 alt' and 104(72%) patients in 'thrice' regimen (Table 3).

Table 3
Percentage of chemotherapy received during phases I and II

Rx received (%)	Number of patients					
	2 alt.		3 alt.		Thrice	
100	19		15		34	
95 – 99	58	68%	57	67%	43	72%
90 – 94	24		27		27	
80 – 89	25		25		19	
70 – 79	9		13		8	
60 – 69	3		5		3	
50 – 59	2		1		1	
< 50	1		0		0	
Continuous FTA > 1m	8		5		10	
Total	149		148		145	

Culture results during treatment: The percentages of culture negativity, based on 3 specimens per month, during 1 - 6 months for patients with initially drug sensitive organisms are given in Table 4.

Table 4
Percentage of culture negativity among patients with initial drug-sensitive organisms

Month after start of chemotherapy	Percentage of culture negative		
	2 alt.	3 alt.	Thrice
1	28	29	26
2	84	80	80
3	99	96	94
4	97	99	96
5	95	97	97
6	99	94	98
Total patients (range)	109-110	100-101	100-101

The proportion with all cultures negative was 26-29% in 1st month, 80-84% in 2nd month, 94-99% in the 3rd and subsequent months. The sputum conversion was very rapid, the range being 80-84% by 2 months.

Similarly the proportion of patients who became culture negative month by month in resistant patients is given in Table 5.

Table 5
Percentage of culture negativity among patients with initial drug-resistant organisms

Month after start of chemotherapy	Percentage of culture negative		
	2 alt.	3 alt.	Thrice
1	14	17	15
2	48	60	58
3	52	79	69
4	57	72	69
5	62	76	62
6	48	72	65
Total patients (range)	21-21	28-29	26-26

The proportion with all cultures negative was 14-17% in 1st month, 48-60% in 2nd month, 52-79% in the 3rd month and 48-76% thereafter.

The study is in progress.

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

(Ongoing study, 1986–95)

As explained in previous annual reports (1988, 1989, 1990 and 1991), a controlled clinical trial is in progress to investigate three fully oral regimens of 6 or 8 months' duration, with varying frequencies of attendance, different rhythms and full, partial or no supervision of drug intake. Patients are 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 1.5 g 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 2.0 g 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 only once a week, when one dose is given under supervision and the other dose is supplied for self- administration (3 or 4 days later).

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.Veerababu). Patients at Madurai were admitted on the basis of smear examination done at Madurai Unit. For patients admitted to the study, multiple sputum specimens are transported to the Centre at Madras, for culture and sensitivity tests. Close liason is 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) have been admitted to the study. Of these, 113 patients (62 at Madras and 51 at Madurai) have been excluded for various reasons: 21 were not eligible for admission, 2 developed pneumo-thorax in the first week, 6 died of non-tuberculous causes, 1 had a change of treatment for toxicity and 83 patients missed 25% or more of their chemotherapy. Of the remaining 1091 patients, 825 had organisms sensitive to rifampicin and isoniazid, 227 had organisms resistant to isoniazid alone, 38 had organisms resistant to rifampicin and isoniazid and one had resistance to rifampicin alone.

The mean age of the patients on admission was 30 years, the mean weight was 39.9 kg and 67% of the patients were males. The response to chemotherapy of these patients has already been presented in 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 777 patients with initially drug-sensitive organisms have completed 16/18 months of follow-up after stopping treatment. Relapse requiring treatment (Table 1) occurred in 13 (4.5%) of 290 patients in regimen 1, 26 (10.1%) of 258 patients in regimen 2, and 20 (8.7%) of 229 patients in regimen 3.

Table 1

Bacteriological relapse rates during 16/18 months follow-up in patients with initially isoniazid sensitive organisms

Regimen	Total assessed	Relapse requiring Rx.		Month of relapse after stopping Rx.			
		No.	%	1-3	4-6	7-12	13-16/18
$2EHRZ_7/6EH^7$	290	13	4.5 (2.1- 6.9)	10	2	0	1
$2EHRZ_2/4EHR_2$	258	26	10.1 (6.4–13.8)	14	7	3	2
$2HRZ_2/4HR_2$	229	20	8.7 (5.1-12.3)	13	3	3	1

1. Figures in parentheses are 95% confidence limits of relapse rates

The 95% confidence limits for the regimen-wise relapse rates are shown in parentheses in Table 1. Confidence limits for the differences between the relapse rates of any two regimens are given in Table 2.

Table 2

Confidence limits for the difference between relapse rates

Regimens	95% confidence limits for the difference between relapse rates(%)	
	Lower limit	Upper limit
2 $EHRZ_2/4EHR_2$ & $2EHRZ_7/6EH_7$	+ 1.2	+ 10.0
2 $HRZ_2/4HR_2$ & $2EHRZ_7/6EH_7$	- 0.2	+ 8.6
2 $EHRZ_2/4EHR_2$ & $2EHRZ_2/6EH_2$	- 3.8	+ 6.6

The difference in the relapse rates between the four-drug intermittent regimen and the daily regimen is statistically Significant (P<0.05) as the lower limit of the confidence interval is positive with a value of 1.2. The other two differences in the relapae rates are not statistically significant, as zero is contained in both the confidence intervals (indicated by the negative lower limit). The upper limits of confidence intervals indicate as to how large the difference could be between the corresponding relapse rates.

The relapse requiring treatment during 16/18 months follow-up of 149 patients with initially isoniazid-resistant organisms is given in Table 3. Six (8%) of 75 patients in regimen 1, 11(23%) of 47 patients in regimen 2 and 4(15%) of 27 patients in regimen3 have relapsed, the majority occurring within 3 months after stopping treatment. The 95% confidence limits for the relapse rates are shown in Darenthesee in Table 3.

Table 3
Bacteriological relapse rates during 16/18 months follow-up in patients with initially isoniazid resistant organisms

Regimen	Total assessed	Relapse requiring Rx.		Month of relapse after stopping Rx.		
		No.	% [1]	1-3	4-12	13-16/18
$2EHRZ_7/6EH_7$	75	6	8 (3.0-16.6)	5	1	0
$2EHRZ_2/4EHR_2$	47	11	23 (12.3-38.0)	11	0	0
$2HRZ_2/4HR_2$	27	4	15 (4.2-33.7)	3	1	0

1. Figures in parenthesea are 95% confidence limits of relapse rates.

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: Such patients are admitted by random allocation to either $3EHRZ_2/6HR_2$, or $3EHRZ_2/9HR_2$, namely ethambutol 1200 mg plus igoniazid 600 mg plus rifampicin 450 mg plus pyrazinamide 2.0 g 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, 107 patients have admitted to these regimens. Of 80 eligible cases for whom information up to one year is available, 22 are excluded (Table 1). Of the remaining 58 (28 in 9m regimen and 30 in 12m regimen), 54 (27 in 9m regimen and 27 in 12m regimen) had a favourable bacteriological response. Four patients showed an unfavourable bacteriological response, without any associated clinical or radiographic changes. Of the 4, three patients (all 12m regimen) had a change of chemotherapy.

Table 1
Distribution of patients with sensitive bacilli to H and R according to their status during and at the end of treatment

Status	No. of Patients	
	9m regimen	12m regimen
No. with information up to 1 year	40	40
Change of Rx due to adverse drug reactions	2	0
Early death	0	1
Non-TB death	0	1
< 75% of chemotherapy	10	8
No. in analysis	28	30
Favourable response	27	27
Unfavourable response	1	3

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 1st month and the latter, from the 2nd month to the 8th month, for the third patient, the regimen was changed in the 6th month as the patient had been persistently culture positive and had developed isoniazid resistance from the 2nd month. The fourth patient (9m regimen) had been persistently culture positive and had developed resistance to isoniazid at the 3rd month.

Patients with bacilli resistant to isoniazid: Such patients are admitted to $6SERZ_2/6ERZ_2$, or $6KERZ_2/6ERZ_2$ and prescribed streptomycin 0.75 g or if the bacilli

are resistant to streptomycin, kanamycin 1.0 g plus ethambutol 1200 mg plus rifampicin 450 mg plus pyrazinamide 2.0 g 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.

$6SERZ_2/6ERZ_2$: So far, 55 patients have been admitted to this regimen. Of 39 eligible cases for whom information up to 1 year is available, 14 are excluded (Table 2). Of the 25 in analysis, 20 had a favourable bacteriological response. The remaining 5. have shown an unfavourable bacteriological response. Three patients required change of chemotherapy. Of these, 1 had positive culture 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 treatment was changed at the end of the specified chemotherapy. The other 2 patients had produced negative cultures from the 1st and 2nd months and positive cultures from the 5th and 10th month onwards, respectively. None of these 5 patients had associated clinical or radiographic deterioration.

$6KERZ_2/6ERZ_2$: So far, 54 patients have been admitted to this regimen. Of the 39 eligible cases, 6 are excluded(Tab1e 2). Of the 33 in analysis, 25 had a favourable bacteriological response. Of the remaining 8 patients, 6 had a change of treatment be tween 5 and 10 months for persistent culture positivity; and 5 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 completed treatment, having been 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: Such 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 1.0 g thrice a week, plus daily ethmbutol 600 mg plus ethionamide 500 mg plus pyrazinamide 1.5 g 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- adminisration.

Table 2
Number of patients according to regimen and their response to treatment

	H resist.	SH resist.	RH resist.	SHR resist.
	$6SERZ_2$/ $6ERZ_2$	$6KERZ_2$/ $6ERZ_2$	$3S_3EmbEthZ_7$/ $9EmbEthZ_7$	$3K_3EmbEthZ_7$/ 9EmbEth7
No. with information up to 1 year	39	39	17	30
Change of Rx due to adverse drug reactions	3	1	4	3
Non-TB death	1	1	0	1
< 75 % of chemotherapy	10	4	5	7
No. in analysis	25	33	8	19
Favourable response	20	25	4	7
Unfavourable response	5	8	4	12

$3S_3EmbEthZ_7/9EmbEthZ_7$: So far, 24 patients have been admitted to this regimen. Of the 17 eligible cases, 9 are excluded (Table 2). Of the remaining 8 patients, 4 had a favourable response. Of the 4 with unfavourable response, 2 persistently had positive cultures and had a change of treatment at the 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.

$3K_3EmbEthZ_7/9EmbEthZ_7$: So far, 34 patients have been admitted to the regimen. Of the 30 eligible cases, 11 are excluded (Table 2). Of the 19 in analysis, 7 had a favourable bacteriological response. Of the remaining 12, 10 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.

The intake to all the regimens is continuing.

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Short course chemotherapy in pulmonary tuberculosis in children

(Ongoing study, 1992-96)

Evaluation of various therapeutic regimens for the treatment of pulmonary tuberculosis has been the major point of focus for more than 3 decades. The accent has been on short course chemotherapy (SCC). Though there are many reports available on SCC in adults, information regarding the same for tuberculosis in children is limited. Hence this Centre has started an SCC study in pulmonary tuberculosis in children, in collaboration with the Institute of Child Health (ICH), Egmore, Madras. Patients are selected from children referred from Tuberculosis Research Centre and ICH. To be eligible for admission, children should be aged between 1 and 12 years and should not have had more than 2 weeks of previous anti-tuberculous chemotherapy. The eligible children are classified into two categories of cases, definite cases (Category A) and probable cases (Category B). The definite cases must have any one of the following abnormalities seen on x-ray before admission :

(a) primary focus plus hilar adenitis,

(b) mediastinal glandular enlargement, or

(c) parenchymal lesion suggestive of miliary tuberculosis or progressive primary tuberculosis.

A history of contact with a known bacillary case of pulmonary tuberculosis or a positive tuberculin test with induration (1 TU) of 10 mm or more will be confirmatory. While definite cases of pulmonary tuberculosis (Category A) are directly started on anti-tuberculosis treatment, those with radiographic abnormality, not conclusive of pulmonary tuberculosis, (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 following two regimens:

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

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 for daily and 15 mg/kg for the thrice-twice weekly phase.

Rifampicin: 12 mg/kg.

Pyrazinamide: 45 mg/kg.

Examinations/investigations on admission : (a) general clinical examination, (b) Mantoux test, (c) A full plate radiograph of the chest, (d) liver function tests, (e) gastric lavage and sputum (wherever possible) cultures for AFB (f) urine tests and (g) haematological investigations. In patients with lymphgland enlargement, lymphnode biopsy will be done for histopathological and bacteriological examination. Chest radiographs will be taken at 2 weeks, 2nd, 6th and 9th month of treatment for both the categories of patients.

It is proposed to admit 200 patients(100 to each regimen). So far, 32 patients have been admitted (15 to Regimen I and 17 to Regimen II).

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Collaborative controlled clinical trial of tuberculous lymphadenitis

(Ongoing study, 1988-93)

Our Madurai Unit is conducting a controlled clinical trial in tuberculous lymphadenitis in collaboration with the Paediatric (Dr.A.J.Thiruthuvathas) and Adult Surgery (Dr.D.Anantharaj) Departments of the Govt.Rajaji Hospital, Madurai. Patients residing in or around Madurai with biopsy proved tuberculous lymphadenitis are admitted to the study. Previous anti-TB therapy is not a contra-indicating factor for admission. The hietopathology slides of the lymph node biopsy are read by the Professor of Pathalogy (Dr.V.Ananthalakshmi), Madurai Medical College and bacteriological investigations are done in Madras at our Centre.

Patients admitted to the study are 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 with pyrazinamide for the first two months ($2RHZ_2/4RH_2$). The drugs are prescribed in uniform dosage for adults, while a weight-adjusted dosage schedule is used for children. Patients are treated on an out-patient basis. Surprise home visits are done for pill counts for patients allocated to the self-administered regimen ($6RH_7$).

Patients are assessed clinically every month upto 12 months, every 3 months upto 24 months and every 6 months thereafter. At the end of chemotherapy, the clinical

response is assessed by an independent assessor. The response is defined as follows:

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

(b) doubtful, if significant residual nodes (>10 mm diameter) are palpable and

(c) unfavourable, if treatment needs to be extended or changed.

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 : A total of 244 patients have been admitted to the study (upto Dec.31, 1992) and 232 have completed treatment. After excluding 11 patients, 221 are available for interim analysis. Of these 221, 147(67%) were adults; 150(68%) were females (Table 1)

Table 1
Distribution of patients by sex and age

	Age (Yrs)	Male	Female	Total
Children	≤6	17	8	25
	7-12	31	18	49
Adults	13-30	17	101	118
	31-40	5	19	24
	> 40	1	4	5
Total		71	150	221

The results of the bacteriological examination of lympnode biopsy specimen for *M.tuberculosis* are shown in Table 2. It is observed that 76% of children and 69% of adults had positive cultures for *M.tuberculosis* prior to start of chemotherapy.

Table 2
Results of bacteriological examination of lymphnode biopsy specimens of M.TB.

	Culture		NTM	Total[1]
	Positive	Negative		
Children	54 *(76%)*	15	2	71
Adults	99 *(69%)*	38	7	144

1.Cultures were not available for 3 children and 3 adults.

Response to treatment : The response was favourable in 32 (89%) out of 36 children in 6 RH_7 and in all 38 (100%) in $2RHZ_2/4RH_2$. In adults, 70 (92%) out of 76 in 6 RH_7 and 69 (97%) out of 71 in $2RHZ_2/4RH_2$ had a favourable response(Table 3). Three adult patients in 6 RH_7 had an unfavourable response, requiring a change or extension of treatment. The response for 9 patients (4 children and 5 adults) was classified as doubtful at the end of treatment. Repeat lymph node biopsy was done in 7 of these 9 patients (3 children and 4 adults). In 6, the lymph node culture was negative though the histopathology was suggestive of tuberculosis and, for the other patient, both histopathology and culture were negative.

Table 3
Response to treatment

	Total pts.	Favourable		Doubtful		Unfavourable	
		No.	%	No.	%	No.	%
Children							
6 RH_7	36	32	*89*	4	*11*	0	*0*
$2RHZ_2/4RH_2$	38	38	*100*	0	*0*	0	*0*
Adults							
6 RH_7	76	70	*92*	3	*4*	3	*4*
$2RHZ_2/4RH_2$	71	69	*97*	2	*3*	0	*0*

The study is in progress.

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Collaborative study of abdominal tuberculosis : follow-up phase

(Ongoing study, 1983 - 2001)

As mentioned in previous annual reports (from 1986 to 1991), the Centre ham carried out a collaborative study on 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 short-course regimen with that of a standard regimen in the treatment of abdominal tuberculosis.

A secondary objective was to develop, from the findings of this study, satisfactory criteria for diagnosis, assessment of response and identification of relapse in abdominal tuberculosis. The study was conducted in collaboration with the Departments of Medicine and Medical and Surgical Gastro- enterology of the Government General Hospital, Madras, and the Govt. Peripheral Hospital, Anna Nagar, Madras.

Adult patients with clinical evidence of tuberculosis of the abdomen were subjected to appropriate diagnostic procedures such as laparoscopy, laparotomy, colonoscopy or liver biopsy and in cases with ascites, percutaneous peritoneal biopsy, for obtaining material for histopathological and bacteriological examinations. Ascitic fluid, when available, was subjected to cytological examination, biochemical investigations and bacteriological examinations. A complete hemogram was done and 3 early morning urine specimens examined by culture for *M.tuberculosis*

A plain radiograph of. the abdomen, barium meal and barium enema series and a chest radiograph were taken. Two sputum specimens were examined by smear and culture in patients suspected to have pulmonary tuberculosis.

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

Regimen 1 (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.

Regimen 2 (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.

Results up to 84 months: Of the 155 patients, who were either symptom free or clinically improved at the end of treatment, 70 patients (40 rifampicin, 30 non-rifampicin) completed 84 months of follow-up. Three patients (1 rifampicin series and 2 non-rifampicin series) among them required retreatment, one following surgery for intestinal obstruction as advised by the surgeon even though there was no histopathological or bacteriological evidence of relapse, one for renal tuberculosis and the remaining one for suspected tuberculous lymphadenitis which was found to be malignancy later. One patient (rifampicin series) died due to tuberculosis and five patients (3 rifampicin, 2 non-rifampicin series) died due to non-tuberculous causes. One patient (non-rifampicin series) who was lost for follow-up in the 16th month was asymptomatic at that time. All the remaining 60 patients are doing well at 84th month and it ie gratifying to note that none of these 70 patients had relapse with abdominal tuberculosis.

These patients will be followed-up up to 120 months from start of treatment.

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Collaborative study of brain tuberculoma : follow up phase

(Ongoing study, 1992-96)

This study was undertaken in collaboration with the Institute of Neurology, Govt.General Hospital (Prof.S.Kalyanaraman), Madras and Railway Hospital (Prof.Zaheer Ahamed Sayeed), Perambur.

The objectives of the study were :

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 upto 60 months after start of treatment.

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

All cases 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)

The detailed findings on the response to the Short Course Chemotherapy of brain tuberculoma patients have been presented in 1991 annual report. This report mainly deals with comparison of the status of the patients at 9th month and 24th month after start of treatment. A total of 144 patients were admitted to the study and 36 patients were excluded for various reasons. Of the remaining 108 patients, 54 patients had no clinical deficit at the start of treatment; at the end of 9 months of chemotherapy, 97 (90%) patients were normal clinically, compared to 94 (87%) at 24 months.

At 9 months, 8 (7%) of 108 patients had residual neurological deficit, 3 with post-papilloedemic optic atrophy alone, 2 with residual hand grip weakness and the other 3 with optic atrophy with hemiparesis. At 24 months, 11 (10%) patients had residual neurological deficit as 3 new patients developed fresh deficit due to convulsions after 9 months.

CT scan progress till the end of Chemotherapy was available in 91 patients as scan could not be taken for 17 patients due to various reasons (total surgical removal (3), death (2), change of treatment (1), scan not available (11)).

Changes as assessed by CT scan at 9 and 24 months are shown in Table 1. At 9 months, CT scan lesions were resolved completely in 78%, decreased in 18%, static in 2%, increased in size in 1% and fresh lesion appeared in 1% (new lesion appeared before 9 months in a female patient who had histopathologically confirmed tuberculoma following craniotomy biopsy. As she was clinically doing well at 9 months, additional chemotherapy was not given and was kept under observation). At 21 months, CT scan was read in 89 patients of whom 78 (88%) had a total resolution of the lesions and 11 (12%) had partial resolution.

Table 1
Changes as assessed by CT scan

Change by CT Scan	9 months		24 months	
	No.	%	No.	%
Complete resolution	71	78	78	88
Partial resolution	16	18	11	12
Static	2	2	0	0
Increased lesion	1	1	0	0
New lesion	1	1	0	0
Total patients	91	100	89[1]	100

In 24 months period, none had a relapse requiring treatment. The 5 year follow-up is continuing.

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Collaborative clinical study of cutaneous tuberculosis

(Ongoing study, 1992–95)

There have been only two studies on cutaneous tuberculosis where drug trials were conducted using multiple drug regimens elsewhere. In view of the paucity of information on these trials, a pilot study on cutaneous tuberculosis was started in collaboration with Department of Dermatology, Govt. General Hospital, Madras (Prof. P. Yesudian) and Department of Dermatology, Govt.Stanley Hospital, Madras (Prof. S.Premalatha) with the, following objectives:

1. to evolve and establish suitable criteria for diagnosis of cutaneous tuberculosis and 2. to study the feasibility of conducting controlled clinical studies in cutaneous tuberculosis to evolve efficacious regimens of treatment.

The 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:

3 Complete hemogram, 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 the end of 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 (below 12 years of age) are treated with a weight adjusted dosage of rifampicin(10 - 12 mg/kg) and isoniazid(5 mg/kg). The drugs are supplied once a week for self administration at home. The patients are assessed at TRC and also at either collaborating hospital at monthly intervals during treatment phase and at 3 monthly intervals upto 24 months.

Table 1
Pre-treatment characteristics

Factor on admission		Patients No.	Patients %
Age(years):			
	< 14	16	27
	15 - 24	19	32
	25 - 34	9	15
	35 - 44	5	8
	≥ 45	10	17
Site of disease:[1]			
	Foot/Sole	25	42
	Arm	10	17
	Knee	6	10
	Gluteal	6	10
	Forearm	5	8
	Finger	4	7
	Thigh	4	7
	Face	3	5
	Others	6	10
Type of disease:			
	Lupus Vulgaris	25	42
	Verrucose cutis	24	41
	Scrofuloderma	5	8
	Tuberculid	5	8
Total		59	

1. 10 patients had disease at multiple sites.

So far, a total of 59 patients have been admitted to the study. The distributions of the pre-treatment characteristics of the patients are shown in Table 1. Thirty-five (59 %) of 59 patients are less than 25 years on admission and only 10(17 %) are aged more than 45 years. Foot is most frequently affected part of the body amounting to 42 %. Forty-nine (83 %) patients have the disease lupus vulgaris or verrucose cutis type.

Table 2
Results of histopathology and culture examinations

Histo-pathology	Culture		Total
	Positive	Negative	
Positive	19	18	37
Negative	3	3	6
Total	22	21	43

Of the 43 patients for whom the results of histopathology examination and bacteriology are available, the diagnosis is confirmed by histopathology only in 37 (86%) whereas bacteriological confirmation of the disease was possible only in 22 (51%) patients (Table 2).

The intake is continuing and the study is in progress.

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Role of bronchoalveolar lavage in the diagnosis of sputum smear negative but x-ray positive pulmonary tuberculosis in adults

(Ongoing study, 1988-93)

Bronchoalveolar lavage studies (BAL) using flexible fiberoptic bronchoscope were carried out in sputum smear negative, but X-ray positive pulmonary tuberculosis patients to assess the role of BAL in the diagnosis of tuberculosis in comparison with sputum culture examination. Patients with respiratory symptoms of less than 6 months duration with radiographic abnormalities suggestive of tuberculosis and without previous anti-tuberculosis treatment were eligible for the study. All patients had 6 sputum smears negative for acid fast bacilli (AFB). Sputa are then processed for culture examination. Informed consent was obtained from each subject and then BAL were done with instilling 100 ml saline in five 20 ml aliquots at each segment and the fluids were aspirated using a clinical suction apparatus. The study was done in two phases.

Phase I: The BAL were done from radiologically abnormal lobe and contralateral middle lobe or lingula. The fluids recovered from both sites were pooled and an aliquot from the pooled specimen was used for bacteriological examination by smear and culture.

Of 49 suspected patients investigated (Table 1), 17 patients had pulmonary tuberculosis as revealed by isolation of *M.tuberculosis* in sputum cultures; 9 had both

sputum and lavage cultures positive for *M.tuberculosis* , whereas 8 were diagnosed by sputum culture alone. Thus BAL specimens, when pooled, provided diagnosis only in 53% of the patients proved to be tuberculosis by sputum culture. BAL smear was positive only in one patient and none had BAL culture positivity without positive sputum culture.

Table 1

Culture results of pooled BAL specimens against routine sputum specimens-phase I

Pooled BAL specimens culture	Sputum culture		All
	Positive	Negative	
Positive	9	0	9
Negative	8	32	40
All	17	32	49

Phase II: Because of poor yield of *M.tuberculosis* from pooled specimens, lavages were done first from radiologically normal lobe and then from contralateral radiologically abnormal lobe during Phase II. An aliquot of BAL fluid from each lobe was separately subjected to bacteriological examination.

Out of 71 suspected patients during phase II (Table 2), 33 patients had pulmonary tuberculosis as revealed by positive culture of *M.tuberculosis* in sputum. Of these 33, 24 (73%) had both sputum and BAL specimens were positive for *M.tuberculosis* whereas 9 were proved to be tuberculosis by growth of *M.tuberculosis* in sputum alone. Thus, when BAL specimens were cultured separately from radiologically abnormal and normal lobes, the yield from BAL was 73% of 33 patients proved to be tuberculous by sputum culture examination. Only 3 patients had BAL smears positive for AFB and none of the cultures had BAL positivity without sputum culture positivity.

Table 2

Culture results of BAL specimens processed separately against routine sputum specimens-phase II

BAL specimens culture	Sputum culture		All
	Positive	Negative	
Positive	24	0	24
Negative	9	38	47
All	33	38	71

In this study, the culture positivity rate is found to be more with sputum specimens. This may be due to the dilution of the epithelial lining fluid by the externally instilled saline during BAL procedure. Though the positivity rata in BAL specimens had gone up when BAL specimen from radiologically abnormal lobe was analysed separately, the positivity rate has not reached the same level as that of sputum culture examination. Therefore, BAL, done by instillation of saline is not superior to sputum culture examination for diagnosis of smear negative but x-ray positive pulmonary tuberculosis.

Further studies utilising centrifuged specimens of BAL, bronchial aspirations without addition of saline, bronchial brush biopsies and post-bronchoscopy sputum specimens are required to assess the role of fiberoptic bronchoscopy in the diagnosis of sputum smear negative pulmonary tuberculosis.

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

(Ongoing 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 and gastric lavages 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 is being carried out in collaboration with the Institute of Child Health and Hospital for Children, Madras (Professor N. Somu as Principal Investigator).

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 are evaluated. Bronchoalveolar and gastric lavages are done at the Institute of Child Health, while bacteriological investigations are carried out at TRC. So far, 38 patients have been admitted.

The study is in progress.

*****

Controlled clinical study of multi-drug therapy for multi–bacillary leprosy

(Ongoing study, 1988-95)

As mentioned in the previous (1988-1991) annual reports, the Centre has undertaken a controlled clinical trial to assess the relative efficacies of pyrazinamide and rifampicin in combination with clofazimine and DDS in the treatment of multibacillary leprosy, at the Govt. Royapettah Hospital, Madras.

The following four regimens are being investigated:

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 fire 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 has been stopped at the end of 24 months irrespective of Bacterial Index (BI) value unless the patient had reactions and was getting steroids (chemotherapy was extended for one more year and reviewed in such cases).

It is proposed to admit 25 patients to each regimen; 80 patients have been admitted so far to the study. In all, 64 patients (16 NLEP, 17 NLEP + Z, 16 NLEP + Ext. of Rif. and 15 NLEP + Ext. of Rif.+ Z) have information upto 24 months. Of these, 9 patients were excluded (3 died due to reasons other than leprosy -carcinoma of the stomach , suicide, late complication (stricture oesophagus) of attempted suicide by swallowing sulphuric acid, treatment was changed in one due to jaundice; 4 due to non-cooperation and one patient migrated) and the findings in the remaining 55 patients (15 reg.I; 13 reg.II; 15 reg.III and 12 reg.IV) are presented here.

Clinical progress: Clinical progress was assessed by an independent asseasor (using scores based on semi-quantitative assessments, as described in the previous annual

reports), who was unaware of the regimen or bacteriological status of the patients. Combining all the four treatment groups, moderate or marked improvement was observed in 19 (37%) of 51 patients assessed during 0-3 months, 28 (55%) of 51 during 0-6 months, 30 (55%) of 55 during 0-12 and 48 (89%) of 54 during 0-24 month.

Bacterial Indices: The mean bacterial indices were 4.55 lor reg.I, 4.35 for reg.II, 3.89 for reg.III and 4.24 for reg.IV on admission and 3.08, 3.38, 2.78 and 2.86 at the end of treatment period respectively. The mean falls in BI are 1.47, 0.97, 1.11 and 1.38, in the 4 regimens, respectively.

Any additional benefit derived from the supplement of rifampicin and pyrazinamide may perhaps be seen in the follow-up period.

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LABORATORY STUDIES - COMPLETED

Early bactericidal action of pulsed exposure to $REHZ_3$ and RE_3HZ_3 (alt.) in pulmonary tuberculosis patients

(Completed study, 1991-92)

In an earlier study (annual report 1990), using an *in vitro* system, it was found that R, E, H and Z together thrice-weekly and RE on one day and HZ on the next day thrice weekly had equally good early bactericidal action (EBA) on *M.tuberculosis* strains. The present study was undertaken in tuberculosis patients admitted to an ongoing study of short course chemotherapy (SCC) regimens. The EBA of administering RE on one day and HZ on the next day, each combination given thrice weekly (RE_3HZ_3(alt.)), in comparison with REHZ given together thrice weekly ($REHZ_3$) was determined by conducting serial sputum viable count examinations in these patients.

Estimation of viable counts of tubercle bacilli was made from a single overnight collection specimen of sputum collected on days 0, 2, 4, 9 and 16 for both regimens, Patients with drug-resistant organisms, all patients with negative cultures at the first time point, and those who failed to attend or whose culture was contaminated, or results were not available for other reasons at any of the time points, were excluded. There remained 49 patients in the analyses (21 in $REHZ_3$ and 28 in RE_3HZ_3(alt.)). The mean log colony forming units (cfu) of *M.tuberculosis* (per ml) in serial sputum samples from the two groups of patients before start of treatment (day 0) and on days 2, 4, 6 and 16 during treatment are presented in Table 1. In both groups of patients, there was a significant reduction ($p< 0.02$) in the cfu of *M.tuberculosis* (per ml) of sputum during the first 2 days of treatment.

The average fall per day in the number of viable bacilli in the sputum of these patients is presented in Table 2.

Table 1
Mean log cfu of M.tuberculosis per ml in serial sputum samples of tuberculosis patients

Regimen	No.of patients	Mean (SD) log cfu/ml on day				
		0	2	4	9	16
$REHZ_3$	21	6.99 (1.68)	5.58 (1.70)	4.53 (2.31)	3.03 (2.20)	2.67 (2.01)
RE_3HZ_3(alt.)	28	6.29 (2.12)	5.37 (2.15)	4.89 (1.87)	3.50 (2.18)	2.65 (1.82)

Table 2
Change in log cfu of M.tuberculosis in sputum of patients treated with $REHZ_3$ and RE_3HZ_3(alt.)

Regimen	No.of patients	Mean (SD) change in log cfu/ml/day between days		
		0 & 2	2 & 16	0 & 16
$REHZ_3$	21	0.71 (0.98)	0.21 (0.19)	0.27 (0.12)
RE_3HZ_3(alt.)	28	0.46 (0.96)	0.19 (0.16)	0.23 (0.16)

In both groups of patients, the EBA of the $REHZ_3$ and RE_3HZ_3(alt.) regimens, estimated in terms of change in log cfu/ml/day during the first two days of treatment was higher (0.71 and 0.46, respectively) compared to the average fall in the subsequent days (2 & 16 days) of treatment (0.21 and 0.19, respectively). The differences between the two treatment groups in the EBA and the fall in counts during 2 - 16 days were not statistically significant ($p>0.3$). Analysis of covariance also showed no relationship between initial counts and the rate of fall in counts ($p>0.3$).

The results of this study-in tuberculosis patients is in conformity with the results obtained from an independent in vitro study. Both regimens had similar early bactericidal action. The results suggest that splitting up REHZ into RE on one day and HZ on the next day in SCC regimens may not affect the bactericidal action of the regimens.

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Methodology for evaluation of early bactericidal effect of different combinations of anti-tuberculous drugs

(Completed study, 1992)

To evaluate the early bactericidal activity (EBA) of different combinations of drugs in *in vitro* system, viable counts will have to be set up from all the cultures treated with the drugs only after passing the cultures through a 0.45 μm membrane filter to remove the drugs and the medium and then resuspending the bacilli in fresh medium. Afterwards, the drugs will have to be added to the medium again in the required concentrations. This procedure of filtration and resuspension has been used in the earlier study on the EBA of RE and HZ on *M.tuberculosis in Vitro* (annual report 1990) and has been found to result in gradual loss of bacilli over the duration of the experiment. In an attempt to avoid the loss of bacilli, a pilot experiment has been carried out after modifying the earlier method. In the earlier method, the medium from the cultures in universal containers was removed by filtration and the bacilli trapped on the filter membrane were recovered by resuspending them in fresh medium. In the modified method, the cultures are first centrifuged to deposit the bacilli. In the next step, the bacilli in the decanted supernatant are also recovered by filtration and resuspended along with the deposit. The earlier method and its modification have been concurrently compared with a third method along with two relevant control methods. In the third method, the cultures in 50ml sterile conical bottom centrifuge tubes are centrifuged and only the deposits obtained are resuspended in fresh medium. A single batch culture of *M.tuberculosis* H37Rv in duplicate aliquots was used for each of the five methods compared. The results are shown in Table 1.

From Table 1, it appears that the modified filtration method, although not showing an increase in the number of bacilli comparable to the controls, is better than the other methods in minimising the loss of bacilli. Hence, starting with *M.tuberculosis* H37Rv, the proposed study for the evaluation of bactericidal effect of ofloxacin in combination with R, E, H and Z *in vitro* will be carried out using the modified method.

Table 1
Log cfu of M.tuberculosis H37Rv per ml medium in cultures treated with different methods for recovery of organisms

	Method	Log_{10} cfu/ml medium on day of culture 0	2	4	6
A.	Culture in universal containers				
A0	Control	5.21	5.48	6.29	6.91
A1	Filtration & resuspension (earlier method)	5.10	5.24	4.92	4.78
A2	Filtration & resuspension with deposit(modified method)	5.21	5.21	5.43	5.92
B.	Culture in 50ml conical bottom centrifuge tubes				
B0	Control	5.18	5.58	6.07	6.74
B1	Centrifugation & resuspension	5.17	5.48	5.35	5.51

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Invitro activity of ciprofloxacin and ofloxacin on South Indian isolates of M.tuberculosis

(Completed study, 1990-92)

Resistance to rifampicin in strains of *M.tuberculosis* resistant to streptomycin and/or isoniazid indicates poor prognosis. For the treatment of such patients, several new classes of chemotherapeutic agents have been suggested. These include the aminoglycoside capreomycin, long-acting rifamycin derivatives, the 4-fluoroquinolones as well as beta-lactam antibiotics combined with betdactamase inhibitors (Allen & others, 1983; Mitchison and others, 1988). *In Vitro* studies with the long-acting rifamycins, rifapentine and rifabutin as well as with capreomycin and ciprofloxacin were presented in the 1989 report. Of these, ciprofloxacin was the most promising. However, ciprofloxacin is poorly absorbed from the gastro-intestinal tract, and, therefore gives lower blood levels than norfloxacin and ofloxacin after oral administration. Further, ofloxacin has a longer terminal half-life in serum (67 hours). Ofloxacin is already being used in this centre on a selective basis for patients with multiple drug resistance. It was therefore proposed to test the activity of ofloxacin on South Indian isolates of *M.tuberculosis* in comparison with that of ciprofloxacin.

A total of 104 strains, comprising of equal numbers of SHR sensitive as well as SHR/HR resistant were tested. In addition, the standard sensitive strain, H37Rv was tested on different occasions. The drugs were incorporated in Lowenstein-Jensen medium in concentrations of 0.5, 1, 2, 4, 8, 16, 32 and 64 mg/l and inoculated with a standard suspension. The slopes were read after 28 days incubation and the minimal inhibitory concentrations (MIC) determined.

The distribution of strains according to MIC for ciprofloxacin is presented in Table 1. It is observed that the distributions were fairly similar, there being not much difference between sensitive and resistant populations. The geometric means of the MIC of sensitive and the resistant groups were 2.00 and 2.17 respectively.

Table 1
Distribution of MIC of ciprofloxacin against South Indian isolates of M.tuberculosis

Strains	No. tested	% strains with MIC of							
		0.5	1	2	4	8	16	32	64
H37Rv	3	*(100)* [1]							
SHR sensitive	52	*2*	*21*	*52*	*25*				
SHR/HR resistant	52	*2*	*7*	*67*	*23*				
Total	104	*2*	*14*	*60*	*24*				

1. Percentage based on small numbers is indicated in parentheses.

The distrubution of MIC to ofloxacin is presented in Table 2.

Table 2
Distribution of MIC of ofloxacin against South Indian isolates of M.tuberculosis

Strains	No. tested	% strains with MIC of							
		0.5	1	2	4	8	16	32	64
H37Rv	2	*(100)* [1]							
SHR sensitive	52	*2*	*15*	*63*	*19*				
SHR/HR resistant	52	*2*	*13*	*63*	*21*				
Total	104	*2*	*14*	*63*	*20*				

1. Percentage based on small numbers is indicated in parentheses.

Here again there was no difference between the two catagories of strains in MIC, the geometric means of the sensitive and resistant populations being 2.00 and 2.05 respectively.

To conclude, both ciprofloxacin and ofloxacin had similar MICs for sensitive as well as resistant strains of *M.tuberculosis.* Also, there Was no cross-resistance between quinolones and streptomycin, isoniazid or rifampicin.

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Isolation of M.tuberculoais from cerebro spinal fluid(CSF) by filtration method - A pilot study.

(Comp1eted study, 1992)

Normally, the CSF specimen is centrifuged and the deposit inoculated for culture of AFB. As it is suspected that tubercle bacilli have the tendency to float up in the supernatant fluid during centrifugation, the rate of isolation of *M.tubcrculosis* by this procedure could be affected to some extent by the loss of bacilli in the supernatant. If this is true, filtration of CSF through a filter membrane and processing of the membrane for culture can yield higher culture positivity. Hence, the objectives of the present experiment were:

1) to detect the presence of tubercle bacilli in the supernatant fluid and (2) to estimate rate of isolation of *M.tuberculosis* from CSF by a filtration method without resorting to centrifugation.

CSF specimens (CSF): A total of 233 consecutive CSF specimens, obtained from as many children with suspected TBM, were investigated. Excess CSF, when available, was collected in a separate bottle (duplicate sample). The specimens were collected at the Institute of Child Health, Egmore, Madras and transported without delay to TRC, Madras where they were processed for culture of mycobacteria by the routine centrifugation method.

Supernatant of CSF (S-CSF): The CSF specimen was centrifuged as a part of the centrifugation method and the supernatant fluid was separated. The supernatant fluid, available from 196 of the above specimens, was processed separately for culture by a filtration method described below and only these specimens were included in the analysis. The CSF from 37 patients were insufficient and the supernatant fluid was not available for filtration method. Of the 37 samples, 31, 4 and 2 were negative, positive and contaminated respectively, by the centrifugation method.

Duplicate CSF specimens (D-CSF): A total of 112 duplicate CSF specimens, collected from as many of the above children, was processed for culture by the filtration method without resorting to centrifugation.

Filtration method: The S-CSF or the D-CSF was filtered, using a syringe, through a membrane filter (Pore size: 0.45 micron; diameter: 25mm; from Advanced Micro Devices Pvt.Ltd., Ambala, India) assembled in a filter holder (Laxbro, India). The membrane filter was then aseptically transferred to about 7 ml of selective kirchner's liquid medium (SKLM) and incubated at 37°C. The SKLM bottles were examined weekly and centrifuged when the growth was suspected or at the end of 6 weeks, and their deposits subcultured onto LJ medium slopes. The LJ slopes were incubated, examined weekly, upto 8 weeks, and the growth of *M.tuberculosis* obtained was confirmed by standard procedures followed in our laboratory.

M.tuberculosis was isolated from 29 of 196 CSF specimens by the centrifugation method. The S-CSF from these specimens, processed by the filtration method, yielded 11 isolates which included 2 additional positives. Thus, 9 (31%) aut of 29 culture positive specimens were found to contain tubercle bacilli in the S-CSF. This confirms the tendency of the tubercle bacilli to float up in the supernatant fluid during centrifugation.

The culture results of the CSF and the D-CSF specimens from 112 children, processed by the centrifugation and the filtration method, respectively are presented in Table 1.

Table 1
Isolation of M.tuberculosis from CSF by filtration and centrifugation methods.

	Culture result	Centrifugation method			Total
		Pos.	Neg.	Cont.	
Filtration method (D-CSF)	Pos.	7	6	0	13
	Neg.	3	81	0	84
	Cont.	1	13	1	15
	Total	11	100	1	112

The rate of isolation was similar by both the methods. Of 112 specimens, 11 were positive by centrifugation method and 13 by filtration method. Although valid conclusions can not be drawn as the CSF and D-CSF specimens were not randomly allocated to the two methods, they indicate that the filtration method can be employed as an alternative method for the isolation of *M.tuberculosis* from CSF without resorting to centrifugation.

The filtration method is a simpler procedure compared to centrifugation method. It is less expensive, as it requires 1 SKLM and 2 LJ slopes when compared to 4 pairs

of 4 different media and an extra pair of LJ slopes required for subculture in the centrifugation method. As the filter holders with the membrane filters are available in sterile packs, the CSF obtained in Clinics/Hospitals can be filtered and the membranes transported, in SKLM, to a Central/Mycobacteriology laboratory for further culture work.

In view of the practical advantages of the filtration method, there is a need for undertaking controlled investigation to confirm the findings of this pilot investigation.

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Isolation and species-level identification of non-tuberculous mycobacteria obtained from the environmental specimens of the BCG Trial area, Trivellore.

(Completed study, 1991-92)

Earlier studies in the South Indian BCG Trial area had shown that, in this population, the prevalence of infection with non-tuberculous mycobacteria (NTM) is very high. However, studies on the NTM in the environment of this area have not yet been done.

The aim of this study was to isolate and identify the strains of NTM prevalent in the environment of the BCG Trial area, estimate the frequency of their occurrence and also to find out any seasonal variations in their occurrence. The environmental specimens included soil, water and house-dust.

Two methods were chosen for processing each type of specimen: Engbaek'e method using 3% sodium lauryl sulphate (SLS) and 1% NaOH with inoculation on LJ and incubation at 30°C for soil and dust specimens, and Engel's method using 3% SLS and 1% NaOH with inoculation on LJ and incubation at 37°C for water specimens.

Fifteen randomly selected villages in one panchayat union (Kadambathur) of the Trivellore taluk were chosen for the study. Ten specimens each of soil and water and 5 specimens of dust were collected from each village. In addition, eight specimens of water were collected from the Poondi reservoir. All the specimens were collected over a period of one week and were brought to the Centre protected from light. The specimens were processed in lots over a period of three weeks. Specimens were collected on two occasions, once in January after the monsoons and once in June in summer. The same sites were sampled on both the occasions.

All the isolates were subjected to preliminary identification testa to classify them according to Runyon's groups. Detailed identification of the isolates up to species-level was done using 19 biochemical tests as described previously.

Table 1 presents the culture results of the different environmental specimens. Of the specimens collected in January, 74.7% for soil specimens, 64.6% for water specimens

and 25.3% for dust specimens produced isolates of NTM, the corresponding figures for specimens collected in June being 63.3% 41.3% and 18.7%, respectively.

Table 1
Number and percentage of exvironmental specimens yielding mycobacteria on culture

Type of specimen	Month of collection	Total No. of specimens	Specimens with isolates of NTM No.	%
Soil	January	150	112	*74.7*
	June	150	95	*63.3*
Water	January	158	102	*64.6*
	June	109	45	*41.3*
Dust	January	75	19	*25.3*
	June	75	14	*18.7*

Soil specimens: A total of 161 isolates belonging to 12 species and 153 isolates belonging to 12 species were obtained from the soil specimens in January and June, respectively. The majority of the soil specimens yielded isolates belonging to the *M.fortuitum* complex (48.0% and 37.3% in January and June, respectively) followed by isolates belonging to the MAIS complex (16.7% in January and also in June). In the majority of cases,one or two species per specimen were obtained in both January and June.

Water specimens: A total of 167 isolates belonging to 16 speciea and 57 isolates belonging to 11 species were obtained from the water specimens in January and June, respectively. The majority of the water specimens yielded isolates belonging to the MAIS complex (50.0% and 29.4% in January and June, respectively). Compared to the isolation profile of soil specimens, the percentage of specimens yielding isolates belonging to the *M.fortuitum* complex were few (3.2% and 2.8% in January and June, respectively). Most of the specimens yielded one or two species in both January and June.

Dust specimens: A total of 22 isolates belonging to 5 species and 17 isolates belonging to 3 species were obtained from the dust specimens in January and June, respectively. The dust specimens predominantly yielded isolates belonging to the MAIS complex (13.3% and 17.3% in January and June, respectively) and the *M.fortuitum* complex (6.7% and 2.7% in January and June$_i$ respectively). A maximum of 2 species per specimen was obtained in both January and June.

Table 2 presents the species-level identification of the mycobacterial isolates obtained from the environmental specimens at the two time-points.

Table 2
Number and percentage of environmental specimens yielding different mycobacterial species

SPECIES	SOIL		WATER		DUST	
	Jan	June	Jan	June	Jan	June
RAPID GROWERS						
M. diernhoferi	5(*3.3*)	1(*0.7*)	13(*8.2*)	2(*1.8*)	1(*1.3*)	0(*0.0*)
M. fortuitum	72(*48.0*)	56(*37.3*)	5(*3.2*)	3(*2.8*)	5(*6.7*)	2(*2.7*)
M.gadium	11(*7.3*)	22(*14.7*)	6(*3.8*)	1(*0.9*)	0(*0.0*)	1(*1.3*)
M.parafortuitum	1(*0.7*)	0(*0.0*)	0(*0.0*)	0(*0.0*)	0(*0.0*)	0(*0.0*)
M.phlei	5(*3.3*)	4(*2.7*)	1(*0.6*)	1(*0.9*)	0(*0.0*)	0(*0.0*)
M. smegmatis	6(*4.0*)	5(*3.3*)	5(*3.2*)	2(*1.8*)	0(*0.0*)	0(*0.0*)
M.thermoresis-tibile	3(*2.0*)	0(*0.0*)	0(*0.0*)	0(*0.0*)	0(*0.0*)	0(*0.0*)
M.vaccae	5(*3.3*)	4(*2.7*)	8(*5.1*)	3(*2.8*)	0(*0.0*)	0(*0.0*)
Others	18(*12.0*)	28(*18.7*)	2(*1.3*)	3(*2.8*)	1(*1.3*)	1(*1.3*)
SLOW GROWERS						
MAIS complex	25(*10.7*)	25(*16.7*)	79(*50.0*)	32(*29.4*)	10(*13.3*)	13(*17.3*)
M. asiaticum	0(*0.0*)	0(*0.0*)	4(*2.5*)	0(*0.0*)	0(*0.0*)	0(*0.0*)
M.flavescens	1(*0.7*)	1(*0.7*)	1(*0.6*)	0(*0.0*)	1(*1.3*)	0(*0.0*)
M.gastri	2(*1.3*)	0(*0.0*)	0(*0.0*)	0(*0.0*)	0(*0.0*)	0(*0.0*)
M.gordonae	0(*0.0*)	0(*0.0*)	6(*3.8*)	2(*1.8*)	0(*0.0*)	0(*0.0*)
M. kansasii	0(*0.0*)	1(*0.7*)	4(*2.5*)	1(*0.9*)	0(*0.0*)	0(*0.0*)
M. nonchromo-genicum	0(*0.0*)	1(*0.7*)	7(*4.4*)	0(*0.0*)	0(*0.0*)	0(*0.0*)
M. szulgai	0(*0.0*)	1(*0.7*)	2(*1.3*)	3(*2.8*)	0(*0.0*)	0(*0.0*)
M. terrae	6(*4.0*)	3(*2.0*)	12(*7.6*)	2(*1.8*)	0(*0.0*)	0(*0.0*)
M. triviale	0(*0.0*)	0(*0.0*)	1(*0.6*)	0(*0.0*)	0(*0.0*)	0(*0.0*)
M.xenopi	0(*0.0*)	0(*0.0*)	0(*0.0*)	0(*0.0*)	1(*1.3*)	0(*0.0*)
Others	1(*0.7*)	1(*0.7*)	11(*7.0*)	2(*1.8*)	3(*4.0*)	0(*0.0*)
Total no. of specimens	150	150	158	109	75	75

Table 3 presents the distribution of specimens according to the number of species obtained.

Table 3
Distribution of specimens positive for mycobacteria according to the number of species obtained per specimen

Type of specimen	Month of collection	Total no.of specimens	Positive for mycobacteria No. (%)	Negative for mycobacteria No. (%)	No. of specimens with isolates per specimen					Total no.of isolates
					1	2	3	4	5	
Soil	January	150	112(*74.7*)	38(*25.3*)	72	33	6	0	1	161
	June	150	95(*63.3*)	55(*36.7*)	52	30	11	2	0	153
Water	January	158	102(*64.6*)	56(*35.4*)	56	29	15	2	0	167
	June	109	45(*41.3*)	64(*58.7*)	35	8	2	0	0	57
Dust	January	75	19(*25.3*)	56(*74.7*)	16	3	0	0	0	22
	June	75	14(*18.7*)	61(*81.3*)	11	3	0	0	0	17

The isolation profile of mycobacteria from water specimens indicates a predominance of MAIS complex organisms. Isolates belonging to *M.asiaticum, M.gordonae* , and *M.triviale* were also obtained only from water specimens. In contrast, there was a predominance of *M.fortuitum* complex organisms in soil specimens. Though the total number of isolates was low for dust specimens, the majority of these specimen yielded isolates belonging to the MAIS complex, as was seen for water specimens.

Control soil specimens: A limited number (10) of soil specimens from England collected in January 1992 were also studied. The 21 mycobacterial isolates from these specimens belonged to 5 species, namely, *M. bovis, M. diernhoferi, M.flavescens, M.gastri* and *M.smegmatis.* No isolates belonging to either the MAIS complex or the *M. fortuitum* complex were obtained.

*****

Restriction fragment length polymorphism (RFLP) analysis of M.tuberculosis isolates from South India

(Completed study, 1992)

,In this study, RFLP analysis has been used as a strain specific marker (1) to match the pretreatment isolates with the isolates at the time of relapse in patients who relapsed after short course chemotherapy and also (2) to study the differences in RFLP patterns in pretreatment (PT) isolates of *M.tubcrculosis* from South Indian patients. For this RFLP analysis, we have used a probe, an Insertion sequence-IS 986 which is found to be specific for *M.tuberculosis* complex.

Two isolates were obtained, one as pretreatment (PT) and the other as relapse culture (R), from each of the 71 patients who relapsed and were coded to conceal their identity. DNA was prepared and analysed from mycobacterial isolates according to the protocol of Hermans et al. (J.Clin. Microbiol. 1990, 28, 2051). In brief, 3-4 week old cultures grown on 7H9 medium were used for DNA extraction. It was digested with Pvu II,subjected to electrophoresis in 1% agarose gel and blotted onto Hybond-N membrane filter. DNA blots were hybridized to the PCR amplified 386 base pair,Xho I-BamH I fragment of IS 986 which was labelled for detection by enhanced chemiluminescence (ECL-Amersham) technique. The molecular sizes of the relevant DNA fragments of mycobacterial isolates were determined from the internal DNA standards size marker (Lambda - Hind III digest).

After decoding, how many individual patients had been idenfified as having the same RFLP pattern was assessed. Out of 71 patients, for 6 patients either pretreatment (PT) or relapse (R) cultures were contaminated. Of the 65 Comparisons of pretreatment with relapse cultures, only 11 (17%) were found to be same. Remaining 54 patients' cultures, either showed no band, a single band pattern or different RFLP patterns, and hence they could not be matched. Among them, totally 13 cultures did not show any IS 986 copy while 47 cultures showed a single copy of IS 986. Other genetic markers will be used to distinguish strains which have shown either none, one or low number of copies with IS 986, before concluding the relatedness of strains.

To study the diversity of RFLP patterns among South Indian isolates, we separately analysed 98 pretreatment cultures (68 PT from relapse group + additional 30 PT cultures). In this analysis, 39 cultures again showed a single copy of IS 986 in the region of approximately 1 kb fragment. It will be of interest to identify and characterize the strains with single band pattern.

Overall, the isolates from 98 patients showed 51 distinct finger prints which is less polymorphic when compared to strains from other countries.

*****

Evaluation of anti-body level to PPD by ELISA for identification of tuberculous infection

(Completed study, 1992)

The tuberculin skin. test with purified protein derivative (PPD), has been the traditional method for identification of tuberculous infection. But, it is known that the skin test is not absolutely specific, since non-specific cross reactivity to other mycobacteria can also lead to positivity. Also, the test is not 100% sensitive, since a proportion of persons with the disease (and therefore definitely infected) show false negativity due to various causes. Further, the skin test has several practical disadvantages like need for well trained field workers for ita administration and reading its reactions. The test cannot also be repeated under the same conditions as its carry-over effect has

been well documented. Therefore, there is need for a serologic test for identification of tuberculous infect ion.

The objective of the present investigation is to evaluate the role of antibody level to PPD by ELISA for identifying tuberculous infection. The rationale of the investigation consists in identifying two groups of subjects, namely

(i) definitely infected with *M. tuberculosis* and

(ii) definitely not infected with *M.tuberculosis* and evolving a criterion for tuberculous infection on the basis of the distribution of antibody levels in the two groups.

While sputum positive tuberculosis cases can be easily identified as a definitely infected group, the uninfected group can be identified only approximately. Nine months to fifteen months old children without any contact history of tuberculosis in the family or in the neighbourhood may provide the closest group for the uninfected. The reason for omission of children below nine months is the possibility that such children might be possessing maternal antibodies. But for practical reasons, children aged 1-3 years with Mantoux reaction (0 - 7 mm) to tuberculin skin test are considered as uninfected group in this investigation, as tuberculin surveys revealed low infection rates in that age group.

Description of study population: A total of 632 finger-prick specimens, were collected during 1992 from the following categories of subjects from Trivellore BCG Trial area. The Mantoux tests were done with 1 TU of RT23 within 6 months prior to collection of finger prick-specimens.

Category A: Children aged 1-9 years with Mantoux induration of 0 to 12 mm to PPD.

Category B: Children aged 1-9 years with Mantoux induration of 16 mm or more to PPD.

Category C: Tuberculosis cases (bacteriologically confirmed).

Category D: Adults aged 15-24 years with Mantoux induration of 0 to 12 mm to PPD.

Category E: Adults aged 15 - 24 years with Mantoux induration of 16 mm or more to PPD.

Assay design: The samples were tested at a dilution of 1 : 100. The specimens within each category were randomly and proportionately divided into group of 40 specimens each to be assayed in 16 different ELISA plates. In addition to the above 632 specimens, 8 specimens were assayed in each of the 16 ELISA plates as standards. These 8 standards consisted of 3 buffers, sera from 3 sputum positive pulmonary tuberculosis patients and sera from 2 healthy subjects. Thus a total of 640 specimens were assayed with 3 different antigens. PPD (PPD-S, 288 and 289), culture filtrate

of *M.tuberculosis* and culture filtrate of *M.scrofulaceum*. The antigen was coated to the plates at a concentration of 5 µg/ml. Forty test specimens and 8 standards to be assayed in a plate were randomly allocated to different wells within the plate and were also coded to conceal the identity of the specimens.

Exclusions: All the specimens in one ELISA plate (because of a processing error) and all specimens obtained from subjects with BCG scars were excluded from analysis.

Analysis of experimental errors: Each specimen was assayed in 2 consecutive wells in each plate. While the standard deviation (with 48 degrees of freedom) of within plate replicate variation ranged from 0.09 - 0.12 among the 3 antigens, the standard deviation of between plate variation ranged from 0.14 - 0.42 among the 5 standard specimens assayed in each of the 15 plates. In spite of the differences that occurred in setting up the assays on different days and in different plates, it is possible to validly compare the optical density(O.D.) distributions of one category with that of another, in view of the design adopted for the assays.

Results: It was found that the O.D. values to any two antigens were positively correlated and the values of correlation of coefficient ranged from 0.7 to 0.9. Therefore, the analysis is confined to O.D. values to PPD only. Table 1 provides the percentage frequency distributions of six groups of subjects. The descriptions of the groups are given in the foot note to Table 1.

Table 1

Percentage frequency distributions according to OD value to PPD in different groups of subjects

OD value to PPD	A1	C	A2	B2	D	E
0.000 -	35.6	3.6	23.3	17.9	10.3	12.8
0.701 -	13.7	0.9	3.3	3.0	3.1	2.1
0.801 -	6.8	0.9	5.0	1.5	3.1	4.3
0.901 -	12.3	4.5	6.7	10.4	6.2	3.2
1.001 -	4.1	8.2	8.3	7.5	9.3	8.5
1.101 -	5.5	2.7	1.7	7.5	8.2	5.3
1.201 -	4.1	7.3	5.0	6.0	6.2	8.5
1.301 -	2.7	9.1	5.0	9.0	5.2	7.4
1.401 -	1.4	8.2	11.7	10.4	8.2	3.2
1.501 -	2.7	4.5	3.3	6.0	3.1	5.3
1.601 -	2.7	5.5	3.3	4.5	5.2	3.2
1.701 -	1.4	5.5	5.0	3.0	4.1	7.4
1.801 -	1.4	6.4	0.0	1.5	7.2	2.1
1.901 -	1.4	6.4	0.0	0.0	3.1	8.5
2.001 -	0.0	10.9	5.0	1.5	6.2	4.3
≥ 2.101	4.1	15.5	13.3	10.4	11.3	13.8
Total	99.9	100.1	99.9	100.1	100.0	99.9
Total No.of subjects.	73	110	60	67	97	94

Description of groups:

A1 : Children aged 1-3 years with induration of 0-7 mm
C : Bacteriologically positive tuberculous cases
A2 : Children aged 4-9 years with induration of 0-7 mm
B2 : Children aged 4-9 years with induration of 16 mm or more
D : Adults aged 15-24 years with induration of 0-12 mm
E : Adults aged 15-24 years with induration of 16 mm or more.

Discriminant analysis (using BMDP package) was carried out between children aged 1-3 years with Mantoux induration of 0 to 7 mm as uninfected group (A1) and bacteriologically positive tuberculosis cases (C) as infected group to arrive at a cut-off O.D. value to classify subjects into ELISA positives and ELISA negatives to indicate infection and non-infection respectively. The cut-off O.D. value was found to be 1.262,i.e., any subject with a O.D. of 1.262 or higher is classified as ELISA positive and otherwise as ELISA negative. The results of application of this cut-off O.D. value in the two groups used in the Discriminant analysis are shown in Table 2.

Table 2

ELISA Positivity in the two groups used for Discriminant analysis

Group	Size of Mantoux induration (mm)	ELISA				Total No.
		Positive		Negative		
		No.	%	No.	%	
Tuberculous patients	0-7	25	*78*	7	*22*	32
	8 - 12	3	*60*	2	*40*	5
	> 12	52	*71*	21	*29*	73
	Subtotal	80	*73*	30	*27*	110
Children aged 1-3 yrs	0-7	15	*21*	58	*79*	73

It is seen (Table 2) that 58 of 73 children are classified as ELISA negative corresponding to a specificity of 79%. It is also seen that 80 of 110 definite cases of tuberculosis were classified as ELISA positive, corresponding to a sensitivity of 73%. Both these expected values of specificity and sensitivity for identifying tuberculous infection are not satisfactory.

Since assays were undertaken independently and concurrently in the other group of subjects, they were classified on the basis of the cut-off O.D. value and tabulated according to age and Mantoux induration in Table 3.

Table 3
ELISA positivity in different groups of subjects by age and Mantoux induration

Age (yrs)	Size of Mantoux induration(mm)	ELISA				All
		Positive		Negative		
		No.	%	No.	%	
4-9	0 - 7	30	50	30	50	60
	≥ 16	32	48	35	52	67
	All	62	49	65	51	127
15-24	0 - 7	34	56	27	44	61
	8 - 12	21	58	15	42	36
	≥ 16	50	60	33	40	83
	All	105	58	75	42	180

It is generally accepted that an induration of 16 mm or more to the standard tuberculin test is a stringent criterion and definitely indicates past tuberculous infection. It is therefore surprising to find that high percentages of such subjects are classified as ELISA negatives; 52% in 49 year age group and 40% in 15-24 age group with induration of 16 mm or more are classified as ELISA negatives. On the other hand, a great majority of subjects with Mantoux induration of 0-7 mm are likely to be uninfected. In such subjects, it is found that 50% in 4-9 age group and 56% in 15-24 age group are ELISA positive. Therefore, in view of the low expected and observed values of sensitivity and specificity, it is concluded that antibody level to PPD has no role in determining the tuberculous infection.

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Histopathological classification of tuberculous lymphadenitis

(Completed study, 1988-92)

The aims, objectives and the methodology of this study have been described in 1989 annual report.

A total of 352 patients remained in the study, after exclusions.

The major findings of this study are as follows :

1. The cases of tuberculous lymphadenitis are classified into four types : non-reactive, hyporeactive, reactive and hyperplastic. The description of these types has been given in detail in the 1989 annual report. There is a histological spectrum of responses ranging from a poor responding granuloma with necrosis to a good response with virtually no tissue destruction. The two extreme types form only about one third of the total cases and the remaining types exhibit a moderate response with varying amounts of tissue destruction.

2. The presence of B-cells, plasma cells and complement C3d in the lesions of most of these histological types indicate the possibility of the participation of humoral immune response in the genesis of these lesions.

3. Clinically, there are no major differences in the child population, but, amongst the adults where nearly three fourths were females, the following was observed; while the male to female ratio was 1:3 or 4 in the non reactive, hyporeactive and reactive types, it was nearly 1 : 1.5 in the hyperplastic variety. Also, the males belonging to this category had lower Mantoux reaction than the other groups

4. There were no major differences in the bacteriological features of the cases belonging to different types.

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LABORATORY STUDIES - IN PROGRESS

Beta Lactamase activity in mycobacteria and its possible role in cefadroxil resistance

(Ongoing study, 1991-93)

An investigation was undertaken to know the susceptibility of NTM to cefadroxil (See 1991 annual report). All the 40 isolates tested were not susceptible to cefadroxil even at 40 mg/l which is much higher than the peak plasma level (28 mg/l) attained in human beings .

Since Beta lactamase plays an important role in Beta Lactam resistance, a qualitative test (ARRD, 1966,94,965) to detect its activity has been standardised in order to understand its role in cefadroxil resistance in mycobacteria. The NTM isolates mentioned above and the clinical isolates of *M.tuberculosis* (annual report, 1991) are being tested for their Beta lactamase activity.

*****

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

(Ongoing study, 1990 —— 93)

Bioluminescence assay of Adenosine triphosphate (ATP) is an useful method for estimation of viable mycobacteria, and unlike conventional method, the results can be obtained within a short period of time. ATP content of viable bacilli is constant and is proportional to colony forming units. This assay has been found to be useful in rapid susceptibility testing of *M.tuberculosis* cultures to various drugs such as Streptomycin (S), Isoniazid (H), Rifampicin (R) and Ethambutol (E), (Nilsson et al, 1988; Beckers et al 1985). In the present study also, this assay was used for drug susceptibility testing of *M.tuberculosis* to S, H, R & E.

Middlebrook 7H9 broth containing S, H, R & E was prepared and distributed in 1 ml quantities. Single colony growth of cultures in 7H9 medium containing approximately 10^4 organisms/ml was inoculated into plain and drug containing 7H9 medium.

ATP assay was done at the end of 5 days of incubation at 37°C after releasing ATP by Nucleotide releasing agent for bacteria (NRB) and relative light units (RLU) were estimated. Using standard inoculum of the same single colony growth, drug susceptibility testing to S, H, R & E was done by conventional method.

The mycobacterial ATP level was calculated as the percentage of the ATP level in an unexposed control culture (ATP index). The discriminating concentration (break point) for S and E was 1 mcg/ml and, for R and H, it was 0.1 mcg/ml (Beckers et al, 1985). Similar discriminating concentration was used for S and E in the present study. For R and H, the break points were 0.5 mcg/ml and 10 mcg/ml respectively which showed better agreement. ATP index had to be less than 1 to classify the strain as sensitive. Culture having an ATP index of 1 or more is taken as resistant.

The crude agreement between ATP assay and conventional method with regard to S susceptibility was 83%. For E, R and H, crude agreements of 74%, 72% and 68% were observed respectively. After correcting for agreement due to chance, moderate agreement was observed only for streptomycin and isoniazid ($\kappa = 0.61$) and not for other drugs.

Using a different ATP extraction method involving boiling TRIS-EDTA, the assay was repeated for 16 cultures. The results by the TRIS method showed better correlation with the conventional method compared to NRB method.

In order to improve this method, it is proposed to test another 40 *M.tuberculosis* cultures by both ATP assay using TRIS method and by conventional method with standard inoculum and also with inoculum containing 10^4 organisms/ml. Sensitivity testing will be done with 0.1 mcg, 1 mcg and 10 mcg/ml for all the four drugs.

The study is to be continued further.

*****

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

(Ongoing study, 1992–93)

It is very well known that pulmonary tuberculosis is caused by the inhalation of tubercle bacilli in the aerosols. The aerosols could get eliminated by the natural process or get deposited in the apical–subapicalregion of the lung to give rise to disease either immediately or afterwards, This exposure to aerosols is likely to be multiple and larger in an endemic area. In such a situation, the implantation of aerosols containing different subtypes (Phage types, Biotypes, Genotypes) of the organism in different sites or at the same site at different time points, is likely to occur. If this is true, and if all the implants or at least few of the implants multiply to produce disease at the

same time, then significantly large proportion of patients are likely to harbour different types of the organism. Hence it will be of interest to demonstrate different subtypes of the organism in the primary isolates from pulmonary tuberculosis patients by studying the clones for their phenotypic/genotypic characters.

To initiate the investigation, 12 primary cultures with atleast 2 plus growth on LJ slopes, obtained from as many pulmonary tuberculosis patients, were selected. A scoop of the inoculum, representative of the entire culture, was suspended in about 7ml of 7H9 liquid medium and homogenised. Then it was filtered through a filter membrane (Pore size: 5.0 micron; diameter: 25mm; from Advanced Micro Devices Pvt. Ltd., Ambala, India) and the filtrate was divided into 3 aliquots of about 2 mil each and stored at - 70°C. Ten fold dilutions of one of the aliquots were made, before storage, and plated onto plain 7H11 agar medium to get individual colonies. The plates were incubated at 37°C in a CO_2 incubator for 3 weeks. From these plates, 10 individual colonies, picked up arbitrarily, were subcultured separately onto 7H9 liquid medium. The 7H9 culture was divided into 2 aliquots and stored at– 70°C for further studies.

This study is in progress to know the RFLP pattern of the clones of these primary isolates. RFLP pattern will be analysed for these 100 clones and further experiments will be planned subsequently.

*****

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

(Ongoing study, 1992 - 93)

In the study carried out to find out the prevalence of NTM in the environment of the BCG Trial area, organisms belonging to the MAIS complex and the *M.fortuitum* complex were among the most frequently isolated organisms from the different types of specimens. In all, a total of 143 isolates belonging to the *M.fortuitum* complex and 184 isolates belonging to the MAIS complex were obtained from environmental specimens in that study. In addition, 23 isolates belonging to the *M.fortuitum* complex and 138 isolates belonging to the MAIS complex were obtained from subjects in the study area during the same time-points.

Studies are in progress to characterize the isolates belonging to these two complexes up to species, subspecies and biovariant levels based on drug susceptibility patterns, resistance to heavy metals, gas-liquid chromatography and plasmid profiles. Studies done elsewhere have shown that these methods are useful in differentiating between clinical and environmental isolates belonging to these species.

*****

Immune response in the guinea pig model to M.avium-intracellulare (MAI) and M.fortuitum complex isolates from clinical specimens and environment

(Ongoing study, 1992-93)

It is proposed to study the immune response to selected MAI and *M.fortuitum* complex isolates from environmental and clinical specimens in animal model experiments. The study has been started using MAI complex isolates. The design of the experiment is given in Table 1.

Table 1
Design of the experiment[1]

Time (Weeks)	Group 1 Control	Group 2 BCG-SIV	Group 3 MAI-BCG-SIV	Group 4 MAIE-BCG-SIV	Group 5 MAIC-BCG-SIV
0			MAI	MAIE	MAIC
6		BCG	BCG	BCG	BCG
11	ST	ST	ST	ST	ST
12	SIV	SIV	SIV	SIV	SIV
14	Score VC	Score VC	Score VC	Score VC	Score VC
18	Score VC	Score VC	Score VC	Score VC	Score VC
24	Score VC	Score VC	Score VC	Score VC	Score VC

1. Five animals in each group are sacrificed at 14, 18 and 24 weeks
MAI, MAIE and MAIC indicate standard strain, environmental isolate and clinical isolate of MAI respectively
SN : *M.tubcrculosis* South Indian low virulent strain (N9431)
ST : Skin tests using PPD-RT 23 and PPD-B
Score : Scores for extent of disease;VC: Viable count in spleen

A total of 75 guinea pigs were taken and divided into five groups, with 15 in each. Group 1 is the control group. Groups 3,4 and 5 were sensitized with a standard strain, an environmental isolate and a clinical isolate of MAI, respectively. Six weeks later the

animals in groups 2,3,4 and 5 were immunized with an ID injection of BCG. In the fifth week after immunization, all the animals were skin-tested simultaneously with PPD-RT 23 and PPD-B. Six weeks after immunization all the animals were challenged with an IM injection of a South Indian Low Virulent (SILV) strain of *M.tuberculosis* . From each group, five animals will be sacrificed at 2,6 and 12 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 animal experiment, the immune response to 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.

*****

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

(Ongoing study, 1992-93)

Rifampicin (R), ethambutol (E), isoniazid (H) and pyrazinamide (Z) are commonly used drugs in the treatment of tuberculosis. Only limited information is available on the pharmacokinetic interactions between these drugs. It is therefore proposed to undertake 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.

An innovative approach to the chemotherapy of tuberculosis has been the introduction at our Centre of the use of 2 different double-drug combinations on alternate days thus making each 2-drug combination intermittent. This approach will reduce the bulk of drugs to be consumed by the patient daily and the incidence of adverse reactions is expected to be appreciably less. Recent investigations *invitro* and in experimental tuberculosis of the mouse have shown that the double-drug combinations of RZ and RE are superior to RH and REHZ in their bactericidal effect. The decrease in the effect of RH and REHZ combinations has been attributed to an antagonism between rifampicin and isoniazid and also to a possible decrease in the bioavailability of rifampicin leading to lower plasma levels of the drug when administered together with isoniazid. This latter phenomenon is yet to be demonstrated in human subjects and it is therefore desirable to obtain information of the plasma levels of rifampicin when administered alone and in combination with E, H, Z or EHZ.

These investigations are being undertaken in a total of 17 healthy subjects (volunteers) with normal hepatic and renal function. Each volunteer will be investigated on 4 occasions with an interval of one week between occasions according to the general design given in Table 1.

The volunteers will be randomly allocated to the different treatment groups (rows) as given above. A cross-over design will be employed and the drugs or their com-

binations to be administered to the individual volunteers on the different occasions (columns) will also be random. On each occasion, about half the volunteers will receive rifampicin or a combination containing the drug (the first 8 serial Nos.) Plasma concentrations of the drugs administered will be determined at 1, 2, 3, 6, 9 and 12 hours; further, excretion of the drugs and their primary metabolites in urine excreted over a 12-hour period will also be determined on each of the 4 occasions. By adopting the design given above, information on plasma levels and urinary excretion will be available on 8 volunteers on rifampicin alone and on 6 each with the other drugs and combinations.

Table 1
Design of the investigation

Sl. No.	Occasion			
	I	II	III	IV
1	R	RH	RZ	RE
2	R	RH	RZ	RE
3	R	RH	RZ	REHZ
4	R	RH	RZ	REHZ
5	R	RH	RE	REHZ
6	R	RH	RE	REHZ
7	R	RZ	RE	REHZ
8	R	RZ	RE	REHZ
9	Z	H	ZH	ZE
10	Z	H	ZH	ZE
11	Z	H	ZH	ZE
12	Z	E	ZE	EH
13	Z	E	ZE	EH
14	Z	E	ZE	EH
15	E	H	EH	ZH
16	E	H	EH	ZH
17	E	H	EH	ZH

R: Rifampicin; E Ethambutol; H: Isoniazid; Z: Pyrasinamide

The dosages of the drugs to be investigated will be approximately 10 mg/kg body-weight for rifampicin, 25 mg/kg for ethambutol, 12 mg/kg for isoniazid and 35 mg/kg for pyrazinamide.

The interactions between the drugs will be studied on the basis of the mean peak concentrations, area under the time concentration curve and half-life of the drug in plasma and on the renal clearance of the drugs.

The study is in progress and the results are being analysed.

*****

Characterization and purification of antigenic components of M.tuberculosis

(Ongoing study , 1988-93)

Isolation of mycobacterial antigen(s) using affinity chromatography was described in the 1991 annual report. It was reported that the yields of these antigens were quite low and also the antigens were not useful for diagnosis. Hence other methods are being tried for isolation of relevant antigen(s) from *M.tuberculosis* . It was decided to use preparative Isoelectric Focussing (IEF) for isolation and purification of antigens. Since it has been planned to use 5 g of *M.tuberculosis* antigens in IEF, efforts have been made to prepare large quantities of these antigens.

Methods of preparation of antigens: The selected *M.tuberculosis* strain was grown in Sauton's medium for 68 weeks. The culture supernatant was removed by centrifugation at 6000 rpm for 30 minutes and sterilized by filtration through Seitz filter. The filtrate was precipitated using 80% ammonium sulphate saturation , dissolved and dialysed extensively against phosphate buffered saline (PBS). The dialysate was termed culture filtrate antigen (CF). Pelleted bacilli were washed thrice in PBS with Tween 80 , suspended in PBS containing protease inhibitors and sonicated for 5 minutes in cold. The sonicate was passed through a French Pressure cell (20000 psi), centrifuged at 6000 rpm for 30 minutes and the supernatant was used as Pressate antigen.

Large quantities (approximately 2 g) of these antigens have been prepared for two strains (H37Rv and South Indians low virulent) and kept frozen at -20°C which will be purified by preparative Isoelectric Focussing.

Further work is in progress.

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Development of DNA probes for M.tuberculosis

(Ongoing study, 1988-93)

In 1991 annual report, the isolation and characterization of pTRC4 was reported. pTRC4 is a circular plasmid with a mycobacterial fragment (2.2 kb) in pGEM4 plasmid vector. Since the previous results obtained on RFLP and specificity experiments were promising, it was decided to continue the work with pTRC4.

This year, we report the complete sequence of this fragment. The sequencing was done as follows. The clone, pTRC4, has Sp6 polymerase primer on one side of the mycobacterial insert and T7 polymerase primer on the other side of the insert. Using these two primers, short stretch of the clone was sequenced. From this short stretch of sequence, oligonucleotides were designed and were used for further sequencing. Thus by designing various oligonucleotide primers, the whale length of the pTRC4 clone has been deduced. Subsequently, pTRC4 clone was subcloned into pP4 and pEP4. The fragments, PST 1 - PST 1 from pP4 and ECOR 1 -PST 1 from pEP4, were sequenced. Thus the original sequence of pTRC4 was confirmed. The deduced sequence is shown in Table 1. The interesting features of the sequence are:

(1) It has 6 inverted repeats (14 base pairs long with 2 mismatches) occurring at bases (a) 5 and 1804, (b) 29 and 1823, (c) 466 and 1136 and (d) 520 and 544 (e) 911 and 1560, and (f) 968 and 1217.

(2) It has 4 direct repeats which are given in brackets in bold face.

(3) A long palindrome is also located just before the putative open reading frame (1031).

(4) It has more than one open reading frame. It will be interesting to know whether this clone is an insertion element.

Table 1

Deduced sequence of pTRC 4

	1-10	11-20	21-30	31-40	41-50
1	GAATTCCCGA	CCTCAGCGCT	GATCGCCTCG	CGCCGCCGCG	CCTACCCCGC
51	AACCCAACCG	AACGGGCCAT	CCATCCCCAC	CATATTGCGG	GCGTTGGCAA
101	TTCACCGCCG	CCCGTCGGCC	AACAGTAAGC	TCGGCCCGGT	AACCACCGAG
151	TTCGACACGT	CCGCCCCCGCG	GATCCTGGAC	CAGCTGCACG	CATCGGACGT
201	CATCCGCCTG	GACGCATCGG	CGCGATGTAT	CCTTTCTCCG	CAGCACCGAA
251	TCCGCATCGT	ATTCAGATCG	CCTATGGTGC	CC(AGGTTTTC	GG) GCTAATCG
301	CAGTTTGGGT	GTAGGGGGGT	CGGGCCGTGTT	CGTTGTTGCC	GGGGTCA(AGG
351	TTTTCGATG) A	TGAAGGTTGG	TTGGAACAAT	CCAGCGGTGA	CGCCCTTGGT
401	GGCCGGCACT	GGGTTTGGGG	TCCACCGCGA	TGGGTGAGTA	TGGGAAGTGT
451	GGCACGTGTG	AGCCGTCGTG	GTGCACACGG	CCAGTGGCAG	CCCGTTGGCG
501	CCGTCGCCCA	ACGTGTTCTG	CGGGCGGAAA	ATCGGGGGGC	TCTGATTCTC
551	CGCCCTCAGT	TCACGCTCGG	TGCCGGTTAG	CCTCACGCGG	TCAACGTGGA
601	CCCTTCGGTT	ACCCCTGCGA	CCCAATGACT	CGGGGTCCGG	GCGGGCGCTC
651	GcGTGTCGTT	TCAGGACCGG	TGCCGATCAG	ACTCTCGAGT	CTCGGTTGGT
701	CCAGGTCACC	ACGTGGAAAG	GATCGACGCG	GCGGACCGCG	TTGGGGCACC
751	GCTGGGCCAC	GACGTCGGCA	ATCCCGGCGG	CAGCATCAGC	TAAGACTACA
801	GTGATCTGCG	CTGGTCCTGT	CCTTTGACAA	GCCAGAAACC	CTAAGCGACA
851	ACGACGTGCG	CCTACTCAAA	CCAGAAGTCC	ACCCACGGAA	GTGTCAGAAG
901	AGCCACTTCT	TCGCAGGCCG	CCAAACCGCG	AAGGCTGGGC	CGCGGCCCAC
951	CTCCGATGTC	AGCGCCATCA	TCGTCGACCT	GACGACTGCA	GTCCGATTGC
1001	GGAGCTACGC	TAATTCGGTC	CCCCTGCTCA	ATGCCTGACC	GAAATGCACA
1051	TTCGGCAGCG	ACCGCTGTGA	TCGCCCCACT	CGACGAAAGT	CACTGAACAA
1101	ATTCGCCCGC	GCGGTGGCCC	AGCATTACGA	TCGTTGCGTC	TGGACCGCGA
1151	CTGGTGATCG	ACGGAAGGAC	AGATCCGTCT	ATCACCCAT(A	GGTTTTCGAT
1201	G) CCGCGGACC	CGACACCTCG	GGTCGACGAC	GGCTCGTGGG	TCATCGTCGC
1251	TGCCCATTGC	GGCACTACCA	CACAGATGCT	CCGATGTCGC	CCATACCGCT
1301	CGACCGATGC	GCGTTGCCGC	ACCGCATAAT	TCGTGGGCCA	ATGCGCTACC
1351	CTGGCGCAGG	CCCCCGACAT	CGACAGGTTC	ACTGTCGTAT	CGGTGCTCGA
1401	TGCGGACTGC	TATCTGGGGA	TCACTCGAGA	CCAACGTGAT	GCGTCCGCGT
1451	GCCCGCGGCT	GCATGAGCGC	CACCCCGATA	TCCGGCCAAT	CACGATACCC
1501	GCCTGTACGA	ACGGTGGCCA	GCGCGTGATG	TGGACCCTAA	GGGCCCGCCC
1551	GCGTGCTCGA	TCGGCCGCTT	GCGAATTGTG	CTCGCCACCC	TCCGGTTGGT
1601	GGCGGTCGCG	GGGTCGTTGT	CGGTGATGC (C	GATTTTCGA) G	CCACCGCAGT
1651	CGACGCTCGC	GTGGGGGCAT	TTTTCGAGCA	TGGGCCGTCG	TCACTAGGGG
1701	TCTGAATGTT	GTTGGGAACC	AACCATTGGT	TGTTGATTTG	ATCAAGGCAT
1751	CCCGCTCAGC	TCTCGTATGC	CCGGACTCAC	CTGCTTCGAA	ATCTTTCTGC
1801	CCATCGCTGA	GGCCGGCAGT	CTTGGCGGCC	CCGCACGCGA	ACTCGGGTTG
1851	ACTCAACAAG	CTGTGTCAAG	GCGGCTCGCA	TCGATGGAGG	GCCCCAGATC
1901	GGTGCGATTG	GCCATCCGGA	CGACACGTGG	CTCCCAACTC	TCTCCTACCG
1951	GCATCGTCGT	CGCCGAATGG	GCGGCCCGCT	TGCTCGAAGT	CGCCGACGAG
2001	ATCGATGCCG	GCCTCGGCTC	GCTGCGCACC	GAAGTCCGCC	AGCGCATCAG
2051	AGTGGTGGCC	AGCCAGACGA	TAGCCGAACA	GCTGATGGCG	CATTGGATGC
2101	TGTGCTTGCG	GGCCGCCGAC	ATGCGC		

From this sequence, several PCR primers will be designed. These primers will be evaluated for their usefulness in diagnosis.

*****

Human leucocyte antigen (HLA) studies in tuberculosis

(Ongoing study, 1990-95)

The main objective of the project is to use a combination of serological and DNA probes to analyse the phenotype and 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. The progress of the studies is reported under four different sections.

*****

I. HLA studies - Screening of parous women sera for HLA-DR and DQ anti-bodies

(Completed study, 1992)

To add to the existing HLA-A, B, C, DR and DQ antisera, attempts were made to procure more HLA-DR and -DQ antisera during the year.

It has been established that the sera of pregnant women are the most suitable and easily obtainable source for HLA antibodies. Further, it is known that HLA-A, -B and -C antisera contain frequently anti-HLA-DR and -DQ antibodies.

A total of 52 HLA-A and -B positive sera of South Indian parous women obtained by Tamil Nadu Forensic Science Laboratory, Madras were screened for the presence of HLA-DR and -DQ antibodies. The sera were absorbed with pooled platelets to remove the HLA-A, -B and -C antibodies in a micro-method and screened against HLA-DR and -DQ typed B-lymphocyte panel (30 staff members of Forensic Science Lab.). Of 52 sera screened, 20 were found to contain HLA-DR and -DQ antibodies. The behaviour or the reaction patterns of these 20 sera were analysed. The 'correlation coefficient' (r) of a given serum with an antigen was calculated using 2 x 2 contingency table using the formula given below:

$r = (ad - bc) / \sqrt{(a+b)(a+c)(c+d)(b+d)}$

where a, b, c and d represent frequencies in 2 x 2 table with following definitions:

a = The Positive (antigen positive/serum positive)

b = False Positive (antigen negative/serum positive)

c = False Negative (antigen positive/serum negative)

d = True Negative (antigen negative/serum negative)

Of the 20 positive sera, 7 sera were found to be specific for well-defined DR and DQ antigens and the remaining 13 sera were multispecific, reacting with more than 2 HLA-DR and -DQ antigens. Table 1 represents the serum reaction pattern of 7 HLA-DR and -DQ positive sera. Only the first 5 sera (1 to 5) are taken for HLA-DR and -DQ typing, as the r-values of the other two sera (CDM 1501 and CDM 1593) are very low.

Table 1

Serum behaviour of the seven HLA-DR and DQ positive sera[1]

Serum identity	Specificity	True Positive (a)	False positive (b)	False Negative (c)	True Negative (d)	r-value
1. CDM 741	DR 2	15	2	2	11	0.73
2. CDM 1111	DR 3	3	0	1	26	0.84
3. CDM 1217	DR 3	3	0	1	26	0.84
4. CDM 1521	DRW 8	2	0	2	26	0.68
5. CDM 1622	DQ 2	6	2	6	16	0.43
6. CDM 1501	DR 2	13	7	4	6	0.24
7. CDM 1593	DR 7 DQ 2	8	7	4	11	0.27

1. parous women screened against a panel of 30 HLA typed B-lymphocytes

*****

II. HLA studies - HLA distribution in population in Madras and its suburbs

(Completed study, 1992)

A total of 113 healthy subjects (mainly from city colleges and staff of Forensic Science Laboratory,) living in Madras city and its suburbs were investigated to know the Class-I (HLA, -A, -B, -C) and Class-II (HLA-DR, -DQ) antigen and gene frequencies in South Indian population. Fifty two individuals were HLA-typed in Tuberculosis Research Centre and 61 individuals were HLA-typed at Forensic Science Laboratory. The phenotype and genotype frequencies of majority of the class-I and class-II antigens were similar to that of North Indian population and Caucasoid population (Table 2). However an increased gene frequency of HLA A2, B17, DR 7, DRW8, DRW10 and a

decreased frequency of B12, BW35, CW4 and DR 1 antigens were seen in the Madras city population (and its suburbs) than the North Indian population.

Table 2
Comparison of percent gene frequencies[1] of selected HLA-class I and class XI antigens from relevant investigations

	Present study	South Indian[2]	North Indian[3]	European[4] Caucasian
A2	20.7	16.4	11.9	26.0
B12	5.5	5.3	9.0	12.1
B17	12.0	10.1	7.8	4.2
BW35	8.8	10.3	14.5	9.5
cw4	14.3	22.5	8.9	22.1
CW6	17.5	13.2	1.8	15.1
DR 1	4.1	3.0	7.3	6.9
DR 2	27.7	25.7	27.2	13.4
DR 3	9.3	5.3	14.0	10.8
DR 7	20.7	15.4	11.9	12.5
DRW 8	5.7	6.1	0.4	2.7
DRW 10	7.4	7.0	1.1	0.7

1. Percent gene frequency is calculated using the following formula. Gene frequency = $1 - \sqrt{1 - PF}$; where PF = Phenotype or antigen frequency
2. Pitchappan et al., 1984; and 1989 ;
3. Mehra et al., 1986;
4. Baur & Danilous, 1980.

****

III. HLA studies - HLA and Immune response

(Ongoing study, 1990-95)

During the year, HLA-A, -B, -DR and-DQ serological typing was carried out in 11 volunteers (TRC-laboratory) in addition to the 40 volunteers mentioned in 1991 annual report. It is well established that the HLA-Class-II antigens (HLA-DR, -DQ and -DP) are known as Ia antigens (Immune region associated antigens) and their genes as Ir genes (Immune response genes). The role of HLA Class-II antigens and their genes on the antibody and cell mediated immune responses against *M.tuberculosis* antigens will be studied in the laboratory volunteers and tuberculosis patients.

*****

N. HLA studies-Investigation in quiescent and relapse cases of pulmonary tuberculosis

(Ongoing study, 1992-94)

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

During the year, serological determination of HLA-A, -B, -DR and -DQ antigen was carried out in 9 quiescent and 9 relapse cases of pulmonary tuberculosis. 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.

*****

Use of monoclonal antibodies for antigen detection assays.

(Ongoing study, 1990 —— 95)

The mouse monoclonal antibody 31.3 which was found to be useful in antigen detection assay is being used in affinity chromatography procedures to isolate specific antigens from crude sonicate extracts and culture filtrate of mycobacteria.

*****

Generation and characterisation of T–Lymphocyte clones in BCG vaccinated individuals.

(Ongoing study, 1 —— 97)

The T-cell clones generated so far need to be expanded for their characterisation.

As sufficient antigen presenting cells (APC) could not be collected from any of the individuals, an alternative source of APC had to be arranged. The B-lymphocytes of the Mononuclear cells (MNC) of each individual kept stored in liquid nitrogen was transformed with Epstein Bar Virus (EBV) and corresponding B–cell lines were produced. These EBV transferred B-cells were used as APC for propagation for the T-cell clone. The initial experiments did not lead to successful expansion of the clones. Attempts are on for propagation of the clones.

* * * * *

The immunopathology of cutaneous tuberculosis

(Ongoing study, 1992-95)

A clinical study on cutaneous tuberculosis has been undertaken by the Centre. Skin biopsies from patients admitted to this study will examined for histopathological diagnosis and also to study the immunopathology of the disease. The methods consist of routine staining and immunoenzymatic staining to pick up the presence of mycobacterial antigens and complement C3d using polyclonal antisera and to pick up B- and T- cells using monoclonal antibodies. So far, a total of 59 patients have been admitted to the study.

*****

Adrenocortical function in children wish tuberculosis meningitis and tuberculous lymphadenitis.

(Study discontinued in 1992)

This study could not be continued in the Institute of Child Health due to frequent change of doctors in the specified units.

*****

EPIDEMIOLOGICAL STUDIES - IN PROGRESS

Longitudinal study of bacteriological quiescence and relapse in pulmonary tuberculosis under programme conditions

(Ongoing study, 1989-93)

An earlier cross-sectional study of the status of a retrospective cohort of smear-positive pulmonary tuberculosis patients in North Arcot district, showed that 31% of these remained bacteriologically positive even 12-36 months after starting anti-tuberculosis chemotherapy (1989 annual report). Among those remaining positive, 66% had resistance to INH and 12% to rifampicin including 29% with resistance to more than one drug. About half the patients who had received less than 50% of Chemotherapy were smear negative. Since, the cohort was assembled retrospectively, no information on pretreatment specimens was available. Hence a longitudinal study was undertaken in order to have a better understanding of sputum conversion and relapse in relation to the amount of chemotherapy received and the pretreatment drug resistance status. The methodology for this study has been described in detail in the 1989 annual report.

Initially, three centres, namely, the District Tuberculosis Centre, Vellore and the Government Hospitals at Arani and Gudiyattam were selected for this study based on their case load. Later, the Government Sanatorium at Adukkambarai was also included.

Table 1
Study population

	No.
Total number of patients with a positive smear	2553
Exclusions:	
Self-reported previously treated patients	985
Found to be previously treated patients on home visit	71
Dead	28
Outside area	463
Migrated	147
New cases (admitted to the study)	859

Any newly diagnosed patient with a positive smear, living within 20 km from these centres and available for follow-up was eligible for this study. Of the patients whose smears were positive in these four centres, 859 were eligible for admission to the study (Table 1).

Of 2,090 cases that came from the study area (i.e., 20 km radius from these centres), only 1034 were found to be new cases. When these were visited within 10 days of their registration, it was found that 28 were dead and 147 had migrated. The remaining 859 formed the study population for longitudinal follow-up (Table 1). Of these, 755 (88%) yielded positive cultures at intake, of which 14(2%) were not examined for sensitivity, 588 (78%) were sensitive and 153 (20%) were resistant to one or more anti-TB drugs.

The pattern of initial drug resistance according to the treatment received later by these patients is given in Table 2.

Table 2

Drug sensitivity status of culture positive new cases

Treatment	No. Eligible	No. Examined	Sensitive to SHR	Resistant to					
				S	H	R	SH	HR	SHR
SCC	488	479	387	6	49	7	19	7	4
Non-SCC	205	201	150	5	31	4	6	3	2
No Rx.	62	61	51	1	5	1	2	1	-
Total	755	741	588	12	85	12	27	11	6

The coverages obtained for the three monthly follow-up examinations are shown in Table 3.

Table 3

Follow-up status of new cases registered

Status	Month of follow-up			
	3	6	9	12
Eligible	859	698	648	610
Available	698	648	610	574
Dead	82	33	18	19
Left	74	17	20	17
Visitors	5	-	-	-

Information was available at 12 months for 574 patients and 152 were dead within one year of starting of treatment. Of these, 82 deaths had occurred within the first

3 months. This gives an early mortality of 10% among new patients who had been started on anti- TB treatment. Within one year, 128 (15%) patients had migrated.

Sputum conversions: Among patients with sensitive organisms started on short course chemotherapy (SCC), 154 (62%) out of 247 examined had converted at 6 months. Of the 154, 27 (18%) had relapsed at 12 months. For those started on standard regimen, 65 (71%) out of 91 converted at 6 months and 10 (15%) out of 65 became sputum positive at 12 months. Among those with resistant organisms put on SCC, 74 (57%) out of 129 converted at 6 months and 12 (16%) of the 74 relapsed at 12 months. Of those who were put on standard regimen, 20 (38%) out of 52 converted at 6 months and 4(20%) out of 20 became sputum positive at 12 months.

*****

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

(Ongoing study, 1990–93)

This study was undertaken in Kadambathur panchayat union with the objective of developing a methodology for periodic screening of children in the community to identify the children likely to be suffering from tuberculosis as early as possible and to investigate such children. The different aspects of this methodology - training and assessing the health workers who could undertake this screening, examining the feasibility of carrying out the investigations at the community level etc. - have been reported earlier (annual reports 1989 and 90). A total of 6049 children of age 0-9 years spread over 15 randomly selected panchayats of Kadambathur formed the study population. The intake and follow-up procedures have been reported last year (see annual report 1991).

Table 1
Status of children on periodic screening for tuberculosis

Status on screening	Number of children at		
	Intake	6 months	12 months
Total screened	6049	6196	6438
Abnormality attributable to TB	757	795	809
Referred for:			
Clinical examination	291	315	154
Investigations	331	210	273
Clinical exam and investigation	78	63	119
Only symptomatic Rx given	786	1383	1317

The intake was completed in December 1991. Complete house to house screening of all children was undertaken at 6,12 and 18 months. The coverages obtained for different examinations at intake, 6 and 12 months follow up have been reported earlier. The status of children on periodic screening for tuberculosis upto 12 months is reported in Table 1.

On screening at intake and follow-up, about 13% of children were identified as having abnormalities attributable to tuberculosis. Of these, 19-40% were referred for clinical examination only, 20-44% for investigations only and 8-15% for both.

X-ray examination: All children screened at intake had a full plate chest x-ray PA view which was read by independent readers. The distribution of the types of shadow and their interpretation is given in Table 2.

Table 2

Status of children on periodic screening for tuberculosis

Type of shadow	Radiographic status				
	Non TB	Inactive TB	Doubtful TB	Definite TB	Total
Hilar adenitis and/or Parenchymal opacity	27	-	612	16	655
Pneumonia/Broncho-pneumonia	290	-	150	3	443
Pleural fluid	1	-	-	-	1
Cavity fibrosis or bronchiectasis		-	3	1	4
Diffused mottling	7	-	5	-	12
Calcification	-	4	-	-	4
Total	325	4	770	20	1119

All children with clinical or radiographic abnormality were re-x-rayed after 3 months. The radiographic status of these children at 3 months in relation to the same at intake is shown in Table 3.

At intake, the x-rays of 20 and 770 children were classified as definite and doubtful for tuberculosis respectively; of these, only 7 (35%) and 153 (20%) respectively were confirmed as having TB (definite or doubtful) at the 3rd monthly examination.

Table 3
Radiographic status of children at 8-months in relation to the same at intake

X-ray status at intake	X-ray status at 3-months						Total
	Normal	Non TB	Inactive TB	Doubtful TB	Definite TB	Not Examined	
Normal	321	14	-	63	3	-	401
Non-TB	236	22	-	51	-	16	325
Inactive-TB	2	-	1	1	-	-	4
Doubtful-TB	546	32	3	147	6	36	770
Definite-TB	12	1	-	5	2	-	20
Total	1117	69	4	267	11	52	1520

Clinical examination: Clinical examination was carried out for all children according to the algorithm and also for those with x-ray abnormalities. In addition, ten percent of normal children were examined as controls. Altogether 1318, 343 and 597 children were examined clinically at intake, 6 and 12 months respectively. Of these 50, 19 and 42 children were suspected to have definite or doubtful TB and were investigated further.

Table 4
Validation of screening method with clinical examination as standard

Screening at Intake	Clinical diagnosis at Intake		
	Abnormal[1]	Normal	Total
Doubtful cases of TB on screening	35 *(8)*[2]	501 *(2)*	536 *(10)*
Normal/other non-TB abnormality	15 *(0)*	745 *(7)*	760 *(7)*
Total	50 *(8)*	1246 *(9)*	1296 *(17)*

1. Abnormal includes doubltful TB or definite TB
2. Figures in parentheses show Histopathology/Bacteriology positive cases

The usefulness of the algorithm to identify children having abnormalities requiring further investigations for tuberculosis is validated with clinical diagnosis as the stan-

dard (Table 4). The sensitivity and specify of screening is 70% and 60% respectively. Of the 760, considered to be normal by the algorithm, 15 have ben considered to be possible cases by the pediatrician. These had, however, been reffered for clinical examination based on an abnormal x-ray.

The usefulness of the algorithm is also evaluated against bacteriological or histopathological investigations (Table 5). Ten out of the cases were identified by screening method. The seven cases that were missed also normal clinically and were picked up by abnormal x-ray.

Table 5
Validation of screening method with bacteriology or histopathology as the standard

Screening	Bacteriology or histopathology		Total
	Positive (cases)	Negative (non cases)	
Doubtful cases of TB on screening	10	399	409
Normal or non-TB abnormality	7	1456	1463
Total	17	1855	1872

It must be noted, that the definition of 'case' used here is very stringent. Other definitions such as persistent clinical or x-ray abnormality should also be considered for validation. It would also be of interest to study the follow-up statue of these children at subsequent rounds.

Further analysis in this direction is in progress.

*****

Development of surveillance methodology for tuberculosis

(Ongoing study, 1990-94)

A long-term community-based epidemiological study has been undertaken with the general objective of identifying a simple, inexpensive tool for the surveillance of the tuberculosis situation in a community (see 1990 annual report). The parameter(s) to be used can be related to infection or disease or both. The following parameters are being studied:

(a) Prevalence and trend in the age-specific infection rates in the community.
(b) Age-sex specific distribution of adult bacillary cases and the trend of this distribution separately for prevalence and incidence cases during follow-up.
(c) The proportion of chronic excretors among prevalence cases and their drug sensitivity, at each round.

The methodology was described in detail in the previous annual reports.

Two panchayat unions viz., Kadambathur and Tiruvelangadu were initially included for the survey. By December 1992, 30 panchayats (15 panchayats in each panchayat union) hade been covered, and a population of 69,777 has been registered. Coverages of 90% or more have been obtained for all examinations except tuberculin testing (81%) in the 15-24 years age group (Table 1).

Table 1
Coverages for different examination at intake

Examination	Age Group (Years)	Population eligible	Population covered No.	%
Symptomatic	≥ 10	54414	50428	*93*
X-ray	≥ 10	54414	49180	*90*
Tuberculin test	≤ 14	22357	20656	*92*
- do -	15-24	12986	10475	*81*
Sputum	≥ 10	8000	7637	*95*

Tuberculin testing among 15-24 years age group and all sputum positive cases was initiated in August 1991. This was done since a review of the distribution of reaction sizes in children below 15 years showed that there was no clear antimode in the age groups 0-4, 5-9 and 10-14.

The curve for the distribution among the age group 15-24 years shows an antimode at 8 mm (Figure 1). Of the 403 sputum positive cases detected at intake, 298 (74%) were tuberculin tested. The distribution of sputum positive cases and population (24 years or less) according to Mantoux reaction (mm) is shown in Figure 2. It is observed that the two curves intersect at 12 mm. All individuals with 12 mm or more induration are defined as positive reactors. The annual risks of infection (ARI) were estimated from the proportion of positive reactors and are given in Table 2. The prevalence of scar is low (18%). Infection rates and annual risks of infection rise sharply with age.

Fig.1. DISTRIBUTION OF TUBERCULIN REACTION BY AGE GROUPS

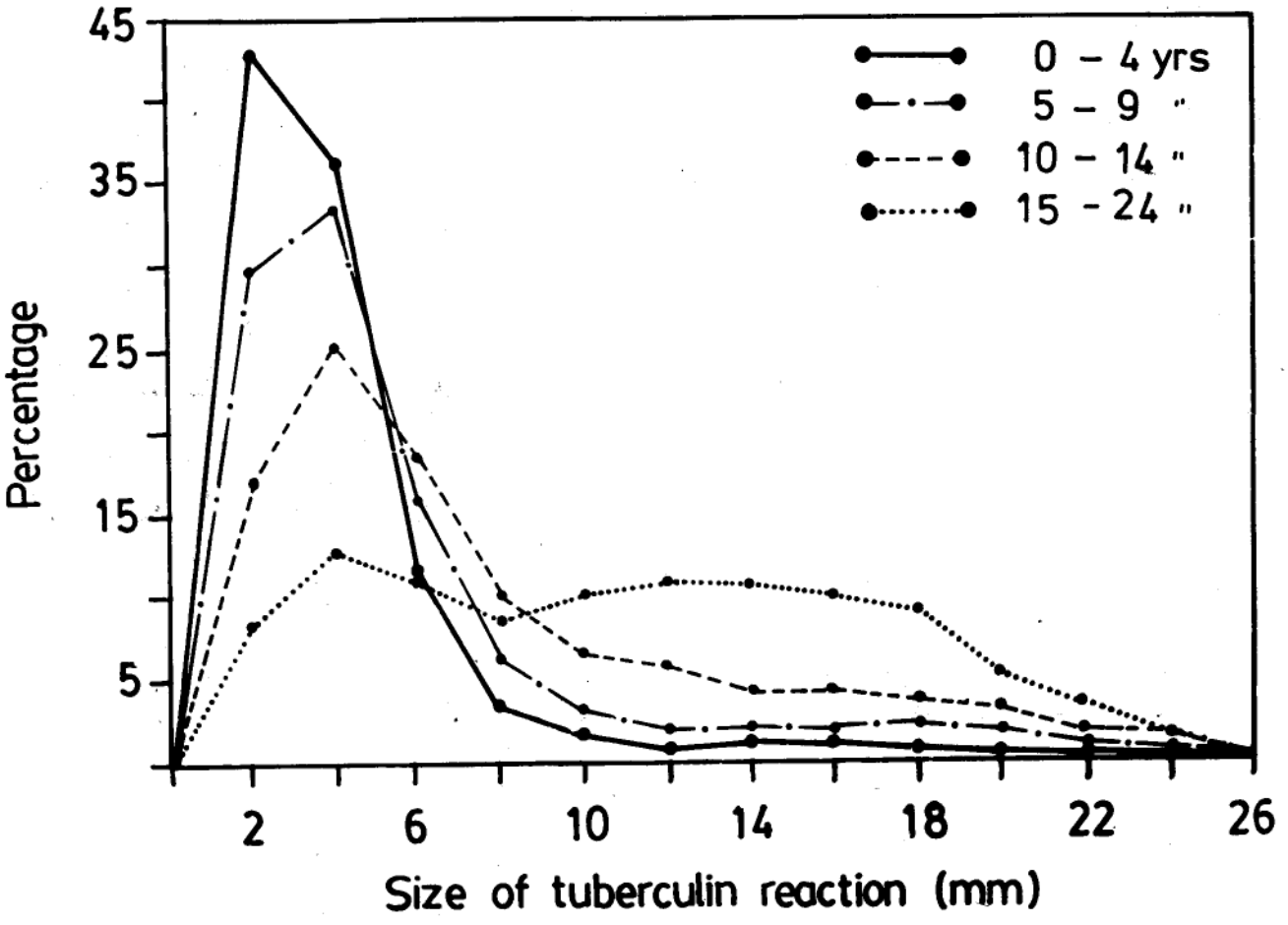

Fig.2. DISTRIBUTION OF TUBERCULIN REACTION SIZE IN THE GENERAL POPULATION AND IN SPUTUM POSITIVE CASES

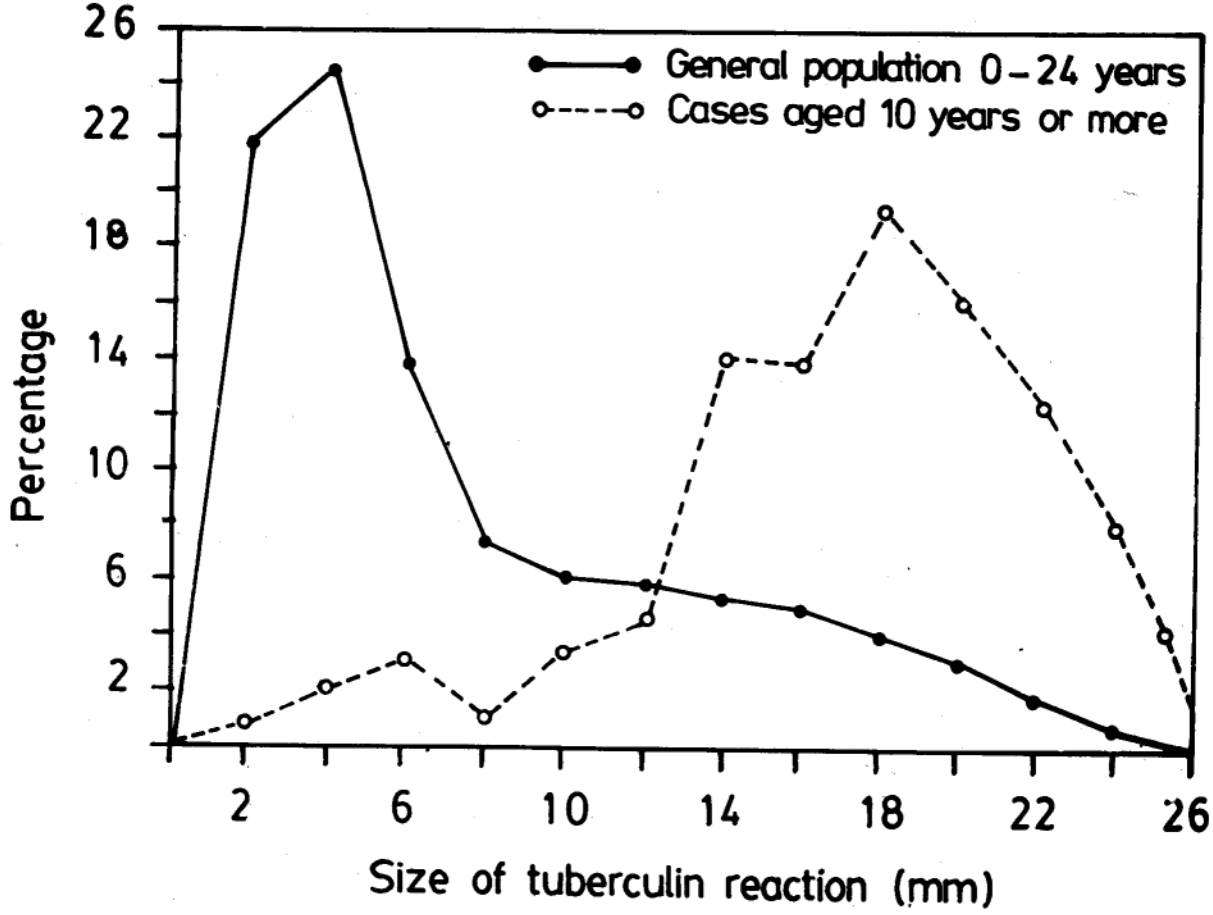

Table 2

Prevalence of infection and the estimated annual risks of infection

Age (in years)	Scar present			Scar absent			
	Number examined	Reactors (≥ 12mm) No.	%	Number examined	Reactors (≥ 12mm) No.	%	ARI[1] (%)
<5	2277	133	*5.8*	4317	170	3.9	*1.6*
5-9	1191	149	*12.5*	6143	696	11.3	*1.6*
10 - 14	677	187	*27.6*	6051	1393	23.0	*1.9*
15 - 24	1594	1030	*64.6*	8881	4365	49.1	*3.3*
All	5739	1499	*26.1*	25392	6624	*26.1*	

1. Annual Risk of Infection

The population eligible for the selective follow up at 6 monthly intervals will be those with chest symptoms or x-ray abnormals in the previous survey (round) and the household contacts of bacteriological positive cases. The persons not x-rayed at intake will be examined only in the first follow up (at 6 months). Sixteen panchayats (all the fifteen from Kadambathur and one from Tiruvelangadu) were covered for 6 month selective follow-up. Fourteen panchayats and 6 panchayats have also been covered for 12 and 18 months selective follow-ups respectively (Table 3).

Table 3

Coverage for different examinations during follow-up

	6 months		12 months		18 month	
	Eligible	Covered %	Eligible	Covered %	Eligible	Covered %
Symptomatic	5561	*90*	4634	*88*	1954	*90*
X-ray	5561	*86*	4634	*87*	1954	*89*
sputum	2652	*94*	2159	*96*	963	*94*

Two more selective follow-ups at 24 months and 30 months will precede next resurvey at 3 years.

At intake and follow up rounds, 1181 x-ray positive cases and 509 sputum positive cases were identified up to December 1992. The distribution of the sputum positive cases by smear and culture is given in Table 4.

Table 4

Bacteriologically positive cases detected

Survey (Round)	Groups covered	Smear + Culture +	Smear - Culture +	Smear + Culture -	All
Intake	30	164	192	47	403
6 month	16	14	28	9	51
12 month	14	4	25	6	35
18 month	6	2	13	5	20
Total	-	184	258	67	509

About half the total number of cases are positive only on culture and 13% are positive only by smear.

Of the 509 bacteriologically positive cases, 442 were culture positive.The results of sensitivity to anti-TB drugs are available for 390. Of these, 47 (12%) had a history of previous treatment. The resistance pattern of cases to streptomycin (S), isoniazid (H) and rifampicin (R) at the time of detection is shown in Table 5.

Resistance to H in persons who have not had anti-TB treatment is 10.2% and to rifampicin is 2.6%.

Table 5

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

History of previous treatment	Number culture positive	Sensitive to SHR	Resistant to					
			S	H	R	SH	HR	SHR
No	343	294	12	18	2	10	4	3
Yes	47	37	1	2	1	5	1	-
Total	390	331	13	20	3	15	5	3

The intake to the study has been temporarily discontinued in December 1992 after the completion of two panchayat unions due to resource constraints.

The follow-up is in progress,

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Surveillance of individuals infected with the Human Immuno–defiency Virus for the development of tuberculosis

(Ongoing study, 1989-99)

A longitudinal cohort study was started in July 1989 with the objective of monitoring the occurrence of tuberculosis among individuals with the Human Immunodeficiency Virus (HIV) infection. The methodology has been described in detail in the 1990 annual report. Briefly, addresses of pereons found to be HIV positive by ELISA are obtained from surveillance centres. These individuals are traced and registered along with their contacts. After initial investigations at TRC, eligible individuals are selected to be followed up every six months.

So far, addresses of 464 individuals had been received from the different centres of which 195 (42%) were traced and registered (Table 1). At the initial examination at TRC, 44 were found to be ELISA negative and another 12 to have tuberculosis. After excluding these 56 individuals from analysis, 139 individuals are available for Cohort analysis, a great majority of them being less than 30 years of age and all of them being HIV positive either by Western Blot (WB) or repeat ELISA. (After Western Blot was discontinued, repeat ELISA on an independent sample was taken to be the confirmatory test as a National Policy from Sept.1992).

These 139 individuals have been followed up at every six month and coverages during the first two years of follow-up have gradually fallen from 80% at 6 months to about 52% at 24 months. During the 24 months period, 8 cases of tuberculosis were found among the 139 individuals (1 at 6 months, 3 each at 12 and 18 months and 1 at 24 months).

Table 1
Study population

Source	Referred	Not trace-able	Died	Traced and registered	Elisa neg	At TRC Elisa positive		
						WB Pos	WB EQ	Twice[1]
STD Clinic	336	251	7	78	23	55	-	-
Vigilance Home	100	1	-	99	13	82	1	3
Others	28	9	1	18	8	9	1	-
Total	464	261	8	195	44	136	2	3

WB: Western Blot; EQ: Equivocal result

1. Positive on two independent samples by ELISA

Chemotherapy of tuberculosis patients with HIV infection: Tuberculosis patients are routinely screened for HIV infection at TRC and some selected Government Health facilities. As a result of this screening and the follow-up of HIV infected individuals described above, a total of 57 cases of tuberculosis were found.

All the 57 patients had had treatment started for TB; 30 at TRC and the others outside. While patients at TRC were started on a nine-month regimen containing Ethambutol, INH, Rifampicin and Pyrazinamide for the first two months followed by INH and Rifampicin for the next 7 months, other patients were treated with routine SCC regimens. Twenty three had completed the treatment, of which one died. For all the 23, the sputum became negative. Of the remaining 34 patients, 11 have died, 11 are sputum positive and 12 sputum negative. Four patients had organism with multiple drug resistance and two of them have died.

Infection among contacts: Among 119 contacts registered, 21 (18%) had HIV infection at intake and 3 were infected during follow up (two within six months).

The study is in progress.

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Surveillance of tuberculosis patients for human immuno deficiency virus infection

(Ongoing study, 1991-93)

This study has been undertaken to study the proportion and trend of HIV infection among tuberculosis patients over time. All tuberculosis patients who reported to the centre (TRC), Madras, or the DTC/TB Sanatorium at Vellore, or at Pondicherry are screened irrespective of their bacteriological status. Extra pulmonary TB cases are also included. At TRC, chest symptomatics are also screened in addition to TB patients. Blood specimen obtained from each patient was tested by ELISA for HIV antibody. For specimens found to be positive, ELISA was repeated; if positive twice by ELISA, the specimens were tested by Western Blot (WB) for confirmation. ELISA tests were done at TRC and JIPMER, Pondicherry. All Western Blot tests were done at CMC, Vellore.

The number screened, the number and proportion positive by ELISA and WB are shown in Table 1. overall, about 1% of TB patients are positive for HIV infection as confirmed by ELISA and Western Blot.

Table 1
HIV infection in TB patients by referral centres

Centre	Number screened	ELISA positive		western Blot		
		Once	Twice	Positive	Equivocal	Negative
TRC,Madras	4464	99	34	13	21	NA
DTC ,Vellore	112	3	5	3	2	-
GTBS ,Vellore	1952	25	39	24	5	10
Chest Clinic, Pondicherry[1]	195	NA	3	3	NA	NA
GTBS, Pondicherry[1]	846	NA	6	6	NA	NA
Total	7569	127	87	49	28	10

1. Figures upto July 92 only; NA indicates data not available

Detailed analysis of 2064 patients screened from Vellore is given in Tables 2 and 3. It can be seen that in every age group, there is a higher proportion of HIV (confirmed by Western Blot) among males as compared to females. Both males and females in the age group 20-29 years appear to be at higher risk compared to other age group (Table 2).

Table 2
HIV infection in TB patients by age and sex

Age (Yrs)	Sex	Total screened	ELISA positive Once	ELISA positive Twice	Western Blot Positive	%	Equivocal	Negative
<10	M	3	-	-	-	-	-	-
	F	8	-	-	-	-	-	-
	Both	11	-	-	-	-	-	-
10-19	M	73	2	3	-	-	2	1
	F	60	1	-	-	-	-	-
	Both	133	3	3	-	-	2	1
20-29	M	245	2	10	7	2.9	1	2
	F	144	6	2	2	1.4	-	-
	Both	289	8	12	9	3.1	1	2
30-39	M	277	5	9	7	2.5	1	1
	F	138	1	1	1	0.7	-	-
	Both	415	6	10	8	1.9	1	1
40-49	M	364	1	9	7	1.9	1	1
	F	105	3	4	1	1.0	-	3
	Both	469	4	13	8	1.7	1	4
50-59	M	330	6	3	-	-	1	2
	F	55	0	-	-	-	-	
	Both	385	6	3	-	-	1	2
> 60	M	233	1	3	2	0.9	1	-
	F	29	0	-	-	-	-	-
	Both	262	1	3	2	0.8	1	-
Total	M	1525	17	37	23	1.5	7	7
	F	539	11	7	4	0.7	-	3
	Both	2064	28	44	27	1.3	7	10

Of twenty-seven positives confirmed by Western Blot, 23 were from those with pulmonary tuberculosis patients (smear or x-ray positive, - Table 3).

Table 3

HIV infection in TB patients by case category

Case category	Number screened	ELSA positive		Western Blot		
		Once	Twice	Positive	Equivocal	Negative
New Smear positive	648	7	20	14	1	5
New x-ray positive, sputum negative	772	10	15	9	3	3
Sputum positive, inspite of adequate chemotherapy	153	3	4	-	2	2
Extra pulmonary tuberculosis	17	1	-	-	-	-
Cases on retreatment	474	7	5	4	1	0
Total	2064	28	44	27	7	10

The study is in progress.

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STATISTICAL STUDY - COMPLETED

Stimulation Ratio : An index for lymphoproliferative response and ita sta–tistical properties

(Completed study, 1992)

Lymphoproliferative assays are carried out in several immunological investigations for different purposes. The proliferative response is basically measured by the radioactive thymidine uptake and expressed as counts per minute (CPM). In the second IUIS/WHO report (Clin.expt.imm., 1988,74: 494), it is recommended that the results are best expressed as total uptake of radioactivity after subtraction of background values but might also be expressed as a relative proliferative response index. As early as 1977, Oppenheim and Billa Schecter (Manual of Clinical Immunology, Chapter 9) suggested that the basic data should be transformed to get a normal distribution for proper statistical assessment because that large changes in the magnitude of ^{3}H-TdR incorporation by cultured lymphocytes with increasing duration of incubation are based on their exponential growth rate and small differences in growth rate become magnified with time and result in non-normal distribution of data. In the same year, Rosanna Dei and Pasqualae Urbano (Journal of Imm. Methods, 1977, 15: 169), after a review of 172 papers published in 1975 in three leading journals, pointed out that the presentation of mean CPM ± SEM (standard error of the mean) in a paper as a clue to an improper statistical handling of the data and considered log-transformation as essential for valid statistical analysis. Another disadvantage of the use of mean CPM ± SEM is that it renders the comparison of the results of one investigation with another difficult. However, untransformed data is still used for presentation of results and statistical analysis in several published papers. For example, the investigations of Fitzpatrick, E.A. et al. (3377), Brodie, C and Gelfand, E.W. (3492), Burstein, H.J. et al. (3687) and Miconnet, I. et al. (3706) from volume 148 of the Journal of Immunology (1992) provide a sample of studies in which mean CPM ± SD/SEM has been used for analysis.

An index designated as Stimulation Ratio and defined as the ratio of the mean of logarithms of CPM in stimulated cultures to the mean of logarithms of CPM in

unstimulated (control) cultures is proposed in this paper as a relative lymphoproliferative response index for general use in statistical analysis. This index is now expressed as a percentage, although it has been used in all earlier studies from our Centre as simple ratio and termed as stimulation index. Since the term stimulation index was used by other investigators with a different definition, it is now called as Stimulation Ratio. The purpose of this paper is to provide theoretical justification for its general use, describe its statistical properties and empirically demonstrate its properties.

Statistical properties of Stimulation Ratio : It is expected that it will be normally distributed with a theoretical mean value of 100. Its value indicates the existence or non-existence of positive proliferative response and also provides a quantitative measure for the response. The estimate also possesses the statistical property of consistency.

Materials and methods : Lymphoproliferative assays were carried out on the lymphocytes of six healthy subjects as described by Paranjape et al. (Ind. J. Tub. 1988, 35: 163). The stimulants consisted of 48 all possible combinations of two concentrations (zero and 5 μg/ml) of PPD with two concentrations (1% and 2.5%) of each of 12 specimens of ascitic fluid, obtained from six tuberculous ascites patients and six non-tuberculous ascites patients. In addition, two sets of controls were set up for each subject, one set with neither ascitic fluid nor PPD and the other with PPD alone. The assay was set up in triplicate and assays for two specimens of ascitic fluid could not be set up for one subject.

The original objective of the assays was to find out whether the tuberculous and non-tuberculous ascitic fluids would produce different levels of proliferative responses in infected healthy subjects. Statistical analysis indicated that the ascitic fluids generally failed to produce positive proliferative response, while PPD produced significant responses. In view of this finding, CPMs in stimulated cultures of each subject have been treated, for the purpose of this analysis, as constituting two sets of replicates, the cultures with PPD in the stimulant as one set and all the other cultures as the second set.

For calculating the index, the CPMs with 2.5% ascitic fluid are taken as values in stimulated cultures while CPMs in 1% of the same ascitic fluid are taken as values in control cultures. This procedure was adopted to empirically demonstrate the properties of Stimulation Ratio. Properties of another index, Reactivity Ratio, defined as the ratio of the mean of CPMs in stimulated cultures to the mean of CPM in control cultures will also be presented for comparison, as it (termed as Stimulation Index) has been used earlier by some investigators (Regina C.C.Dorea et al. Clin.Exp.Immunol. 1988, 71: 26; Nelson et al., Cellular Immunology, 1987, 104: 99). Specimen values of CPM with corresponding values of Stimulation and Reactivity Ratios are shown in Table 1.

Table 1
Specimen values of CPM and corresponding stimulation and reactivity ratios

PPD Conc.	Ascitic fluid Conc.(%)	Identity No.	Replicate values (CPM)			Stimulation Ratio	Reactivity Ratio
Nil	1	TB1	2169	1476	874	-	-
	2.5	TB1	3896	1206	746	101.03	129.41
	1	TB2	1702	1119	2416	-	-
	2.5	TB2	461	1854	1828	95.15	79.11
	1	NON-TB1	749	520	456	-	-
	2.5	NON-TB1	381	517	1050	100.80	112.93
	1	NON-TB2	794	606	1238	-	-
	2.5	NON-TB2	442	880	610	95.44	73.24
5 μg	1	TB1	4486	4837	4530	-	-
	2.5	TB1	6611	3128	6732	101.37	118.90
	1	TB2	242	5782	6090	-	-
	2.5	TB2	3864	4906	3850	97.22	78.32
	1	NON-TB1	6312	3162	3236	-	-
	2.5	NON-TB1	4909	3556	3912	100.22	97.38
	1	NON-TB2	3848	4460	3678	-	-
	2.5	NON-TB2	5398	4358	3558	101.13	111.08

Results: The material, as described earlier and illustrated in Table 1, gave rise to 140 observations for each ratio. The frequency distributions of these ratios are presented in Tables 2 and 3.

The distribution of Stimulation Ratio is unimodal with a mean of 99 and very nearly symmetric around its mean. Ninety four per cent of the observations are concentrated in the narrow range of 89 to 112, with a standard deviation (SD) of 6.08. As for the Reactivity Ratio, it is spread over a wide range (22 to 271) with a mean of 101.2 and an SD of 43.5..

Table 2
Frequency distribution Ratio of Stimulation Ratio

Stimulation Ratio (percentage)	Frequency	
	No.	%
83 - 85	3	*2.1*
86 - 88	3	*2.1*
89 - 91	4	*2.9*
92 - 94	18	*12.9*
95 - 97	22	*15.7*
98 - 100	41	*29.3*
101 - 103	25	*17.9*
104 - 106	10	*7.1*
107 - 109	4	*2.9*
110 - 112	7	*5.0*
113 - 115	2	*1.4*
116 - 123	1	*0.7*
Total	140	*100.0*

Table 3
Frequency distribution of Reactivity Ratio

Reactivity Ratio (percentage)	Frequency	
	No.	%
22 - 49	11	*7.9*
50 - 59	10	*7.1*
60 - 69	10	*7.1*
70 - 79	17	*12.1*
80 - 89	11	*7.9*
90 - 99	16	*11.4*
100 - 109	14	*10.0*
110 - 119	18	*12.9*
120 - 129	9	*6.4*
130 - 139	4	*2.9*
140 - 149	3	*2.1*
150 -159	1	*0.7*
160 - 169	2	*1.4*
170 - 179	5	*3.6*
180 - 271	9	*6.4*
Total	140	*99.9*

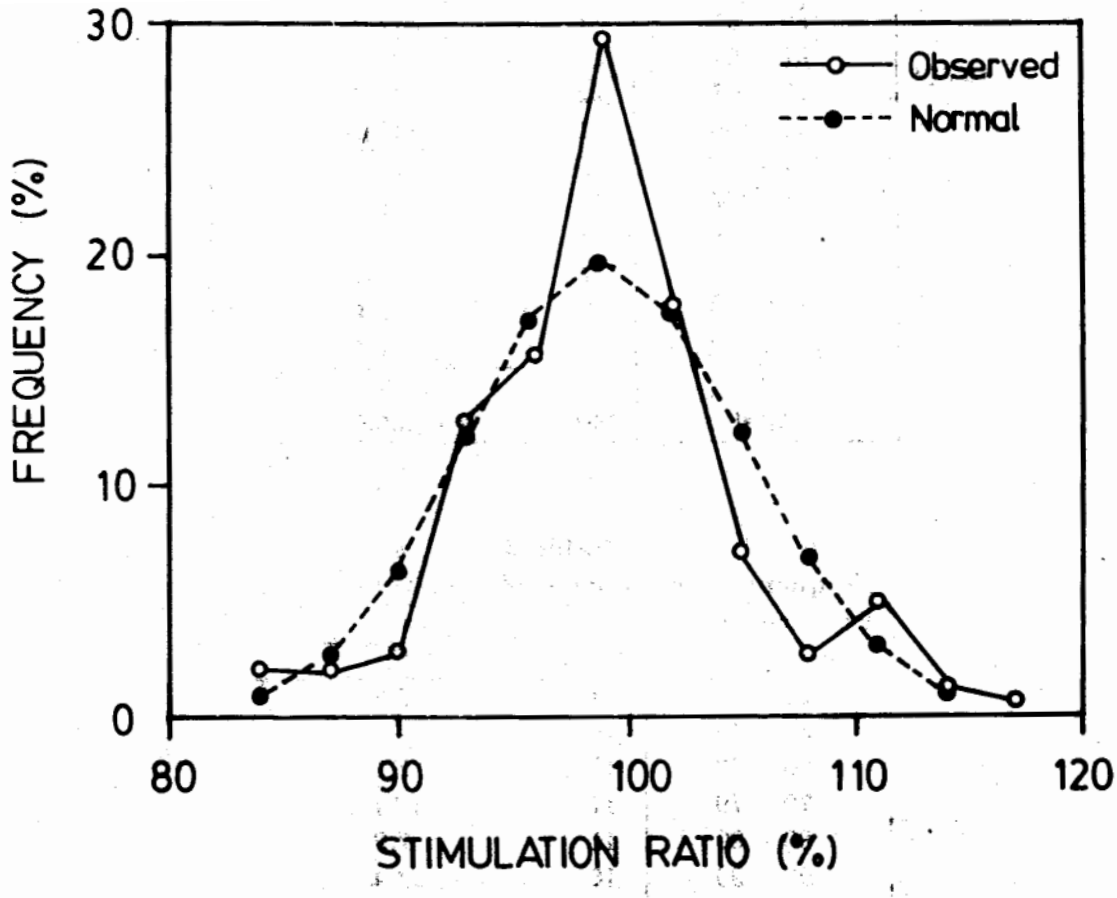

Fig. 1 Observed and expected percentage frequency curves for stimulation ratio.

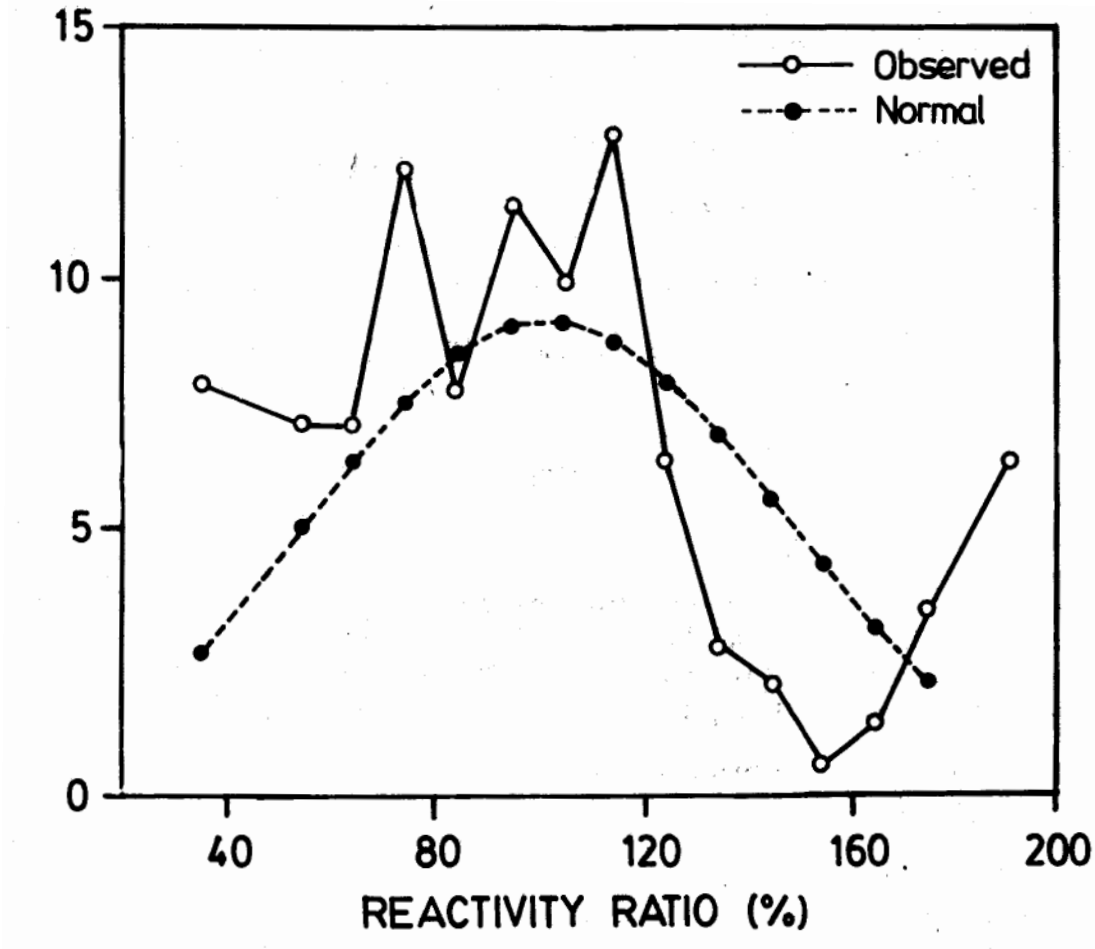

Fig 2 **Observed and expected percentage frequency curves for reactivity ratio**

The percentage frequency curves along with expected Standardized normal curves are presented in Figures 1 and 2. Comparison of the observed and expected frequency curves in Figure 1, clearly indicates that the normal approximation for the distribution of Stimulation Ratio fits in nicely and is justified. In fact, the observed frequency curve for the Stimulation Ratio is more peaked than the expected distribution. On the contrary, it is seen from Figure 2 that the observed and the expected frequsncies for the Reactivity Ratio do not fit well and it is therefore concluded that Reactivity Ratio does not follow normal distribution, implying its unsuitability as relative response index.

Although the assays are not generally carried out in such a way that the individual CPMs in stimulated and their corresponding control cultures could be paired, such a possibility can be assumed for theoretical discussion. If $(y_1, x_1),(y_2,x_2), ...(y_n,x_n)$ represent the logarithms of CPMs of the stimulated and the corresponding control cultures respectively, the following two estimates of Stimulation Ratio

$$T_1 = 100(1/n)(\Sigma y_i/x_i)$$

$$T_2 = 100(\bar{y}/\bar{x})$$

where $\bar{y}$ and $\bar{x}$ denote the respective mean values can be considered. Of these two estimates, T_1 is biased and does not admit a simple correction for bias, where as T_2 was found to be a consistent estimator (C.R.Rao, Advanced Statistical Methods in Biometric Research, 1952, p 154). Hence T_2 is to be preferred.

Interpretation of Stimulation Ratio : With very weak stimulants, it has been empirically shown that Stimulation Ratio is normally distributed with a mean of 99. and an SD of 6.08. But, a hypothetical normal distribution with a mean of 100 and an SD of 6.08 can be considered for arriving at a criterion for positive proliferative response. Since larger values of Stimulation Ratio provide evidence of positive response, the 95th percentile of this hypothetical distribution can be chosen as the criterion for classification of proliferative response. Thus the response of a subject is classified as positive if Stimulation Ratio is higher than 110 (95th percentile) and otherwise as negative.

Empirical evaluation : The Stimulation Ratio criterion for positive proliferative response evolved above is evaluated in a study independently carried out earlier at our Centre (R.S.Paranjape et al. 1988). In that investigation, the assays were carried out with four replicates for each specimen by different technicians following same methodology with two concentrations (10 and 25 μg/ml) of PPD in a total of 105 tuberculosis and non-tuberculous patients. To classify the response in those patients, unpaired student's *t* -test (with 6 degrees of freedom) was carried out for each patient to test the equality of means in stimulated and control cultures after logarithmic transforma-

tion of CPMs. The results of 210 such t-tests are presented in Table 4 against Stimulation Ratio criterion.

Table 4
Comparison of Stimulation Ratio criterion against the result of Student's t-test.

Stimulation Ratio	Student's t-test		All
	Significant > 1.934 [1]	Not significant ≤ 1.934	
> 110.0	71	10	81
≤ 110.0	3	126	129
All	74	136	210

1. 95th percentile of Student's t- distribution with 6 degrees of freedom.

It is seen from Table 4 that the two classifications agree in 197 (94%) out of 210 comparisons. It is also important to note that 10 of the 13 disagreements are in one direction, i.e. with non-significant t-value but significant Stimulation Ratio. While it is difficult to attribute definite reasons for this finding, there can be two possibilities: one is the smaller number of degrees of freedom for the t-test and the other is the two means compared in the t-test are correlated but the covariance could not be taken in to account in the test procedure. Therefore it is gratifying to note that Stimulation Ratio criterion has been confirmed in as high as 94% of the cases by t-test.

Discussion: This paper proposes Stimulation Ratio as a relative index for interpreting the results of lymphoproliferative assays. A simple criterion based on the value of Stimulation Ratio has been evolved to classify the proliferative response. The reliability of the criterion has been empirically evaluated from an independent investigation and found to be as high as 94%. Experimental data with 140 observations have been presented to show that Stimulation Ratio follows normal distribution, although experimental data did not consist of genuine replicates as some very weak stimulants have been used.

Ziegler et al (Journal of Immunology, 1974, 113: 2035) reported that logarithmic transformation of CPM provided better approximation to normal distribution over square or cube root transformations. For the validity of t-test, the log. transforms of CPM in stimulated and corresponding control cultures should follow normal distribution. If so, the question arises whether Stimulation Ratio (defined as the ratio of the means of log. transforms of CPM in stimulated and control cultures) also can follow normal distribution. In other words, if x_1 and x_2 follow bivariate normal distribution, how is the ratio, x_1/x_2, distributed? Fieller (1932) and Hinkley (1969)

derived the mathematical form of the distribution of this ratio and showed that it is approximately normal when the coefficient of variation of the denominator variable is small and tends to zero. Other conditions under which this ratio can be approximately normally distributed have been studied in greater detail, theoretically and also by simulation, by Shanmugalingam (The Statistician, 1982, 31: 251). One important conclusion of his study is : The distribution of the ratio is symmetric about μ_1 / μ_2 and approximately normal when p = C2/C1, where μ_1 and C1 denote the mean and coefficient of variation of x_1, μ_2 and C2 the mean and coefficient of variation of x_2 and p the coefficient of correlation between x_1 and x_2. It can be easily seen that, in the absence of any stimulation, the numerator (x_1) and denominator (x_2) of the Stimulation Ratio will have the same mean and same Standard deviation with correlation coefficient very nearly equal to unity. The values of the relevant parameters in the current experimental data are : μ_1 = 23.5, μ_2 = 23.8, C1 = 14.0, C2 = 13.7 and p = 0.92. Thus Shanmugalingam's study also provides justification for our assumption that Stimulation Ratio follows normal distribution.

Further, if the logic of Oppenheim and Bill Schecter is followed, the log. transforms of CPMs represent the growth rates of blast transformation of cells. Then either the difference or the ratio between the mean values of log. transforms of CPMs in stimulated and control cultures can be chosen as response index. As growth rates might vary from individual to individual, it is thought that the ratio of growth rates provides a better measure than the difference in the growth rates.

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ELECTRONIC DATA PROCESSING

During the year further progress was made in developing new computer programs for the studies, (a) Development of Surveillance methodology for tuberculosis, (b) Follow-up of tuberculosis cases detected and put on treatment in surveillance study and (c) Short -course chemotherapy under District Tuberculosis Programme. Necessary modifications were carried out in previously written computer programs to suit the study requirements precisely. Regular support is being given by the Electronic Data Processing (EDP) unit to transfer files (draft texts) from IBM compatible personal computers to Apple Macintosh computer, in order to obtain neat laser print outputs of final versions of papers to be sent for publication.

Development of Surveillance methodology for tuberculosis : For this study, since 18-month follow-up of individuals was newly introduced during the year, computer programs were specially developed to provide the required printouts to field teams and to process the data on the VAX-11 computer. Data base was created for 18-month follow-up and integrated with the main database for the Surveillance study. Computer printouts were provided for 9 panchayats during the year. In addition computer printouts were provided for 18 panchayats in which 6- months follow-up was due and 14 panchayats in which 12-month follow-up was due.

District Tuberculosis Programme : Four new computer programs were developed to take print outs according to performances of PHI's.

Payroll : System designing and system analysis were carried out during the year to computerise the Pay-roll of the Centre including the Epidemiology Unit. Programming support was obtained from a private software vendor to get tailor-made menu driven screen-oriented software developed on IBM compatible computers to meet our specific requirements. The administrative staff were trained to directly use the software to update the database and obtain the required print-outs. Paybills, payslips for staff members and various schedules are being prepared directly from the computer databases.

For editing and printing the annual report on research activities of the Centre, personal computers are being used as described in the 1991 annual report.

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LIBRARY & INFORMATION SERVICES

The library (and Documentation Centre) of TB Research Centre has a fairly large collection of books and bound volumes of scientific journals on different aspects of tuberculosis and other related subjects. The library also gets a number of (103 Indian and 107 foreign) scientific journals. In addition the resources of the British Council Library, Madras are also made use of by renewing the institutional membership facility. A major and significant development during the year has been the introduction of several new Information and Documentation Services to meet the ever growing needs of the scientific community of the Centre.

I. Information and documentation services

(a) Current awareness service : As part of a computerised Selective Dissemination of Information (SDI) service, the library is bringing out 'Tuberculosis Alert' an in-house fortnightly publication. This contains bibliographic details of recent articles published in Indian and foreign journals and about monograph on tuberculosis, mycobacterial and allied diseases.

(b) Literature search : For scientists of the Centre, literature searches were conducted by utilising the MEDLARS databases available at National Information Centre (NIC) State Unit, Madras and CD-ROM data bases such as Medline, Bookfind, Book Bank etc. at British Council Library, Madras.

(c) Library resource sharing: With a view to improve library's resource facilities, sharing of periodicals of mutual interest has been introduced on a weekly basis with Vector Control Research Centre (VCRC), Pondicherry. A total of 41 periodicals are shared mutually. 'Current Contents' on Discs are also shared from VCRC.

(d) NUCSSI, CAPS, ADONIS: The National Union Catalogue of Scientific Serials in India (NUCSSI) of Indian National Scientific Documentation Centre (INSDOC) has been introduced. This would facilitate the location and procurement of micro documents (scientific articles) published in journals not subscribed by the Centre. The library has commenced the Contents, Abstracts and Photocopy Services (CAPS) of INSDOC. The ADONIS CD-ROM database of NIC, New Delhi is also made use of for obtaining the full-text of recent articles.

II. Computerisation activities

(a) The library has commenced the computerisation of the Book-Catalogue (Current & Retrospective) using CDS/ISIS software.

(b) For monitoring the receipt of journals and periodicals, payment of subscriptions etc., computers are being used.

(c) A database of TRC publications and an exclusive bibliographic database on 'TUBERCULOSIS' have been created and are being updated regularly.

III.Regional Sophisticated Information Centre (RSIC) on communicable diseases

The Scientific Advisory Committee (SAC) has recommended the proposal for developing TRC as a Regional Sophisticated Information Centre on Communicable Die eases with emphasis on TUBERCULOSIS (RSIC-T) with assistance from WHO; The proposal has been approved by ICMR and is under the consideration of WHO.

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APPENDICES

TRAINING PROGRAMMES

WHO Fellows

Mr.Yin Kim Tye and Miss Tong Swee Sin, Malaysia from 26.10.92 to 6.11.92.

Trainees

The following underwent training in different departments as follows:

Bacteriology

Dr.V.P.Myneedu, Microbiologist and a technician, Lala Ram Sarup Institute of Tuberculosis and Allied Diseases, New Delhi, for 4 weeks from 1.1.92.

Three M.D. (Microbiology) and 4 M.Sc.(Microbiology) students from B.A.L.M. Post-graduate Institute of Basic Medical Sciences, Taramani, Madras, from 17.1.92 to 22.1.92.

Eighteen students of Diploma Course in Medical Laboratory Technology, Voluntary Health Services, Adyar, Madras, from 4.5.92 to 16.5.92.

Cardio-Pulmonary Medicine

Dr.Aloke Gopal Goshal, North Bengal Medical College, Siliguri, West Bengal, from 10.4.92 to 29.4.92.

Dr.B.Jayakrishnan, P.S.G. Institute of Medical Sciences and Research, Coimbatore, from 6.7.92 to 10.7.92.

Immunology

Mr.Sanjiva Bimal, M.Sc., from Rajendra Memorial Research Institute of Medical Sciences, Patna, from 17.8.92 to 27.8.92.

Pathology

Mr.Kannadasan,Ph.D student, from Zoology department, Nandanam Arts College,. Madras for 1 month, from 1.3.92.

General

Ms.Brenda Henry and Ms.Teresa Lappanan, students from School of Social Work, Michigan State University, U.S.A., from 30.6.92 to 13.8.92.

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 - 2 batches.

Medical College, Calicut - 1 batch.

Post-graduate students

Mrs.Nalinashi, Ph.D. student from Central Leprosy Teaching and Training Institute, Chengalpattu.

Dr.Prem Kumar, M.D.(TB & CD) student from TB & ID Hospital, Visakapatnam.

Dr.Pravin Bhat, MD(TB & CD) student from Goa Medical College, Goa.

Thirteen M.Sc.(Microbiology) final year students from Gulbarga University, Gulbarga.

Four M.Sc.(Microbiology) students from Christian Medical College, Vellore.

M.D.(TB & CD) and DTRD students from Institute of Thoracic Medicine, Madras.

Nursing and para-medical students

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

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

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

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

B.Sc.(Nursing) students from Kalyani Hospital, Madras - 1 batch.

ICMR-WHO WORKSHOP

A 2-day Regional workshop organised on 14th and 15th March 1992 at Puri, Orissa (covering Puri, West Godavari and Raichur districts), was inaugurated by Dr.S.P.Tripathy, DG, (ICMR). The subjects covered and the names of the speakers are given below :

Lectures for Medical Officers

Subject	Speaker
National Tuberculosis Programme	Dr.S.P.Gupta
Case finding - Clinical aspects	Dr.I.Ranga Rao
-d0- Bacteriological aspects	Dr.C.N.Paramasivan
Short course chemotherapy	Dr.M.S.Jawahar
SCC in DTP - Review of 18 districts	Dr.R.Prabhakar
Case holding	Dr.N.M.Sudarsanam
Management of health services programme	Dr.Jagdish Bhatia
Documentation	Mr.P.V.Krishnamurthy
Community participation	Dr.K.Thilakavathy

Panel Discussion

Moderator :	Dr.R.Prabhakar
Members :	Dr.C.N.Paramasivan, Dr.N.M.Sudarsanam, Dr.I.Ranga Rao, Dr.J.Bhatia, Dr.N.Srinivasa Rao, Dr.Brahmanandam and Dr.R.K.Nath

Lectures for Lab Technicians, Treatment Organisers and Drug Distributors

Subject	Speaker
Role of paramedical workers in DTP	Dr.N.M.Sudarsanam
Case finding	Mr.B.N.Gopalan
Chemotherapy in DTP	Dr.M.S.Jawahar
Case holding	Dr.K.Thilakavathy
Documentation	Mr.B.Janardhanam

Panel Discussion (KAP Questionnaire)

Moderator : Dr.R.Prabhakar

Members : Dr.C.N.Paramasivan,
Mr.N.M.Sudarsanam,
Mr.B.Janardhanam and
Mr.A.S.L.Narayana

STAFF DEVELOPMENT PROGRAMME

1. Mr. K. Sankaran attended a Workshop on "Common Laboratory Equipment - Their Theory, Practice and Maintenance" at Physiology Department, Dr.A.L.M. Poet-Graduate Institute of Basic Medical Sciences, Taramani, Madras, from 13-20, March, 1992.

2. Mrs. Jayalakshmi Vadivel was awarded a 3-month TCTD Fellowship under British ODA programme for training in Nursing Education in Queen Margaret College, Edinburgh,U.K., from March, 1992.

3. Dr. M.Kannapiran was awarded Ph.D. in Biochemistry by the University of Madras, Madras, during 1992.

4. Dr. Daniel Herbert was awarded Ph.D. in Microbiology by the University of Madras, Madras, during 1992.

5. Mr.V.Sundaram underwent a 6-week part-time computer training course on "UNIX and C programming" conducted by the Ramanujan Computing Centre, Anna University, Madras, from 28th September, 1992.

PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

Name of conference, venue and date	Title of paper	Name of staff member
International Conference on Respiratory Medicine, Apollo Hospitals, Madras, 31 January - 1 February, 1992	Tropical eosinophilia - Recent developments	Dr.V.K.Vijayan (Chair person for a scientific session)
-do-	Tuberculous Iympha-denitis	Dr.M.S.Jawahar
International Academy of Chest Physicians & Surgeons (East India Chapter), V Zonal Conference on Cardio-Pulmonary Medicine, Calcutta, 15-16 February, 1992	Tropical eosinophilia-Recent advances	Dr.V.K.Vijayan
-do-	Chronic airflow limitations Broncholitis obliterans	-do-
XI National Congress on Respiratory Diseases, Kozhikode, 19-22 February, 1992	Clinical application of flow-volume loops	-do- (Chair person for a scientific session)
-do-	Alveolitis in victims of toxic gas leak at Bhopal	-do-
-do-	Effect of cortico-steroids on alveo-litis of chronic tropical eosinophilia	-do-

Name of conference, venue and date	Title of paper	Name of staff member
XI National Congress on Respiratory Diseases, Kozhikode, 19-22 February, 1992	Effect of treatment on transfer factor and its subdivisions in tropical eosinophilia	Dr.V.K.Vijayan
Joint Annual Conference of Association of Physicians of India & Cardiological Society of India,Southern Zone, Bangalore, 10 June 1992	Drug resistent tuberculosis	Dr.MS.Jawahar
2nd National Update on Pulmonology, Kovai Medical Centre and Hospital Ltd., Coimbatore, 12-13 September, 1992	Interstitial lung disease, diagnosis and management	-do- (Chair person for a scientific session)
XIII Annual Conference of the Indian Association of Biomedical Scientists, Trivandrum, 19-20 September, 1992		Dr.K.V.Kuppurao (Delegate)
First Conference and National Update on Allergy, Asthma and Applied Immunology, Indian Academy of Allergy, Bangalore, 16-18 October, 1992	Recent advances in tropical eosinophilia	Dr.V.K.Vijayan (Chair person for a scientific session)

Name of conference venue and date	Title of paper	Name of staff member
X Annual Conference of Indian Society for Medical Statistics and National Seminar on Bio-statistical and Demographical Aspects of AIDS and AIDS Control in India, Bombay, 1-3 November, 1992	Modelling the spread of AIDS in India	Dr.P.Venkatesan
- do -	Analysis of spinal tuberculosis survival data : Cox and Weibull models with covariates	-do-
-do-	Exploring nominal, ordinal and block variables in the path analysis of medical data	-do-
33rd Annual Conference of Association of Microbiologists of India, Goa, 5-7 November, 1992	Use of cetylpyridinium chloride for the storage of sputum samples and isolation of tubercle bacilli	Dr.N.Selvakumar
- do -	Bioluminescence assay in mycobacteria	Dr.Vanaja Kumar
4 th National Conference of the Respiratory Chapter of the Indian Academy of Paediatrics, Bangalore, 7-8 November,l992	Pulmonary function testing in children	Dr.Soumya Swaminathan
First Conference of Tamil Nadu Science Congress,26-28 November, 1992	Survival analysis of censored data	Dr.P.Venkatesan

Name of conference, venue and date	Title of Paper	Name of staff member
47th National Conference on Tuberculosis & Chest Diseases, Bombay 26-28 November, 1992	Application of Restriction Fragment Length Polymorphism (RFLP) analysis to the origins of relapse and isolated positive cultures from tuberculous patients in Hong kong	Dr.D.Sulochana
- do -	Serial CT Scan follow up of brain tuberculoma treated with short course chemotherapy	Dr.Rajeswari Ramachandran
- do -	Response of pulmonary TB patients with initially drug resistant organisms to treatment with short course chemotherapy	Dr.Rema Mathew

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

Name of the event, venue and date	Title of paper	Name of staff member
IAL Workshop on "Advances in Laboratory Techniques with reference to patient care in leprosy". Bhilai, 5th January, 1992	Development of immuno-histological techniques for understanding nerve damage in leprosy	Dr.V.D.Ramanathan
Tamilnad Hospital Ltd. Cheran Nagar, Madras, 17 January, 1992	Flow volume loops (Guest lecture)	Dr.V.K.Vijayan
CME Programme, Dr.A.L.M. Post-graduate Institute of Basic Medical Sciences, Taramani, Madras, 1-8 February, 1992	Basic applied immunology and immunological techniques (4 lectures)	Dr.Ramesh S.Paranjape
XI National Congress on Respiratory Diseases, Kozhikode, 19-22 February,l992	-	Dr.A.M.Reetha

Name of the event, venue and date	Title of paper	Name of staff member
Brain Storming Meeting on Trends in Laboratory, Animal Science and Technology, Indian Perspective , Laboratory Animal Information Service Centre (LAISC), National Institute of Nutrition, Hyderabad, 21-22 February, 1992	-	Dr.Ramesh S.Paranjape
CME programme, Tamilnad Hospital Ltd., Cheran Nagar, Madras, 27 February,l992	Current concepts in behavioural immunology	Dr.V.D.Ramanathan
Indian Medical Association, Badagara, 1 March,1992	Adult respiratory distress syndrome (Guest lecture)	Dr.V.K.Vijayan
- do -	Management of patients with interstitial lung disease complicated by diabetes mellitus and pulmonary tuberculosis	-do-
Meeting of the Dept. of Bio-Technology, Ministry of Science & Technology, New Delhi, 3 June, 1992	-	Dr.Alamelu Raja

Name of the event, venue and date	Title of paper	Name of staff Member
Expert Committee meeting convened by Principal Secretary (Gas Relief) for establishing a Pulmonary Medicine Centre, Bhopal, 4-6 June, 1992	-	Dr.V.K.Vijayan (Expert member)
Meeting on the development of new treatment for tuberculosis, World Health Organisation, Washington D.C, 29 - 30 June,1992	-	Dr.T.Santha Devi
Association of Physicians of India Madras Chapter), Madras, 26 July,1992.	Pulmonary function tests in clinical practice (Guest lecture)	Dr.V.K.Vijayan
Expert Commit tee meeting to finalise the equipment and staff for the proposed Pulmonary Medicine Centre at Bhopal, Madras, 7-8 August,1992	-	-do- (Expert member)
Annual Meeting of the Association of Physicians of India(Madras Chapter), Madras, 23 August,1992	-	Dr.A.M.Reetha
WHO National Workshop on External Quality on Assurance Scheme Network, Madras Medical College, Madras,27-30 September,l992	Internal and external quality control measures as practised at TRC in the isolation and identification of mycobacteria	Dr.C.N.Paramasivan

Name of the event, venue and date	Title of paper	Name of staff member
WHO/Govt. of India, Comprehensive Review Programme of National TB Programme, New Delhi, 1-20 September, 1992	-	Dr.C.N.Paramasivan Dr.Manjula Dutta Dr.V.Kumaraswami Dr.R.Balambal Dr.K.C.Umapathy Mr.A.S.L.Narayana and Mr.V.Chandrasekaran
Indian Association of Medical Microbiologists (Pondicherry & Tamilnadu Chapter), JIPMER, Pondicherry , 27 September,1992	Indirect tests in the diagnosis of TBM	Dr.N.Selvakumar
Indian Association of Medical Microbiologists (Pondicherry & Tamilnadu Chapter), JIPMER, Pondicherry , 27 September, 1992	-	Dr.Vanaja Kumar
ESI Hospital, K.K.Nagar, Madras, 28 September, 1992	Bronchial asthma-management (Guest lecture)	Dr. V.K.Vijayan
National Symposium, Heart and Lung Diseases - Current Approach, Institute of Integral Health Studies, Madras, 3-4 October, 1962	Occupational lung diaeases	-do-
- do -	-	Dr.A.M.Reetha

Name of the event, venue and date	Title of paper	Name of staff member
Indian Academy of Paediatrics,Coimbatore Branch, Coimbatore, 15 November,l992	Simple pulmonary function testing in children	Dr.Soumya Swaminathan
Madurai Kamaraj University and Merieux Course on Molecular Immunology and self and non-self reactivity, Madurai Kamaraj University, Madurai, 16-22 November, 1992	Antigenic cross reactivity in mycobacteria and its relevance to immune response in experimental system and in human	Dr.C.N.Paramasivan
Seminar on Respiratory Medicine, Association of Physicians of India (Madras Chapter), Apollo Hospitals ,Madras, 29 November,l992	-	Dr.V.K.Vijayan (Expert member)
CME Programme,Child Trust Hospital, Madras, 8 December, 1992	Recombinant DNA technology and its application in clinical medicine	Dr.Sujatha Narayanan
Refresher Course on "Recent advances in Statistics for College Teachers", University of Madras, Madras, 18-19 December, 1992	Statistical methods for analysis of cross-tabulated data	Dr.P.Venkatesan (Resource person)
- do -	Non -linear regression models	-do-
CME Programme, Apollo Hospitals, Madras, 20 December,1992	Basic techniques in mycobacteriology	Dr.N.Selvakumar

LIST OF PUBLICATIONS

Papers published

1. Raghupati Sarma, G., Chandra Immanuel, Krishnamurthy, P.V., Rani Balasubramanian, Geetha Ramachandran and Prabhakar, R. Effect of administration of rifampicin on the adrenocortical function in patients with pulmonary tuberculosis *Indian Journal of Tuberculosis,* 1992, **39,** 21-28.

2. Sujatha Narayanan, Sahadevan, R., Ramanujam, S., Prabhakar, R. and Narayanan, P.R. Development of DNA probes for M.tuberculosis . *Indian Journal of Tuberculosis,* 1992, **39,** 99-105.

3. Theresa Xavier. Strategies to improve case finding in tuberculosis programme. *Indian Journal of Tuberculosis,* 1992, **39,** 125-126.

4. Vijayan, V.K., Reetha, A.M., Jawahar, M.S., Sankaran, K. and Prabhakar, R.Pulmonary eosinophilia in pulmonary tuberculosis. *Chest,* 1992, **101,** 1708-1709.

5. Chandra Immanuel, Raghupati Sarma, G., Krishnamurthy, P.V., Geetha Ramachandran and Kumaraswami, V. Salivary cortisol in the assessment of adrenocortical function in patients with pulmonary. tuberculosis. *Indian Journal of Medical Research (A),* 1992, **95,** 1-7.

6. Selvakumar, N., Vanaja Kumar, Acharyulu, G.S., Fathima Rahman, Paramasivan, C.N. and Prabhakar, R. Susceptibility of South Indian strains of *Mycobocterium* tuberculosis to tuberactinomycin. *Indian Journal of Medical Research (A),* 1992, **95,** 101-104.

7. Prema Gurumurthy, Raghupati Sarma, G., Jayasankar, K., Thyagarqjan, K., Prabhakar, R., Muthusethupathy, M.A., Sampath Kumar,P. and Shivakumar, S. Single-dose pharmacokinetics of isoniazid and rifampicin in patients with chronic renal failure. *Indian Journal of Tuberculosis,* 1992, **39,** 221-228.

8. Hong Kong Chest Service/Tuberculosis Research Centre, Madras/British Medical Research Council. A double-blind placebo- controlled clinical trial of 3 antituberculosis chemoprophylaxis regimens in patients with silicosis in Hong Kong. *American Review of Respiratory Diseases,* 1992, 145, 36-41.

9. Herbert, D. and Prabhakar, R. Observations on the cultivation of *M.Leprae* and *M.tuberculosis* in medium "V" and "V1". *Indian Journal of Leprosy,* 1992, *64,* 341-347.

10. Padma Ramachandran and Prabhakar, R. Defaults, defaulter actions and retrieval of patients during studies on tuberculous meningitis in children. *Tubercle and Lung Disease,* 1992, **73,** 170-173.

11. Manjula Datta. Tuberculosis control in the developing world : A review. *In: Epidemiology in Medicine,* Ed.: G.N.Menon, Interline Publishing, Bangalore, 1992, 99-118.

12. Manjula Datta, Samdani, P.G., Udani, P.M., Bermejo, A., costello, A., Crofton, J., Cundall, D., Cutting, W., Hone, N., Miller, F. and King, M. Tuberculosis in children in India - I. *National Medical Journal of India,* 1992, **5,** 226-234.

13. Manjula Datta, Samdani, P.G., Udani, P.M., Bermejo, A., Costello, A., Crofton, J., Cundall, D., Cutting, W., Hone, N., Miller, F. and King, M. Tuberculosis in children in India - II. *National Medical Journal of India,* 1992, **5,** 281-285.

14. Sanjeevi, C.B., Narayanan, P.R., Prabhakar, R., Charles, N., Thomas, B.E., Balasubramanian, R. and Olerup, O. No association or linkage with HLA-DR or -DQ and genes in South Indian with pulmonary tuberculosis. *Tuberculosis and Lung diseases,* 1992, **73,** 280-284.

15. Subramanian, V.S., Selvaraj, P., Narayanan, P.R., Prabhakar, R., Damodaran, C. and Chandrasekaran, P. HLA-DR and -DQ antibodies in the sera of South Indian parous women. *Indian Journal of Forensic Sciences,* 1992, **6,** 109-113.

16. Sulochana Das, Vallishayee, R.S., Shuk Han Cheng, Lowrie, D.B. and Narayanan, P.R. The pattern of mycobacterial antigen recognition in sera from Mantoux-negative individuals is essentially unaffected by BCG vaccination in either South India or London. *Clinical and Experimental Immunology,* 1992, **89,** 402–406.

17. Rajeswari Ramachandran, Rani Balasubramanian and Santha, T. Short - course chemotherapy in neuro-tuberculosis - Brief review of clinical trials undertaken at the Tuberculosis Research Centre, Madras. *Progress in Clinical Neuro Sciences,* 1992,869-881.

18. Venkatesan, P. Survival analysis of censored data. *In: Mathematical, Statistics and Computer Science.* Ed: Ponnuswamy, K.N. University of Madras, Madras, 1992, 139-149.

19. Kuppu Rao, K.V., Vijayan, V.K., Venkatesan, P and Sankaran, R. Maximal respiratory flow rates in tropical eosinophilia. *Bio- medicine,* 1992, **12,** 59-62.

20. Rajajee, S. and Narayanan, P.R. Immunological spectrum of childhood tuberculosis. *Journal of Tropical Paediatrics,* 1992, **38,** 1-3.

21. Rajajee, S. and Alamelu Raja. Immunodiagnosis of tuberculous meningitis. *Journal of Tropica1 Paediatrics,* 1991, **37,** 266-268.

22. Sanjeevi, C.B., Vivekanandan, S. and Narayanan, P.R. Fetal response to maternal ascariasie as evidenced by anti-ascariasis lumbricoides IgM antibodies in the cord blood. *Acta Paediatrics Scandinavica,* 1991, **80,** 1134-1138.

Papers accepted for publication

1. 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.*

2. Vijayan, V.K., Sankaran, K., Venkatesan, P. and Kuppu Rao, K.V. Prediction equations for maximal voluntary ventilation in non- smoking normal subjects in Madras. *Indian Journal of Physiologv and Pharmacology.*

3. Vijayan, V.K. Drug-induced respiratory diseases. Medicine Update. *Association* of *Physicians of India, Ed: Dr. S. Chandrasekaran.*

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

5. Vijayan, V.K. Tropical eosinophilia: aetiology, pathology and pathogenesis. *In: Respiratory Diseases, Ed: Pandey, J.N., Ozford University Press,* New Delhi.

6. Vijayan, V.K. Tkopical eosinophilia: Indian scene. *Indian Journal of Clinical Practice.*

7. Vijayan, V.K. and Kuppu Rao, K.V. Early clinical, pulmonary function and blood gas studies in victims of Bhopal tragedy. *Biomedicine.*

8. Vijayan, V.K. Current status of bronchoalveolar lavage as a diagnostic aid. *Indian Journal of Clinical Practice.*

9. Thomas, A., Paulin Joseph and Prabhakar, R. 'Flu' syndrome associated with other systematic manifestation with once a month rifampicin in the treatment of multi-bacillary leprosy. *Indian Journal of Leprosy.*

10. Paramasivan, C.N., Chandrasekaran, V., Sudarsanam, N.M., Santha Devi, T and Prabhakar, R. Bacteriological investigations for short-course chemotherapy under District Tuberculosis Programme in two districts in India. *Tubercle and Lung Disease.*

11. Venkataraman, P., Paramasivan, C.N. and Prabhakar, R. Invitro activity of rifampicin, rifapentine and rifabutin against South Indian isolates of *M.tuberculosis. Indian Journal of Tuberculosis.*

12. Venkataraman, P., Paramasivan, C.N. and Prabhakar, R. Invitro activity of capreomycin and ciprofloxacin against South Indian isolates of *M.tubercuosis* *Indian Journal of Tuberculosis.*

13. Selvakumar, N., Vanajakumar, Narayana, A.S.L., Suryanarayanan, D., Umapathy, K.C. and Paramasivan, C.N. Use of cetyl pytridinum chloride for the storage of sputum specimens and isolation of *M.tuberculosis .Indian Journal of Tuberclosis.*

14. Kamala, T., Paramasivan, C.N., Daniel Herbert, Venkatesan, P. and Prabhakar, R. Evaluation of procedures for isolation of mycobacteria from soil and water. *In: Proceedings of the IWGMT Colloquium, International Journal of systematic bacteriology. Ed: Prof. Portaeles,* Antwerp, Belgium.

15. 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.*

16. Swaminathan, S., Venkatesan, P. and Mugundan, R. Peak respiratory flow rates in South Indian children. *Indian Paediatrics.*

17. Venkatesan, P. Mathematical models in biomedical sciences. *Bio- medicine.*

18. Vijayan, V.K., Kuppu Rao, 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.*

19. Paton, J.Y., Swaminathan, S., Sargent, C W., Hawskworth, A. and Keens, T.G. The ventilatory response to exercise in children with congenital central hypoventilation syndrome. *American Review of Respiratory Diseases.*

20. Thilakavathy.S., Jemima Shiela Fredricks, Fredricks, K.G., Parthasarathy, R.,Santha Devi, T.,Somasundaram, P.R. and Prabhakar, R. High Coverage for long-term follow-up of patients with spinal tuberculosis. *Indian Journal of Tuberculosis.*

J.OURNAL 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 depart–ments 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, 39 such meetings were conducted during the year.

LECTURES BY VISITING SCIENTISTS

Subject	Speaker
Introduction to health economics	Dr. Henry Glick, Health Economist, University of Pennsylvania, Philadelphia.
Skin tuberculosis	Prof. Patrick Yesudian, Prof. & Head of the Department of Dermatology, Madras Medical College, Madras.
Cultural research methods and qualitative analysis	Dr. Mitchell Weiss, University of Toronto, Canada.
Leishmaniases and phlebotomine sandflies in India	Shri K. Ilango, Assistant Zoologist, Zoological Survey of India, Madras.

DISTINGUISHED VISITORS

1. Dr. Christopher Murray, Harvard School of Public Health, WHO Steering Committee on Tuberculosis Operational Research, Geneva.
2. Dr. Juraj Ivanyi, MRC Unit, Royal Post-graduate Medical School, Hammersmith Hospital, London, U.K.
3. Dr. Fabio Luelmo, Tuberculosis Unit, WHO, Geneva.
4. Dr. Rose W.Pray, TB Consultant/WHO/IUAT, Geneva.
5. Dr. Richard O'Brien, Medical Officer, WHO TB Programme, Geneva.
6. Dr. Mitchell Weiss, Culture, Community and Health Department, The Clarke Institute, University of Toronto, Canada.
7. Dr. C.P.Ramachandran, Secretary, Steering Committee, Filariasis, TDR, WHO, Geneva.
8. Dr. E.A. Ottesen, Chief, Section of Clinical Parasitology, Allergy and Infectious Diseases, National Institute of Health, Bethesda, Maryland, U.S.A.
9. Dr. Gavin Boyd, Consultant Respiratory Physician, Glasgow Royal Infirmaray, U.K.
10. Dr. Stephen Burke, Senior Registrar in Respiratory Medicine, Freeman Hospital, New castle Upon Tyne, U.K.
11. Dr. Astrid Brundin, University Hospital, Linkoping, Sweden.
12. Dr. C.P.Singh, Consultant in Medicine and HOD Medicine, Dr.R.M.L. Hospital, New Delhi.
13. Dr. R.C. Jain, Director, Lala Ram Sarup Institute of TB and Allied Diseases, New Delhi.
14. Dr.(Mrs.) Shibani Bandyopdhya, Asst. Director, NICD, New Delhi.

STAFF MEMBERS ON ADVISORY COMMITTEES OF OTHER INSTITUTIONS

Staff member	Name of committee
Dr.R.Prabhakar	Temporary Adviser, WHO, Geneva.
- do -	Fellow, International Academy of Chest Physicians and Surgeons of the American College of Chat 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 -	Scientific and Technical Committee for Vaccines against Bacterial Diseases, 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 -	Project Review Committee for Tuberculosis, ICMR, New Delhi.
- do -	Editorial Board, *Indian Journal of Tuberculosis,* New Delhi.

Staff member	Name of committee
Dr.R.Prabhakar	Scientific Advisory Committee, Regional Medical Research Centre, ICMR, Port Blair, Andamas.
- do -	Planning and Research - Medical Research Committee of the University of Health Sciences, Viayawada.
- do -	Research Advisory Panel, Schieffelin Leprosy Research and Training Centre, Karigiri.
- do -	Planning Board, Dr. M.G.R. University of Medical Sciences, Madras.
- do -	Senate, Dr.M.G.R. University of Medical Sciences, Madras.
- do -	Board of Management, Vision Research Foundation, Madras.
- do -	Research Sub-Committee, Vision Reaearch Foundation, Madras.
- do -	Editorial Advisory Committee, *Lung India,* Madras.
-do-	Steering Committee, Advanced Centre for Clinical Epidemiological Research and Training, Madras.
- do -	Board of Studies - D.M.(Clinical Epidemiology) Course, University of Madras, Madras.
Dr.G.Raghupati Sarma	Editorial Board, *Indian Journal of Chest Diseases and Allied Sciences,* V.P.Chest Institute, New Delhi.
- do -	Editorial Board,*lndian Journalof Tuberculosis* New Delhi.
- do -	Research Committee, Drug Addiction Reaearch Centre, Madras.

Staff member	Name of committee
DR.P.R.Narayanan	Editorial Board, *Indian Journal of Tuberculosis,* New Delhi.
Dr.C.N.Paramasivan	Editorial Board, *Indian Journal of Tuberculosis.* New Delhi.
- do -	Editorial Board, *Indian Journal of Medical Microbiology,* Madras.
Dr.V.K.Viayan	Central Crisis Group for Chemical Disasters, Ministry of Environment and Forest, Government of India, New Delhi.
- do -	Project Advisory Committee of ICMR on Clinical and Broncho-alveolar Lavage studies on MIC-exposed people at Bhopal, Bhopal Gas Disaster Research Centre, ICMR, Bhopal.
- do -	Consultant to Government of Madhya Pradesh, for establishing a super speciality hospital for pulmonary medicine at Bhopal.
- do -	Editorial Board, *Indian Journal of Chest Diseases and Allied Sciences,*. V.P.Chest Institute, New Delhi.
- do -	Member, Panel of Judges to select the best paper, Indian Chest Society, 1992.
- do -	Member, Select Committee, ICMR, 'Smt.Kamal Satbir Award, 1992'.
- do -	Treasurer, International Academy of Chest Physicians and Surgeons (South India Chapter), Madras.
- 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	Respiratory Medicine Specialists panel, Institute of Integral Health Studies, Madras.
Dr.Padma Ramachandran	State Resource Faculty, Continuing Medical Education in Paediatric Update, Indian Academy of Paediatrics, Tamil Nadu State Branch, Madras.
Dr.Manjula Datta	Task Force for the National ARI Control Programme, Government of India, New Delhi.
- do -	Member, Scientific Advisory Committee, Regional Medical Research Centre, Jabalpur.
- do -	Task Force for the ARI Control Programme in Tamil Nadu, Government of Tamil Nadu, Madras.
- do -	Curriculum Development Committee for Clinical Epidemiology, Dr.M.G.R.Univemity of Medical Sciences, Madras.
Dr.V.Kumaraswami	WHO Expert Committee on Control of Lymphatic Filariasis, WHO, Geneva.
- do -	Steering Committee, Filariasis, TDR/WHO, Geneva.
- do -	Expert Advisory Panel, Parasitic Disease (Filariasis), WHO, Geneva.
Dr.Soumya Swaminathan	Member, Journal Committee, Indian Academy of Paediatrics (IAP), *Journal of Practical Paediatrics,* Madras.
Dr.Manjula Datta, Mr.P.R.Somasundaram, Mr.P.V.Krishnamurthy	Steering Committee, Advanced Centre for Clinical Epidemiological Research and Training, Madras.

PRIZES AND AWARDS RECEIVED BY STAFF MEMBERS

1. Dr. P.R. Narayanan was visiting scientist in the Laboratory of Prof.Bary R. Bloom, Dept. of Immunology and Microbiology, Albert Einstein College of Medicine, Bronx, New York, U.S.A

2. Dr. V.K. Vijayan was awarded membership of the National Academy of Medical Sciences (India) in recognition of significant contribution for the advancement of medical sciences.

3. Dr. C.N. Paramasivan was awarded the University of Madras/Tamil Nadu Tuberculosis Association Endowment Lectureship in medicine for the year 1991-92.

OBITUARY

Dr.Nallapat Kesava Menon, FRCP (Edin)

The sad and sudden demise of Dr Menon on 30th January, 1993 is recorded with deep sense of sorrow.

Dr.Menon had the distinction of being the first National Director of this Centre (formerly known as the Tuberculosis Chemotherapy Centre) appointed by the Indian Council of Medical Research, in 1965, He was endowed with an excellent clinical acumen along with a rare blend of aptitude for research, qualities which made him a good Research Director. During his tenure as Director, the Centre continued to excel in clinical research in tuberculosis. The Centre initiated collaborative controlled clinic trials with National Tuberculosis Institute, Bangalore, during his tenure as Director of National Tuberculosis Institute, Bangalore.

Earlier, he acquired rich experience in the tuberculosis control programme while in the Medical Service of Andhra Pradesh Government. He joined the galaxy of doyens in tuberculosis when he was elevated to the post of Advisor in Tuberculosis, Government of India, in 1969. He had served in various capacities in the World Health Organisations as its Temporary Advisor, Short Term Consultant, etc. and was often approached for advice in tuberculosis control by many Government and Non-Governmental organisations.

We pay our respectful homage to the departed soul with a prayer for it to rest in eternat peace.

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