TUBERCULOSIS RESEARCH CENTRE

CHETPUT MADRAS-600 031

REPORT ON RESEARCH ACTIVITIES DURING 1991

INDIAN COUNCIL OF MEDICAL RESEARCH

NEW DELHI

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PREFACE

The Centre had continued its research activities during the year in the areas of thrust with an accent on operational studies and applied research, to strengthen the National Tuberculosis Programme. These studies conducted in rural and urban areas, while focussing mainly on improving different aspects of the District Tuberculosis Programme (DTP), are also expected to identify defects, if any, in the health services management ot tuberculosis and help in finding remedial measures.

In the rural areas, two strategies are being tried. Among tribal populations in a remote hilly area with inadequate medical facilities, educated youth from the community are imparted basic training in identifying chest symptomatics, collecting sputum specimens and transporting them to a PHC. The ongoing operational research study of utilising "Dais" at the village level tor case-finding for tuberculosis has been yielding promising results and will be tried out in a larger area in Sriperumbudur taluk.

In an urban situation, NSS volunteers from colleges are being utilised for health education of the public and mobilisation of the chest symptomatics for seeking medical help at the health post near their homes in Madurai City. The NSS volunteers perform street plays and folk songs on tuberculosis in slums and in other crowded localities. Some of these programmes are broadcast by the local AIR station. Posters are put up in several places to spread the message. These strategies have yielded good results in diagnosing cases of active pulmonary tuberculosis. It should be possible to utilise this student force in rural areas also, it the villages are adopted by the NSS volunteers.

A prospective long-term follow-up study is being carried out in one district to find out the fate of sputum positive patients treated under the DTP. In addition to providing reliable data on mortality and relapse rates in such patients, if is hoped that this study would throw light on the minimum chemotherapy required by patients tor attaining and maintaining bacteriological quiescence.

Controlled clinical trials with short course regimens are being continued in pulmonary and extra-pulmonary forms of tuberculosis. The regimens in the current trial on sputum-positive pulmonary tuberculosis have been evolved to improve drug compliance under field conditions. In these regimens, four oral SCC drugs, which are usually given together in a single dose, are split up into two-double drug combinations which are administered to the patients on alternate days. Besides reducing the bulk of drugs taken in a single dose, the study would indicate whether toxicity and adverse reactions would be lower with these regimens. It successful, the drug combinations could be introduced in the DTP in the torm of sealed packs, for self-administration by tuberculosis patients.

The outcome of a collaborative study of tuberculoma of the brain suggested that the great majority of patients could be treated successfully with 9 months of chemotherapy, avoiding the need for specialised and expensive surgery. The findings are promising but long-term follow-up results are awaited. Another collaborative study showed that it was possible to treat any form of abdominal tuberculosis with short course chemotherapy. The patients are being followed up for 10 years to confirm that the risk of relapse is low. A study of tuberculous lymphadenitis in children and adults in Madurai is continuing.

An investigation to study the mycobacterial flora in soil, dust and water in the BCG trial area in Chengalpattu district has been initiated. This could possibly explain non-specific sensitisation of the population in the trial, that could have intertered with the efficacy of BCG vaccination. A study was undertaken for screening an indigenous drug claimed to be active against mycobacteria for its activity against **M.tuberculosis** in vitro.

A study of circulating immune complexes suggested that reaction to antigens of molecular weight less than 47 KDa could help to distinguish tuberculosis patients from healthy controls. The study is being extended further. Studies to develop specific and sensitive immunodiagnostic tests are being continued. Studies are also in progress for evolving DNA probes for the diagnosis of tuberculosis. Monoclonal antibodies are being used to evolve antigen detection assays for the diagnosis of tubercutosis.

New methodology to demonstrate mycobacterial antigen in the tissues using immuno-peroxidase technique is being tried out for tissue diagnosis in tuberculosis. Biochemical studies on macrophage functions in mice dealing with mycobactericidal activity under different situations have been completed. A study to understand the adreno-cortical function in children with tuberculous meningitis and lymphadenitis has been initiated.

The Epidemiology Unit is continuing the study for developing a methodology for the surveitlance of tuberculosis. The study relating to the diagnosis of childhood tuberculosis at the primary health care levet using basic health workers is in progress. The Unit had trained health workers in Karhal block in Madhya Pradesh and scientists of the RMRC (ICMR), Jabalpur, in methodologies to conduct a survey for the prevalence of tuberculosis in a tribal population (Saharias).

The long term follow-up study of individuals with HIV infection is being continued; HIV-infected individuals found to have active radiological or bacteriological pulmonary tuberculosis are being treated with a 9-month short course regimen. Their family members are also screened for tuberculosis and other intercurrent illnesses, besides sero-surveillance for evidence of HIV infection. This study could provide valuable information on the incidence of extra pulmonary forms of tuberculosis among HIV infected individuats.

The Electronic Data Processing Unit has simplified the monitoring ot the District Tuberculosis Programme by developing software tor processing the data received from the districts, tor assessing the functioning efficiency of various components ot the DTP. The printing ot the report on Research Activities of the Centre during 1991 was done using IBM PCs, an Apple Macintosh and a laser-printer at the Centre.

The 80th anniversary ot the ICMR and the 35th anniversary ot the Centre were celebrated together in a titting manner. An Exhibition on Tuberculosis and the Research Activities of the Centre, a puppet show on tuberculosis in the local language (Tamil) and Ihe distribution of health education pamphlets on tuberculosis were arranged. Shri K.Inbasagaran, Secretary and Commissioner of Health, Government of Tamil Nadu, inaugurated the exhibition; Prof.K.V.Thiruvengadam spoke on the occasion. There was good response trom the public in spite of highly inclement weather conditions. In addition, the Highlights ot the Research Activities of the Centre were telecast by the Madras Doordarshan Kendra to mark the occasion.

The Scientific Advisory Committee, which met on the 18th and 19th July 1991 under the Chairmanship ot Dr.S.P.Pamra, and the Epidemiology Sub-Committee, which met on 17th July 1991, gave valuable guidance and helpful suggestions regarding the research activities of the Centre.

The Centre will continue to contribute substantially towards research and development tor strengthening the National Tuberculosis Programme. Finally, I wish to gratetully acknowledge the unstinted cooperation and enthusiasm shown by all the staff members ot the Centre towards the pursuit ot high quality research.

R.Prabhakar
Director

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AS ON 31.12.1991

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* Deceased during the year

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OPERATIONAL RESEARCH STUDIES

STUDIES IN PROGRESS

Short course chemotherapy under District Tuberculosis Programme

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 regimens being prescribed are:

1. **$2RHZ_2/4RH_2$:** Rifampicin 600 mg plus isoniazid 600 mg plus pyrazinamide 2.0 g 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 1.5 g 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 1.5 g daily for 2 months, followed by rifampicin 600 mg plus isoniazid 600 mg twice a week for 4 months; in the first two months, 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 make periodic monitoring visits to the districts; during 1991, 6 districts were visited at least once by a team from the Centre.

Sputum examination and intake to SCC: The average sputum examinations per month from the inception of SCC ranged from 762 to 3572 in the Policy A districts, 645 to 2279 in the Policy B districts and 584 to 1629 in the Policy C districts; the percentage of positivity ranged from 4% to 14%, 7% to 9%, and 4% to 9%, respectively. The percentage of eligible patients started on SCC ranged from 48-79 (median 62) in the Policy A districts, 31-72 (median 54) in the Policy B districts and 42-82 (median 66) in the Policy C districts. Considering all the 18 districts, the admissions ranged from 40-59% in 8 districts and 60-89% in 9 districts. In the other district, only 31% were started on SCC.

Detailed analyses on smear positivity in the districts over the years have shown that there was some variation in some districts but there was no clear-cut trend. Considering all the 18 districts, the smear positivity in 1991 was less than 5% in 2 districts, 5-9% in 10 districts and 10-15% in the remaining 6 districts.

Considering the intake to SCC, there seems to be an improvement over the years in most of the districts. In 1991, 80% or more were put on SCC in 5 districts, 70-79% in 6, 60-69% in 3, 50-59% in 3 and less than 50% (42%) in 1 district.

Contribution of PHIs to case finding: The total number of sputum examinations at the DTC during 1991 (see Table 1) ranged from 2048 to 8597 in the 6 policy A districts (median 4813) , 1017 to 6242 in the 6 policy B districts (median 2294), and 1734 to 8839 in the 6 policy C districts (median 4443). The corresponding figures for the PHIs are 2678 to 42865 (median 11320), 5495 to 29269 (median 8126), and 3658 to 18215 (median 14124), respectively. The sputum positive cases diagnosed at the DTC ranged from 271 to 1431 (median 596) in the policy A districts, 198 to 653 (median 431) in the policy B districts, and 322 to 840 (median 586) in the policy C districts. The corresponding figure for the PHIs are 126 to 2521 (median 1112), 209 to 2890 (median 558) and 119 to 1111 (median 550), respectively. The over-all positivity rate at the DTCs is 13% and at the PHIs, 6% . The PHIs examined 17 sputum specimens for every positive obtained whereas at the DTCs, it was 8 specimens for every positive. Of the total 25308 sputum positive cases diagnosed during the year 1991, 60% were diagnosed at the PHIs and 40% at the DTCs.

Table 1

	DTC			PHIs		
	New sputum examined	Positive No.	%	New sputum examined	Positive No.	%
Policy A						
Total	29855	3986	**13**	111371	6776	**6**
Median	4813	596	**14**	11320	1112	**6**
Range	2048-8597	271-1431	**8-17**	2678- 42865	126-2521	**3-18**
Policy B						
Total	18450	2555	**14**	71770	5242	**7**
Median	2294	431	**18**	8126	558	**7**
Range	1017-6242	198- 653	**9-22**	5495- 29269	209-2890	**4-10**
Policy C						
Total	28165	3495	**12**	74686	3254	**4**
Median	4443	586	**13**	14124	550	**4**
Range	1734-8839	322- 840	**8-19**	3658- 18215	119-1111	**1 - 6**

Treatment completion rate: From the inception of SCC, treatment completion rates for 6 different cohort periods (ending June, 1990) are available. In the current cohort, the SCC treatment completion rate is 53% (median 53%) and ranges from 40 to 71% in the Policy A districts, 54% (median 52%) and 33 to 67% in the Policy B districts, and 54% (median 56%) and 30 to 74% in the Policy C districts.

Considering the treatment completion rate according to the regimen during the 6th cohort period (July 1989 to June 1990), 50% of 1478 patients on the fully supervised regimen had received 80% or more of chemotherapy, compared with 54% of 10062 patients on the unsupervised regimen and 55% of 1215 patients on the partially supervised regimen. The treatment completion rates for the previous 5 cohort periods were 49% of 4129, 53% of 1719, 47% of 1716, 46% of 1351, and 51% of 1446 patients for regimen 1, 48% of 2834, 50% of 4598, 57% of 6509, 54% of 5601 and 55% of 6985 patients for regimen 2, and 76% of 631, 55% of 1258, 63% of 1043, 60% of 1051 and 61% of 1325 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.

For 3 concurrent cohort periods, comparison of treatment completion rates for SCC and standard regimens are also available, and are given in Table 2.

The treatment completion rate with SCC for the 3 cohort periods has been consistent for Policy A and B districts, the medians being 51%, 54% and 55% in Policy A districts and 52%, 48% and 50% 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% in the 3rd cohort period. 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

Table 2

	SCC admissions during:						Standard Rx admissions during:					
	7/86-6/87*		7/87-6/88		7/88-6/89		7/86-6/87		7/87-6/88		7/88-6/89	
	No. of pts.	≥80% Rx (%)	No. of pts.	≥80% Rx (%)	No. of pts.	≥80% Rx (%)	No. of pts.	≥80% Rx (%)	No. of pts.	≥80% Rx (%)	No. of pts.	≥80% Rx (%)
Policy A												
Total	4482	**59**	3298	**54**	3008	**54**	2023	**27**	1812	**36**	2097	**33**
Median	787	**51**	500	**54**	524	**55**	363	**26**	364	**35**	258	**34**
Range	270-1215	**44-75**	256-915	**44-94**	332-939	**47-60**	113-503	**14-35**	218-498	**29-45**	95-909	**30-47**
Policy B												
Total	2391	**59**	2160	**51**	2969	**49**	2439	**24**	2250	**44**	2305	**52**
Median	306	**52**	324	**48**	426	**50**	486	**24**	332	**43**	340	**32**
Range	196-902	**24-75**	171-792	**25-76**	187-830	**32-64**	333-708	**17-37**	162-651	**16-68**	190-986	**17-73**
Policy C												
Total	2395	**46**	2545	**53**	3779	**61**	2514	**27**	2663	**40**	1827	**52**
Median	342	**52**	400	**51**	632	**60**	442	**28**	438	**44**	289	**52**
Range	200-837	**26-68**	304-677	**22-86**	356-979	**28-82**	159-650	**15-49**	267-696	**13-57**	160-576	**19-66**
Median	342	**51**	375	**52**	524	**56**	445	**27**	365	**36**	302	**39**

* Month/year

2nd, and was 39% in the 3rd. Detailed analysis has shown that between the 2nd and 3rd cohorts, there was an increase in the treatment completion rate by 30% in 1 district, 10-20% in 5 districts, and by 1% and 5% in 2 districts; in 1 district, the treatment completion rate remained the same; in 6 districts, the treatment completion rate was less by 2%, 5%, 7%, 15%, 16% and 19%, respectively. In the other 3 districts, information was not available for the 2nd or 3rd cohort period.

(started: 1983; expected year of completion: 1992)

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

The National Service Scheme is a major youth programme in the country with the objective of personality development through community service. NSS activities provide students and teachers with opportunities to participate in the development programmes in various sectors including health, both in rural and urban areas. Hence a task force consisting of NSS volunteers was used to motivate the community for the Madurai City TB Programme (see 1990 annual report). At the same time, some of the health posts available in the city were activated for TB work, with a view to provide medical facilities near patients' homes.

Aims and objectives:

1. Activation of corporation dispensaries in Madurai for anti-TB work.

2. To study the application of SCC under DTP conditions through the corporation dispensaries.

3. To activate case-finding and case-holding components of Madurai City TB Programme, utilising the NSS task force.

4. Utilising NSS volunteers for health education and sensitisation of the community by street plays, villu pattu, etc.

Study area and personnel involved: The personnel involved in this study are NSS student volunteers from Lady Doak College, Sri Meenakshi College, Mannar College and American College, staff of two corporation dispensaries in Sellur area, staff of the Thoracic Medicine department, Rajaji Hospital, Madurai and District TB Centre, Periakulam. This study was carried out in Sellur area ORS zones 1 and 3.

Work done: In the year 1991, two sputum camps were conducted in the above area, in addition to 'case-holding' work of cases detected in 1990. The details of the two camps and post-camp activities are summarised in Table 1.

Table 1

	First camp in 1991	Second camp in 1991
	Sellur ORS Zone 3	Aruldaspuram
Sensitisation methods	Door to door campaign, street play, villu pattu	Hand bills, radio, wall posters, street play, villu pattu
Population	18000	25000
No. of symptomatics who attended	275	237
No. of sputum examinations done	445*	232*
No. of patients with a positive smear	15	17
No. of sputum examinations during post-camp period	366	273
No. of patients with a positive smear	19	8

* If negative for AFB, a second sputum was collected.

Case-holding: A total of 108 patients were started on treatment, of whom 63 were sputum positive and 45 were only X-ray positive. Once in 15 days, students visit the patients receiving treatment to check regularity in drug collection and drug consumption, and for toxicity. A total of 286 visits were done by students in this year.

The study is ongoing.

(started: 1990; expected year of completion: 1992)

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

The Jawadhu hills area is thickly wooded and situated about 900 m. above mean sea level, with a population of about 90,000. Some of the villages and hamlets (consisting of 15-20 huts each) are remote from the roads (2-10 km) and have to be reached on foot. During the rainy season, landslips and swollen streams are likely to make them unapproachable. Health care delivery for this population needs to be strengthened, more so because of the physical inability of health workers to reach the interior villages. Regarding the National Health Programme, the coverage is about 50% for family planning and 60% for triple vaccine. With reference to the Tuberculosis Programme, there were no facilities available for sputum microscopy at Jamnamarudur PHC. Awareness about tuberculosis is poor in these villages and quite a number of patients (diagnosed during a survey by the Centre's Epidemiology Unit) who were started on treatment, had stopped treatment prematurely.

A pilot study in Jawadhu Hills is being undertaken to augment the case finding and case holding components of the District Tuberculosis Programme.

The aim is to find out the feasibility of involving 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.

Nineteen roadside hamlets situated around Jamnamarudur were selected and one literate youth from each hamlet was identified with the help of oorans.

An exhibition on tuberculosis was conducted, and training in case finding and drug delivery was given to literate youths already identified. A handbook on tuberculosis was also issued to all the literate youths. The pharmacist of Jamnamarudur PHC was also instructed to help the literate youths. Literate youths identify chest symptomatics, collect sputum specimens from them and bring the sputum specimens to Jamnamarudur PHC on a specified day. The Centre's Lab. Technician examines the sputum specimens by smear and reports the results the same day or the next day. All smear positive patients are started on a Short Course Regimen—2EHRZ/6TH in the village the next day by the literate youth, in the presence of the Centre's physician. Literate youths were asked to collect 15 days' drugs from the PHC and supply it to the patients.

The Centre's Medical Officer and Social Worker do the following during their visit:

(a) visit the patients started on treatment, at random, and do pill count to check whether the patient takes the drugs regularly;

(b) question a few symptomatics identified by literate youths, to check whether they are true chest symptomatics;

(c) visit a few households in the hamlet, at random, to find out whether there are any symptomatics 'missed' by the literate youths, and collect a sputum specimen from them.

Work carried out in the field: The literate youths had done enumeration of the population in 19 hamlets. A total of 3715 persons have been screened. Out of this, the population aged 15 years and above is 2278 persons in a total of 819 households.

Case-finding by literate youths: The literate youths had identified 80 chest symptomatics and sputum was collected from 61. The rest of them did not give sputum for examination.

To ascertain whether literate youths had identified all the chest symptomatics in the community, a sample of the population inclusive of all chest symptomatics, in 19 hamlets was interviewed by a Medical Officer (MO)/Social Worker (SW). The findings are given in Table 1.

A total of 1483 individuals inclusive of 78 chest symptomatics were interviewed. The time gap between identification by literate youths and the interview by the Centre's personnel was about 2 months; 12 additional chest symptomatics, who presented with symptoms at the time of the second interview, were detected. Of the 80 chest symptomatics identified by the youth, two were not available at the time of interview. Of the rest, 73 had been correctly identified as chest symptomatics, though 22 of them were suffering from non-tuberculous

Table 1

		Chest symptoms, assessed by literate youth		
		Present	Absent	Total
Chest symptoms, assessed by MO/SW	Present	73	0	73
	Absent	5	1405	1410
Total		78	1405	1483

respiratory illnesses, while 5 were not considered to be chest symptomatics by the MO/SW. The remaining 1405 individuals of the general population did not have any symptoms suggestive of tuberculosis.

At the time of the interview, a statistician checked a sample to find out whether the enumeration of the households and the population was done properly as many of the huts did not have door numbers.

Smear examination for AFB was done at the PHC by the Centre's Laboratory Technician and 4 of the 61 sputum specimens were found to be positive. The sputum samples were also cultured and one patient, whose sputum was negative by smear, yielded a positive culture for **M.tuberculosis**. All five patients were started on the short course regimen of 2EHRZ/6TH by the Centre's physician and the literate youth concerned. The literate youths were instructed to collect 15 days' supply of drugs from the PHC and supply to the patients for the scheduled period.

Case-holding: The literate youths had collected drugs only twice out of 80 collections due. However, the village nurse had supplied the drugs to the patients at their doorstep.

Reasons for not collecting the drugs at the proper time were elicited by the Medical Officer/Medical Social Worker during four visits to the tribal area. The youths did not express any difficulty in following instructions or contacting people in the village or the PHC staff or in collecting and transporting sputum to the PHC. A few mentioned that the PHC pharmacist was not available when they visited.

Surprise drug checks were done on 3 occasions by the Medical Officer /Social Worker. Four patients had taken drugs fairly regularly and they were very happy as they got relief of symptoms with treatment. One patient had not taken the drugs as she had vomiting after taking the drugs. The Social Worker advised her to attend the PHC and consult the Medical Officer.

Conclusions: This pilot study has shown that literate youths were efficient in identification of chest symptomatics in the community and this force could be utilised for case-finding activity in the tribal area. However, the literate youths were not useful in collecting drug supplies from the PHC and supplying to the patients.

It is proposed to extend the study to more villages, including those in the interior, training the literate youths to identify chest symptomatics in the community, collect sputum and transport it to the PHC, but entrust the village health nurses with drug supply to sputum positive patients.

(started: 1990; expected year of completion: 1992)

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

"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 with the aim to explore the feasibility of utilising the services of Dais for 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 348 sputum specimens were collected from symptomatics identified till December 1991 and examined by smear for AFB and by culture for **M.tuberculosis** (see Table 1). Of these 348 specimens, 42 (12%) were found to be positive by smear for AFB and 11 (3%) were negative by smear but positive by culture for **M.tuberculosis.** Sensitivity tests to isoniazid and rifampicin were done on all positive cultures. Results are available for 45 cultures (31 pretreatment and 14 during treatment); 8 (18%) cultures were resistant to isoniazid and one (2%) resistant to isoniazid and rifampicin.

Chemotherapy: All patients positive by smear or culture were prescribed ethambutol 600 mg plus isoniazid 300 mg daily for 12 months. Treatment was initiated by a Medical Officer of the Centre, and monthly supplies of drugs were issued to the Community Health Assistants, to be handed over to the respective Dais, who supplied the drugs to the patients with instruction to take the drugs daily at night after food.

Out of 53 patients who were positive by smear or culture, 2 died before starting treatment, 1 patient refused chemotherapy in spite of repeated

Table 1

		No.	%
Population catered to by Dais (according to 1991 census)		26413	
Population aged > 15 years		16740	63
Total symptomatics identified by Dais		348	2
Sputum positives			
- by smear alone	19	42	12
- by smear and culture	23		
- by culture alone		11	3
- Over-all positivity (smear and culture)		53	15

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 48 patients. During treatment, 3 patients died and 2 patients migrated. One patient complained of giddiness after taking treatment in the first month of treatment but with reassurance, he felt better.

So far, 25 patients have completed one year of treatment. At the end of treatment, sputum was collected from 22 patients by the Dais. Two specimens were positive by smear and culture; the organisms were sensitive to isoniazid and rifampicin and retreatment was initiated with ethambutol, isoniazid, rifampicin and pyrazinamide thrice a week, the Dais administering the drugs under supervision.

A Health Visitor does surprise drug check at least once a month for all patients on treatment. Efforts are also being made to identify and collect sputum samples from chest symptomatics missed by 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.

The study is in progress.

(started: 1989; expected year of completion: 1992)

Estimation of case potential in taluk hospital (XC) and PHC (MC) by passive case finding

The aim of the study was to estimate the case potential from the Out-Patient Department (O.P.D.) of a Taluk Headquarters Hospital (XC) and a PHC (MC).

Centres with outpatient attendances of about 20,000 per month in XC and 1000 in MC according to two consecutive quarterly reports were selected. Symptomatics as defined in the District Tuberculosis Programme (those with cough for more than 15 days, chest pain for more than one month, haemoptysis at any time) among those aged more than 15 years were the subjects. The symptomatics were identified by the Centre's Medical Officer in the routine O.P.D. on four consecutive days in XC and three consecutive days in MC. At the XC, the Medical Officers of the institution were requested to identify symptomatics and refer them for sputum examination as usual. The Centre's Medical Officer looked after only male outpatients, aged 15 years or more, on one day and only female outpatients, aged 15 years or more, on another day; all the other outpatients were looked after by the PHI Medical Officer in XC. At the MC, all new OPD cases were seen by both TRC and PHI Medical Officers. The visits were without prior intimation and the sputum specimens were examined by the Centre's Laboratory Technician. The study had been carried out at Gudiyatham (XC) and Odugathur (MC) in the previous year (see 1990 annual report).

The study was carried out during the year at Thiruppathur (XC), Arakkonam (XC), Ambur (XC), Vettavalam (MC) and Punnai (MC). The routine data from these PHIs, obtained from the quarterly report of October - December 1990, are presented in Table 1, and the findings of the present study in Table 2.

Table 1

PHI	New OP	New sputum examined		Sputum positive	
		No.	%	No.	%
Thiruppathur(XC)	98,889	1480	**1.5**	17	**1.1**
Arakkonam(XC)	74,491	1211	**1.6**	4	**0.3**
Ambur(XC)	1,27,384	653	**0.5**	6	**0.9**
Vettavalam(MC)	5,870	87	**1.5**	2	**2.3**
Punnai(MC)	4,104	32	**0.8**	0	**0.0**

At Thiruppathur, the percentage of new sputum examined among those aged 15 years or more, seen by TRC M.O. is 3.0. In the quarterly report, where all the new OPD cases are included irrespective of age, the percentage is 1.5. The sputum positivity percentages observed are14.3 and 1.1, respectively, among symptomatics identified by TRC M.O. and according to the quarterly report. A similar trend was observed at Arakkonam and Ambur.

Table 2

PHI	Exam by M.O. of:	New OP No.	New sputum examined No.	%	Smear positive No.	%
Thiruppathur(XC)	TRC	938	28	**3.0**	4	**14.3**
	PHI	2042	15	**0.7**	1	**(6.7)***
	Total	2980	43	**1.4**	5	**11.6**
Arakkonam(XC)	TRC	479	12	**2.5**	2	**(16.7)**
	PHI	1868	17	**0.9**	0	**(0.0)**
	Total	2347	29	**1.2**	2	**6.9**
Ambur(XC)	TRC	737	19	**2.6**	2	**(10.5)**
	PHI	2823	19	**0.7**	1	**(5.3)**
	Total	3560	38	**1.1**	3	**7.9**
Vettavalam(MC)	TRC	231	4	**1.7**	1	**(25.0)**
	PHI	231	9	**3.9**	0	**(0.0)**
	Total	231	13	**5.6**	1	**(7.7)**
Punnai(MC)	TRC	207	3	**1.4**	0	**(0.0)**
	PHI	207	7	**3.4**	0	**(0.0)**
	Total	207	10	**4.8**	0	**(0.0)**

* Figures in parentheses indicate percentages based on fewer than 25 observations.

At Vettavalam and Punnai, the new O.P. attendances, new sputum examined and smear positives observed were too few to draw valid conclusions.

These results suggest that proper and careful selection of symptomatics could help in estimating the true case potential, as seen from enhanced sputum examination rates and higher percentage of smear positivity during the period when the TRC physician was identifying the symptomatics as compared to the routine identification done by PHI M.Os.

(started: 1990; expected year of completion: 1993).

Awareness about tuberculosis in a South Indian rural community before and after sensitisation

Case finding in the District Tuberculosis Programme is passive and the persons with chest symptoms are expected to seek diagnosis and treatment from the available health facilities. For the success of the programme, it is therefore essential that the chest symptomatics in the community are aware of the symptoms of tuberculosis and are motivated enough to get this condition diagnosed and treated. In order to find out the basic awareness about tuberculosis and the outcome of community sensitisation for increasing this awareness, a study was undertaken in a rural community (villages in Sriperumbudur Taluk). Sensitisation was done through health education on tuberculosis.

'Prepare' is a voluntary organisation in Sriperumbudur taluk rendering comprehensive welfare services including health. In this area, 24 villages were selected at random and 466 households were visited by a Medical Social Worker. The heads of the households were interviewed to assess the existing level of awareness about tuberculosis, by using a questionnaire. Their knowledge was reasonably good (67%) about the free investigation/ treatment and the need for investigation of family members of patients (Table 1). Regarding the category of people affected, area of prevalence and the type of people prone to illness, 45%, 37% and 38% answered correctly. With regard to the duration of treatment, precautions while coughing and preventive measures, their knowledge was very poor (14-15%).

After finding the basic awareness, a one-page hand-out containing important facts on tuberculosis in the local language (Tamil) was distributed, with a view to sensitise the community on tuberculosis. The utility of the hand-out in spreading the message was assessed and reported (see 1990 annual report). In addition, film shows, exhibitions and role plays on tuberculosis were arranged in a

few villages. Dais of all the villages, community health attendants, animators and youths were educated periodically on tuberculosis through lectures with flash cards, slides, film shows, role plays, group discussions and field demonstrations.

After 2 years, the heads of the selected households were interviewed again, using the same questionnaire, to find out the long-term effect of sensitising the community and the current awareness of tuberculosis. Out of the total 466 persons interviewed initially, 33 could not be interviewed; of these, 10 had died, 15 had migrated and 8 were not available when their house was visited. An interim analysis for the 433 (93%) respondents for whom data is available, is given in Table 1.

Table 1

Expected right answer	Answer before sensitisation			Among(a),answered correctly after sensitisation	
	Correct No.	%	Wrong No. (a)	No.	% of (a)
1.Category of people affected by TB-both rich and poor	196	**45**	237	204	**86**
2.Area of prevalence- both rural and urban	161	**37**	272	260	**96**
3.Prone to get illness-both adults and children	165	**38**	268	242	**90**
4.Need not pay for investigation and treatment at Govt.Hospital	289	**67**	144	143	**99**
5.Duration of treatment 4-6 months	65	**15**	368	143	**39**
6.Precaution while coughing is to cover the mouth	66	**15**	367	150	**41**
7.Family members need to be investigated - yes	288	**67**	145	140	**96**
8.Preventive measure- vaccination	60	**14**	373	110	**29**

Analyses were done separately for each question; the persons who gave correct answers at present but had answered wrongly on the earlier occasion are presented in the table. Among these people, their knowledge had improved and the proportion of correct answers ranged from 86% to 99% , except for duration of treatment (39%), precautions while coughing (41%) and about preventive measure (29%). However, their knowledge regarding these three aspects had been much poorer when questioned initially.

(started: 1989; expected year of completion: 1992)

CLINICAL STUDIES

STUDIES COMPLETED

Collaborative study of abdominal tuberculosis

As mentioned in previous annual reports (1985-86; 1989 and 1990) the Centre has carried out a collaborative study of abdominal tuberculosis. The objectives of this study were:

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

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

A subsidiary objective was to develop, from the findings of this study, satisfactory criteria for diagnosis, assessment of progress, 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 allocated at random to either of the following regimens:

2RHZ/4RH (Regimen 1- rifampicin series): Rifampicin 10 mg/kg plus isoniazid 300 mg plus pyrazinamide 30 mg/kg daily for 2 months, followed by

rifampicin 10 mg/kg plus isoniazid 300 mg daily for the next 4 months.

SEH/EH (Regimen 2 - 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.

Between August 1983 and January 1991, 898 patients were registered and of these, 193 patients were admitted to the study; information up to the end of treatment is available for 193 patients (96 2RHZ/4RH, 97 SEH/EH).

Characteristics on admission: The mean age was 30 years (range 13-72 years); there were 85 males and 108 females. An initial induration of 10 mm or more to 1 TU of PPD (RT23 with Tween 80) was seen in 75% of the patients. Of the 193 patients, 134 (69%) had intestinal lesions, 84 (44%) had peritoneal tuberculosis, 27 (14%) had hepatic tuberculosis, 17 (9%) had mesenteric tuberculosis and 2 had retroperitoneal tuberculosis; 60 patients had combined lesions.

In all, 149 (77%) patients had presented with pain in the abdomen, in association with tenderness of the abdomen or alteration in bowel habits or increased borborygmi or distension of the abdomen or anorexia.

Confirmation of diagnosis: Among 193 patients, bacteriological confirmation of the biopsy specimen was obtained by culture in 16 (8%) patients and by smear in 4 (2%) patients. Ascitic fluid culture examination for ***M.tuberculosis*** yielded positivity in 9 (5%) patients and smear alone was positive in 2 (1%) patients. In addition, histopathological confirmation was obtained in 78 (40%) patients. Indirect evidence of tuberculosis, that is, sputum culture positivity was available in 27 (14%) including 1 patient with a positive urine culture also. Thus, in all, confirmation was obtained in 136 (70%) of 193 patients. Of 134 patients with intestinal tuberculosis, 111 (83%) had radiographic evidence by barium contrast studies, of whom 64 had either direct histopathological or bacteriological confirmation or tuberculosis elsewhere in the body (indirect evidence). For details of confirmation of diagnosis in other forms of abdominal tuberculosis, please see 1990 annual report.

Population in analyses: Out of 193 patients admitted to the study, 31 patients were excluded for various reasons; 10 (4 Reg 1, 6 Reg 2) patients died of causes other than tuberculosis and 12 (3 Reg 1, 9 Reg 2) had received less than 75% of treatment; 3 patients (all Reg 1) had interruption of 25% more of allocated chemotherapy for toxicity management and 6 (2 Reg 1 ,4 Reg 2) had the allocated chemotherapy terminated for severe toxicity. Thus there remained 162 patients (84 Reg 1 and 78 Reg 2) for efficacy analyses.

Response at the end of treatment: The response at the end of treatment is shown in table 1.

Table 1

Status at the end of Rx	2RHZ/4RH		SEH/EH	
	No.	%	No.	%
Symptom free	79	94	68	87
Clinically improved but still symptomatic	3	4	5	6
Change of Rx for clinical deterioration	1*	1	2	3
Surgical intervention	1	1	1	1
TB death	0	0	2	3
Total patients with assessable response	84	100	78	100
Response not assessable				
Non-tuberculous death	4		6	
Received less than 75% of Rx	3		9	
Interruption of 25% or more of Rx due to side-effects	3		0	
Termination of Rx due to severe toxicity	2		4	
Total patients	96		97	

* Continuation of Rx beyond the prescribed period because of discharging sinus.

Out of 84 patients in the rifampicin series and 78 in the non-rifampicin series, 79 and 68 respectively were symptom-free at the end of treatment. Three patients in the rifampicin series and five patients in the non-rifampicin series had improved clinically but were symptomatic at the end of treatment. One in the rifampicin series and 2 in the non-rifampicin series had a change of chemotherapy for clinical deterioration, one in each series had surgery (in the 6th month for

acute obstruction and in the 2nd month for perforation, respectively) and 2, both in the non-rifampicin series, had died of tuberculosis.

Results up to 60 months: The patients are being followed up routinely up to 10 years from the start of treatment. Among 155 patients who were symptom free or clinically improved at the end of treatment, information is available upto 60 months on 85 (44 rifampicin, 41 non-rifampicin) patients. Four patients required retreatment for various reasons - two for renal tuberculosis, one for tuberculous meningitis and one patient following surgery for intestinal obstruction as advised by the surgeon even though there was no histopathological or bacteriological evidence of relapse. None of the other 81 patients had a relapse of abdominal tuberculosis.

Adverse reactions to drugs: Patients were not questioned about symptoms of drug toxicity but any spontaneous complaints were recorded after careful questioning. All the 193 patients admitted to the study are considered for the analysis (Table 2).

Table 2

Type	Rif. series n=96			Non-rif. series n=97		
	Incidence	Interruption	Termination	Incidence	Interruption	Termination
Hepatic	16	16	0	4	4	0
Peripheral neuritis	2	0	2	1	0	1
Visual	0	-	-	2	0	2
Cutaneous	1	0	0	1	0	1
Arthralgia	7	0	0	0	-	-
Vertigo	0	-	-	2	0	0

Hepatotoxicity: Hepatitis occurred in 16 (17%) of 96 patients in the rif. series and 4 (4%) of 97 patients in the non-rif. series ($P < 0.01$). Of these, 12 patients had clinical jaundice. The onset of jaundice was within 2 months of the start of treatment in all 16 patients in the rifampicin series but after 6 months of

treatment in all 4 patients in the non-rifampicin series. Rifampicin, isoniazid and pyrazinamide in the rifampicin series and isoniazid in the non-rifampicin series were withheld for periods varying from 7 days to 58 days, and substituted with daily streptomycin and ethambutol in the rifampicin series and streptomycin in the non-rifampicin series. Treatment was resumed uneventfully in all the patients.

Peripheral Neuritis: Two patients developed peripheral neuritis in the rifampicin series, in the first and the fourth month, respectively. The dosages of isoniazid they received were 8.0 mg/kg and 9.4 mg/kg body-weight, as calculated from the uniform dosage of 300 mg of isoniazid. Isoniazid was terminated for both. Both patients recovered after a period of 4 months. One patient in the non-rif. series developed peripheral neuritis in the fifth month of treatment; he was receiving isoniazid 8.5 mg/kg and the drug was terminated. He recovered after a period of 3 months.

Visual: Two patients, both in the non-rif. series, developed dimness of vision after 6 months of treatment. Ophthalmological examination showed constriction of visual fields and ethambutol was terminated. The field of vision became normal subsequently for both the patients.

Cutaneous: One patient in the rifampicin series developed itching in the first week of treatment which subsided with antihistamines; antihistamines were given along with anti-tuberculosis drugs throughout the period of treatment. One patient in the non-rifampicin series developed fixed drug eruptions on the 76th day of treatment and isoniazid was terminated. The drug eruption completely subsided within one week of stopping isoniazid which was substituted with pyrazinamide.

Arthralgia: In all, 7 (7%) of 96 patients in the rifampicin series and none in the non-rifampicin series developed arthralgia. The onset was in the first 6 weeks of treatment in 6 patients and in the 8th week in the other. All patients responded to analgesics.

Vertigo: Two (2%) of 97 patients in the non-rifampicin series developed mild giddiness on the 3rd and 12th day of treatment, respectively, and were managed with symptomatic treatment.

The overall incidence of adverse reactions was 26 (27%) in the rifampicin series and 10 (10%) in the non-rifampicin series. The difference is statistically significant ($P < 0.01$).

Conclusions: The short course regimen of 6-month duration appears to be as effective as the standard regimen in the treatment of abdominal tuberculosis. The rifampicin-pyrazinamide-isoniazid combination was significantly more hepatotoxic than ethambutol-isoniazid but could be managed by withholding the offending drugs during the episode of jaundice. Our study has

shown that the management of any form of abdominal tuberculosis with anti-tuberculosis chemotherapy, especially with a highly potent combination of bactericidal drugs, is a logical and rational approach. Only 2 patients (1 in each series) needed surgical intervention -- one for perforation and one for acute obstruction. Another encouraging fact is that none of the patients who have completed 5 years has had a relapse of abdominal tuberculosis, so far. However, long-term follow-up results (up to 10 years from the start of treatment) are essential for drawing definite conclusions.

(started: 1983; efficacy study completed: 1991)

Collaborative study of brain tuberculoma

Brain tuberculoma is now being suspected much more often than in the past, probably due to increased awareness of the disease among physicians and the greater availability of CT scan. There are reports which suggest that chemotherapy alone may be effective even for large brain tuberculomas with increased intracranial tension; however, no reports are available on the use of short-course chemotherapy for brain tuberculoma. A controlled study was undertaken in collaboration with the Institute of Neurology (Prof. S. Kalyanaraman), Govt. General Hospital, Madras, to evaluate the efficacy of short-course chemotherapy in the management of brain tuberculoma and determine indications for surgery (see 1987 annual report). The study was later extended to the Railway Hospital, Perambur (Prof. Zaheer Ahmed Sayeed).

A circumscribed hyperdense lesion compared to the surrounding brain, with a volume of 1000 cu.mm. or more, enhancing with contrast and having adjacent oedema on CT scan, was taken as tuberculoma for admission to the study.

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)

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

The following investigations were done on admission: CT scan, radiographs of the chest and skull, CSF culture, culture of sputum and urine,

mantox, liver function tests and haematological examinations. CT scan was repeated at 1 month, 2 months and every 2 months thereafter till 2 consecutive scans were normal. If the size of the mass at the second month scan was more than 80% of the mass on admission, a biopsy of the mass was done for histopathology and culture examinations.

In all, 144 patients (121 from General Hospital and 23 from Railway Hospital) have been admitted to the study. The following analysis is based on 113 patients who have completed treatment, after excluding 31 for various reasons, including 13 who were diagnosed as having a non-tuberculous lesion or where the scan criteria was not fulfilled, and 5 who had chemotherapy terminated for serious toxicity in the first 3 months. Table 1 gives the characteristics on admission.

Table 1

Factor on admission		$3RHZ_7/6RH_2$		$3RHZ_3/6RH_2$		Both	
		No.	%	No.	%	No.	%
Sex	Male	23	**40**	21	**38**	44	**39**
	Female	35	**60**	34	**62**	69	**61**
Age (years)	< 15	21	**36**	28	**51**	49	**43**
	15-24	16	**28**	15	**27**	31	**27**
	25-34	13	**22**	4	**7**	17	**15**
	35-44	7	**12**	4	**7**	11	**10**
	≥45	1	**2**	4	**7**	5	**4**
Lesion	Single	43	**74**	45	**82**	88	**78**
	Multiple	15	**26**	10	**18**	25	**22**
Mantoux (mm)	0- 9	25	**43**	28	**51**	53	**47**
	10-19	16	**28**	11	**20**	27	**24**
	≥20	11	**19**	11	**20**	22	**19**
	Not tested	6	**10**	5	**9**	11	**10**
	Total	58	**100**	55	**100**	113	**100**

Of the total 113 patients, 58 were allocated to the initially daily regimen and 55 to the intermittent regimen; 44 (39%) were males, 43% were less than 15 years of age, and only 4% were above the age of 44; and 88 (78%) patients had a single lesion in the CT scan. The characteristics in the 2 regimens were similar.

Clinical presentation: Of the 113 patients, 20 presented with

Table 2

Symptoms and signs	Daily		Intermittent		Both	
	No.	%	No.	%	No.	%
Convulsions	47	81	45	82	92	81
Headache	40	69	31	56	71	63
Vomiting	20	34	19	35	39	35
Limb weakness	21	36	13	24	34	30
Fever	13	22	11	20	24	21
Visual disturbances	14	24	12	22	26	23
Unsteady gait	8	14	6	11	14	12
Others	6	10	5	9	11	10
Normal	26	45	29	53	55	49
Papilloedema	11	19	9	16	20	18
Deficit	10	17	9	16	19	17
Papilloedema plus deficit	11	19	8	15	19	17
Total patients	58	100	55	100	113	100

papilloedema only (Table 2), 19 had papilloedema with neurological deficit, 19 had only neurological deficit and 55 had only symptoms. Convulsions (81%) and headache (63%) were the main presenting symptoms (Table 2); 35% complained of vomiting and 30% of limb weakness.

The urine culture on admission was positive for **M.tuberculosis** in 2 patients (1 in each series). The initial chest radiograph was suggestive of pulmonary tuberculosis in 11. Of these, 2 were culture-positive by sputum examination, with drug-sensitive organisms.

Surgery: Of 113 admitted cases, histopathological examination following craniotomy biopsy was done in 7 patients; all were positive for tuberculosis. In 6 of these, the surgery had been undertaken prior to the start of chemotherapy and in the other patient, in the 3rd month.

Among the 31 cases excluded from analysis, histopathological examination of craniotomy biopsy specimens was done in 7 patients -- in 1 prior to the start of chemotherapy and in 6 after the start of chemotherapy (range: 19 days to 12 months); all the latter 6 were reported to have non-tuberculous pathology.

Table 3 shows the progress between 0 and 9 months as assessed by CT scan in 92 patients (45 daily, 47 intermittent) for whom readings were available. It was observed that in both series taken together, tuberculomas had totally

Table 3

Progress (0-9 months)	Daily No.	Daily %	Intermittent No.	Intermittent %	Both No.	Both %
Lesion disappeared	36	77	34	76	70	76
Lesion decreased						
by ≤ 50%	2	4	3	7	5	5
by > 50%	6	13	6	13	12	13
Static	2	4	0	0	2	2
Lesion increased						
by ≤ 50%	0	2	1	2	1	2
by > 50%	1		0		1	
New lesion appeared	0	0	1	2	1	1
Total patients	47	100	45	100	92	100

disappeared in 70 (76%) of the patients. In 17 (18%), a decrease in the size of the lesion was observed. In 2 patients, the lesion remained static. In 2 (2%) other patients, the lesions had increased in size and in another patient, a new lesion had appeared. Of the two patients with increased lesions, one refused surgery and continued to have hemiparesis and papilloedema. The remaining patient with increased lesion, the two patients whose lesions remained static and the patient who had a new lesion were clinically doing well.

Table 4 shows the incidence of untoward reactions to drugs. Jaundice was the main side-effect, and occurred in 22% in the daily regimen and 5% in the intermittent regimen ($P < 0.01$). The incidence of other side-effects was low and equally distributed in the two regimens. In all, drugs were interrupted in 16 patients (13 daily, 3 intermittent). For 7 patients (4 daily, 3 intermittent) rifampicin had to be terminated -- for jaundice in 2 patients, 'flu' syndrome in 2, vasculitis in 2 and toxic epidermal necrolysis in 1.

The patients are being followed up till 60 months, and the clinical status at 24 months for 100 patients available for follow-up is given in table 5. Of these, 48 had been normal at the start of treatment and 52 patients had clinical signs - 19

Table 4

Nature of complaint	Daily No.	Daily %	Intermittent No.	Intermittent %
Jaundice	15	22	3	5
'Flu' syndrome	1	1	1	2
Vasculitis	1	1	1	2
Arthralgia	0	0	1	2
Others	7	10	9	11
Total patients in analysis	68		63	

with papilloedema, 16 with neurological deficit and 17 with papilloedema plus deficit. At 24 months, 91 patients were normal clinically; 8 patients continued to have papilloedema and/or neurological deficit. None of the patients had a relapse of tuberculoma. One patient had treatment restarted for positive urine culture.

Table 5

Clinical status	Initial		At 24 months	
Normal	48		91	
Papilloedema	19	52	2	9
Neurological deficit	16		2	
Papilloedema plus deficit	17		5	
Total patients	100			

Conclusion: At the end of chemotherapy, the results with the daily and intermittent regimens were similar. In all, 77% and 76%, respectively, showed total scan clearance. Clinically, 90% in the intermittent regimen and 91% in the daily regimen were normal. Residual deficit was seen in 6% in the intermittent

regimen and 5% in the daily regimen. But jaundice was less with the intermittent regimen. The results of SCC of 9 months' duration in the treatment of brain tuberculoma appear encouraging from the available data; long-term follow-up findings are awaited.

(started: 1986; efficacy study completed: 1991).

Pulmonary function in healthy children (7-14 years) in South India

Since wide changes in pulmonary function in normal subjects are known to occur due to ethnic variation, physical activity, environmental conditions, altitude of dwelling, tobacco smoking, age, height, sex and socio-economic status, a comprehensive study of pulmonary function to establish regression equations for predicting normal pulmonary functions was carried out in South Indian subjects residing at Madras. The subjects are ethnic South Indians of Dravidian stock, live in a tropical climate at sea level and rice is their staple diet. In all, 208 subjects (104 boys and 104 girls) aged between 7 and 14 years were studied. Although the study population is not random, attempts were made to obtain a representative cross-section of normal subjects of Madras city. To achieve this, the subjects included relatives of patients attending the Centre; the subjects were eligible for the study if they were ethnic South Indians, with no structural deformity of the thoracic cage, who had been free from respiratory infection for at least three months. None of the subjects had any cardio-respiratory disease, as assessed by detailed history, physical examination, full plate chest radiograph and 12-lead electrocardiogram. Multiple regression equations based on age, height and sex will be established.

All pulmonary functions were carried out using P.K.Morgan Transfer Test Model C. They were as follows:-

1. Forced Vital Capacity (FVC)
2. Forced Expiratory Volume in 1 sec (FEV_1)
3. (FEV_1 x 100)/FVC %
4. Total Lung Capacity (TLC)
5. Functional Residual Capacity (FRC)
6. Residual Volume (RV)
7. RV/TLC %
8. Effective Alveolar Volume (VA)
9. Single Breath Carbon Monoxide diffusing capacity (TLCO)
10. Transfer Co-efficient (KCO)

Pulmonary function tests were also done in 64 elderly subjects (44 males and 20 females).

The data are being analysed.

(started:1985; completed:1991)

Follow-up studies in Tropical Eosinophilia (TE)

Earlier broncho-alveolar lavage and pulmonary function studies such as spirometry (FVC, FEV_1), lung volumes (TLC, FRC and RV) and diffusing capacity measurements (VA, TLCO and KCO) had shown that there was intense eosinophilic alveolitis with diffusion defect in Tropical Eosinophilia (J.Clin.Invest,1987,80:216-225). As it had been shown that untreated TE patients presenting with symptoms of long duration could develop interstitial fibrosis, this study was planned to observe the course of TE in patients presenting with symptoms of shorter duration (less than 6 months) who had been treated for 3 weeks with diethylcarbamazine citrate (6 mg/kg body weight). In all, 171 patients have been admitted to the study and are being followed up at 1, 3, 6, 12, 24, 36, 48 and 60 months. Out of a total of 104 patients due for the 60th month follow up, 80 attended. At the 24th month, of 131 patients due for follow up, 109 attended. The data are being analysed.

(started:1984; intake completed:1991)

Controlled clinical trial of fully oral short course regimens in Madras and Madurai

As explained in previous annual reports (1988, 1989 and 1990), 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 is being undertaken at the Centre and at the Centre's unit at the Government Rajaji Hospital, Madurai (Dean:Dr. Veera Babu). Patients at Madurai are admitted on the basis of smear examination done at the Madurai unit. For patients admitted to the study, multiple sputum specimens are transported to the Centre at Madras, for culture and sensitivity tests. Close liaison 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 Madras and 51 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.

Three sputum specimens are collected every month till the end of treatment and 2 specimens thereafter up to 24 months; they are examined by smear for AFB and by culture for **M.tuberculosis**, and sensitivity tests for rifampicin, isoniazid and ethambutol are done on positive cultures. Month by month culture negativity based on multiple specimens in patients with initially drug-sensitive organisms is given in table 1.

Table 1

Month	Percentage culture negative		
	$2EHRZ_7/6EH_7$ (1)	$2EHRZ_2/4EHR_2$ (2)	$2HRZ_2/4HR_2$ (3)
1	**32**	**23**	**28**
2	**88**	**73**	**75**
3	**90**	**91**	**89**
4	**94**	**97**	**91**
5	**96**	**97**	**89**
6	**95**	**98**	**91**
7	**95**		
8	**94**		
Total patients (range)	304 - 305	261 - 263	254 - 257

At 2 months, 88% of the patients in regimen 1 and 73% and 75% in regimens 2 and 3 had all three cultures negative. The difference between regimen 1 and regimens 2 and 3 is statistically significant ($P < 0.001$). From the 4th month onwards, culture negativity is similar in regimens 1 and 2; the differences between regimens 2 and 3 are statistically significant ($P<0.01$), while the differences between regimens 1 and 3 are not statistically significant.

An unfavourable response at the end of chemotherapy is defined as a total of 2 or more positive cultures in the last 2 months, including at least one in the last month of treatment and at least one having a growth of 20 colonies or more. Patients who had a change of treatment for persistent bacteriological positivity or radiographic deterioration or clinical deterioration and those who died of tuberculosis are also classified as having an unfavourable response. Thus, 11 (3.6%) of 305 patients with initially drug-sensitive organisms in regimen 1, 1 (0.4%) of 263 patients in regimen 2, and 24 (9.3%) of 257 patients in regimen 3 had an unfavourable response (see table 2); the differences between regimens 1 and 2, 2 and 3, and 1 and 3 are statistically significant ($P=0.02$, <0.0001, and $=0.01$, respectively).

Table 2

Regimen	Total assessed	Unfavourable response No.	%	95% confidence limits
1) $2EHRZ_7/6EH_7$	305	11	3.6	1.8 - 6.5
2) $2EHRZ_2/6EHR_2$	263	1	0.4	0.0 - 2.2
3) $2HRZ_2/4HR_2$	257	24*	9.3	6.2 - 14.0

* Including one tuberculous death

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 690 patients with initially drug-sensitive organisms have completed 16/18 months of follow-up after stopping treatment. Relapse requiring treatment (table 3) occurred in 13 (5.1%) of 253 patients in regimen 1, 26 (11.3%) of 231 in regimen 2 (a significant difference : $P=0.02$), and 20 (9.7%) of 206 patients in regimen 3. The differences between regimens 2 and 3, and

Table 3

Regimen	Total assessed	Relapse requiring Rx No.	%	95% confidence limits	Month of relapse after stopping Rx 1-3	4-6	7-12	13-16/18
1) $2EHRZ_7/6EH_7$	253	13	**5.1**	**2.8- 8.8**	10	2	0	1
2) $2EHRZ_2/4EHR_2$	231	26	**11.3**	**6.9-14.9**	14	7	3	2
3) $2HRZ_2/4HR_2$	206	20	**9.7**	**6.2-15.0**	13	3	3	1

regimens 1 and 3 are not significant. Ten of 13 relapses in regimen 1, 14 of 26 in regimen 2, and 13 of 20 in regimen 3 occurred within 3 months after stopping chemotherapy.

The response and relapse during 16/18-month follow-up of 227 patients with initially isoniazid-resistant organisms is given in Table 4.

Table 4

Regimen	Total assessed	Unfavourable response No.	%	Total assessed for relapse	Relapse requiring Rx No.	%	Month of relapse after stopping Rx 1-3	4-12
1) $2EHRZ_7/6EH_7$	94	16	**17**	63	5	**8**	4	1
2) $2EHRZ_2/4EHR_2$	59	12	**20**	39	10	**26**	10	0
3) $2HRZ_2/4HR_2$	74	46	**62**	22	4	**(18)***	3	1

*The parentheses indicate that the percentage is based on fewer than 25 observations.

Seventeen percent of 94 patients in regimen 1, 20% of 59 patients in regimen 2 and 62% of 74 patients in regimen 3 had an unfavourable bacteriological response. The differences between regimen 3 and each of the other 2 regimens is statistically significant ($P<0.0001$ for both). Relapse occurred in 8% of 63 patients in regimen 1, 26% of 39 patients in regimen 2 ($P=0.03$) and 18% of 22 patients in regimen 3.

There were 38 patients with organisms resistant to rifampicin and isoniazid on admission (13 in regimen 1, 12 in regimen 2 and 13 in regimen 3). All except 3

had an unfavourable response and change of treatment; of these 3, one (regimen 3) had a bacteriological relapse in the first month of follow-up and treatment was restarted. One other patient (regimen 2) had organisms initially resistant to rifampicin but sensitive to isoniazid; he also had an unfavourable response and change of treatment.

Adverse reactions: The commonest adverse reaction encountered was arthralgia, which occurred in 23% of 334 patients in the daily regimen as against 5% of 586 patients in the twice weekly regimens combined ($P<0.001$). Gastro-intestinal upsets occurred in 8% of patients in the daily regimen and 10% of patients in the twice weekly regimens. Jaundice occurred in 4% of patients in the daily regimen and 1% in the twice weekly regimens; in all, rifampicin, isoniazid and pyrazinamide were withheld temporarily and reintroduced uneventfully when jaundice subsided. Isoniazid had to be terminated in one patient because of convulsions, rifampicin had to be terminated in 2 patients due to vomiting, 2 patients were successfully desensitized to pyrazinamide for cutaneous hypersensitivity and one patient, who had vomiting due to hypersensitivity to isoniazid, was also successfully desensitized. In all other patients, adverse reactions could be successfully managed with symptomatic treatment.

Interim findings of the study show that with the regimen where rifampicin, isoniazid and pyrazinamide were given twice a week for the first 2 months followed by rifampicin and isoniazid twice a week for the next 4 months ($2RHZ_2/4HR_2$), 9% of the patients with organisms initially sensitive to rifampicin and isoniazid had an unfavourable response and 10% had a bacteriological relapse requiring treatment within 18 months of completing treatment. The corresponding figures among patients with initial resistance to isoniazid were 62% and 18%, respectively. With the twice weekly regimen containing ethambutol in addition, the corresponding figures were 0.4% and 11%, respectively, in patients with initially drug-sensitive organisms, and 20% and 26%, respectively, in patients with initially isoniazid-resistant organisms. Thus neither of the intermittent regimens appears to be satisfactory, even in patients with initially drug-sensitive organisms; the former regimen is also highly unsatisfactory with respect to response to treatment in patients with initial isoniazid resistance, while the latter regimen is unsatisfactory with respect to relapse in such patients.

Considering the regimen where rifampicin, ethambutol, isoniazid and pyrazinamide were given daily for the first 2 months, followed by ethambutol and isoniazid daily for the next 6 months, 4% had an unfavourable response and 5% had a relapse requiring treatment among patients with initially drug-sensitive organisms; the corresponding figures were 17% and 8%, respectively, among patients with initial isoniazid resistance. Thus, this regimen seems to be effective, irrespective of the initial isoniazid-sensitivity status. However, findings over a longer period of follow-up are necessary to draw firm conclusions; the patients are being followed up till 5 years from the start of treatment to obtain this information.

Also, the drugs are self-administered, and the possibility of patients not ingesting the collected drugs regularly, especially under programme conditions, has to be kept in mind.

(started: 1986; expected year of completion of follow-up: 1995)

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

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

The dosages are the 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 isoniazid is 600 mg, irrespective of body weight.

A total of 298 patients (156 at Madras and 142 at Madurai) have been admitted by the end of December 1991.

The distribution of patients according to the regimen is given in table 1.

Table 1

Centre	2 alt.	3 alt.	Thrice	Total
Madras	53	54	49	156
Madurai	48	44	50	142
Both	101	98	99	298

So far, 238 patients have completed phase I (127 at Madras and 111 at Madurai). Four patients were excluded from analysis due to early death (1), pretreatment culture negativity (2) and infection with non-tuberculous mycobacteria on admission (1). Of the remaining 234 patients, 173 received 90% or more of chemotherapy, i.e. , 58 patients in "2 alt", 52 in "3 alt" and 63 in "thrice" regimen (Table 2).

Regarding adverse reactions among the patients who completed phase I (Table 3), 11 patients in "2 alt", 19 patients in "3 alt" and 22 patients in "thrice" regimen had some complaint(s). One patient (3 alt) had change of treatment, because of anaphylactic reaction; all others were managed symptomatically. Gastro-intestinal intolerance seemed to be more common in "thrice" regimen, where all the four drugs were given together in a single dose.

Table 2

Rx received(%)	2 alt.		3 alt.		Thrice	
100	17		11		38	
95 - 99	21	58	29	52	13	63
90 - 94	20		12		12	
80 - 89	11		13		7	
70 - 79	6		6		3	
60 - 69	0		5		2	
50 - 59	1		0		3	
< 50	0		0		1	
Continuous FTA > 1m	0		1		2	
Total	76		77		81	

Table 3

Regimen	Total No. of patients	Patients with complaints				
		Any	Arthralgia	Gastro - intestinal	Giddiness	Others
2 alt.	76	11	4	3	2	3
3 alt.	77	19	9	8	3	4
Thrice	81	22	4	15	4	3

The study is in progress.

(started: 1990; expected year of completion of intake: 1994)

Collaborative controlled clinical trial of tuberculous lymphadenitis

A controlled clinical trial of tuberculous lymphadenitis is in progress at the Centre's Unit in Madurai in collaboration with the Paediatric (Dr.A.J.Thiruthuvathas) and Adult Surgery (Dr.M.N.Kamaluddin) Departments of the Government Rajaji Hospital. Patients residing in and around Madurai, irrespective of any history of previous chemotherapy, are considered for the study, provided the clinical diagnosis of tuberculous lymphadenitis is confirmed by either histopathology or culture of lymph node biopsy. The histopathology slides are read by the Professor of Pathology (Dr.V.Ananthalakshmi), Madurai Medical College, and bacteriological investigations are done at the Centre, at Madras.

Patients admitted to the study are treated as out-patients, and randomly allocated to either a 6-month daily self-administered regimen of rifampicin and isoniazid ($6RH_7$) supplied twice a month, or a 6-month fully supervised twice-weekly regimen of rifampicin, isoniazid and pyrazinamide for the first 2 months, followed by rifampicin and isoniazid for the next 4 months ($2RHZ_2/4RH_2$). The drugs are prescribed in uniform dosages for adults, while a weight-adjusted dosage schedule is used for children. Surprise home visits for pill counts are done for patients allocated to the self-administered regimen ($6RH_7$).

The patients are examined clinically every month up to 12 months, every three months up to 24 months and every six months thereafter at the Centre's Unit and also by the surgeons. At the completion of 6 months of chemotherapy, the response is assessed by an independent observer. The response is defined as favourable if the lymph nodes have regressed in size to 10 mm or less and if any sinuses present on admission have healed. The response is considered as doubtful if significant residual nodes (>10 mm diameter) are present at the end of treatment; repeat lymph node biopsy is done for such patients. The patients will be followed up for 5 years from the start of treatment.

A total of 210 patients have been admitted to the study (71 children and 139 adults); 171 patients have completed treatment and 164 are available for interim analysis (at the end of treatment). Of these, 53 (32%) are males (Table 1) and, 41 (25%) were aged less than 10 years; 156 (95%) had lymph node histology suggestive of tuberculosis while in 5, the results were negative and in 3 patients lymph node histology was not available. Lymph node culture was positive for **M.tuberculosis** in 118 patients (72%) and negative in 35 patients (21%).

In all, 76 (92%) of 83 in the $6RH_7$ regimen (self-administered) and 79 (98%) of 81 in the $2RHZ_2/4RH_2$ regimen (supervised) had a favourable response at the

Table 1

Factor on admission		Patients No.	Patients %
Sex			
Male		53	32
Female		111	68
Age (years)			
< 10		41	25
10 - 29		98	62
≥ 30		25	13
Histopathology			
Positive		156	95
Negative		5	3
Not tested		3	2
Bacteriology			
Culture positive		118	72
Smear positive	16		
Smear Negative	102		
Culture negative		35	21
Smear positive	2		
Smear negative	33		
Non-TB mycobacteria		6	4
Not done		5	3
Total		164	100

end of treatment. In 6 and 2 patients respectively, the response was doubtful; the post-treatment repeat biopsy was negative by histology and culture in all the patients. One patient ($6RH_7$ regimen) had an unfavourable response (clinical deterioration during treatment).

The intake is continuing.

(started: 1988; expected year of completion: 1993)

Long term follow-up of children treated for tuberculous meningitis with short course chemotherapy

The detailed findings of the short course chemotherapy study on tuberculous meningitis patients have already been presented (1988 annual report). In brief, patients aged between 1 and 12 years were randomly allocated, after stratification according to clinical severity, in equal proportions 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 patients (27%) died of tuberculous meningitis, 52 (35%) had neurological sequelae and 58 (39%) had a complete recovery. The response was similar in the two regimens.

The survivors at the end of treatment are being followed up to find out the relapse rates and the course of the lesions. They are 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 include (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 become normal, and (c) cerebro-spinal fluid (CSF) examination for cell count, biochemical characteristics and bacteriological examination for **M.tuberculosis** every three months 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) radiographs of skull for evidence of calcification.

Of the 110 survivors at the end of chemotherapy, 16 patients had a persistent abnormality in the chest radiograph. In 13 patients it became normal (in 6 patients between 10-12 months, in 4 between 13-24 months, and 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 sequelae) up to 60 months; 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 cerebrospinal(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 last CSF examination done at the 48th month was still abnormal ; the examination could not be repeated thereafter 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 and 57 with complete recovery) have completed the 48th monthly examination, i.e., 39 months of follow-up after stopping treatment. Table 1 shows the status at 48 months compared to the status at 9 months.

Table 1

Status at 9m	No. of pts.	Death after 9m due to		Status at 48 months				
		TBM sequelae	Non TB causes	No.eligible	Sequelae			Complete recovery
					Severe	Moderate	Mild	
Severe sequelae	5	5	0	0	-	-	-	-
Moderate sequelae	30	1	1	28	0	26	1	1
Mild sequelae	17	2*	1	14	0	2	10	2
Complete recovery	58	0	1	57	0	4	2	51
Total	110	8**	3	99	0	32	13	54

* Including one patient who had a relapse

** 5 patients died between 10 and 24 months, 2 between 25 and 36 months and 1 patient between 37 and 48 months.

Of the 28 patients with moderate sequelae, in 26 the status remained the same, 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 the lower and upper limbs and the other developed secondary epilepsy as a late sequelae), and in 10 there was no change, while the remaining 2 patients recovered completely.

Of the 57 patients with complete recovery, in 4 the status changed to moderate sequelae (all developed secondary epilepsy) and in 2 to mild sequelae; in the remaining 51, the recovery was maintained.

One patient, who had mild sequelae at the end of treatment, had a relapse within 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 the 31st month..

The 5-year follow-up is continuing.

(started: 1982; expected year of completion: 1993)

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

Case holding is an important component of the National Tuberculosis Programme. The level had been 30% with standard regimens of 12-18 months' duration and has increased to around 50% with 6 or 8- month short course regimens.

Motivation on admission and during treatment is an important factor which helps to improve case holding. In the field of health, motivation is generally the responsibility of the provider system, consisting of doctors, nurses, social workers and other para-medical staff. This has an impact on patient compliance. However, making an additional effort at motivation by using a patient previously treated successfully as the motivator, a kind of "satisfied customer" approach, could possibly further improve patient compliance.

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.

So far, 98 patients have been admitted to these regimens. Of 71 eligible cases for whom information up to one year is available, 20 are excluded (Table 1). Of the remaining 51 (26 in 9m regimen and 25 in 12m regimen), 47 (25 in 9m

Table 1

	9m reg	12m reg
No. with information up to 1 year	36	35
Change of Rx due to adverse drug reactions	2	0
Early death	0	1
Non-TB death	0	1
<75% of chemotherapy	8	8
No. in analysis	26	25
Favourable response	25	22
Untavourable response	1	3

regimen and 22 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. 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 to the 8th month. In one 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 other 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 $6SEmbRZ_2/6EmbRZ_2$ or $6KEmbRZ_2/6EmbRZ_2$ 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 ritampicin plus pyrazinamide in the same dosages twice a week for the next 6 months (total 12

In this study, all the patients are treated with a 6-month fully supervised regimen with twice weekly attendance. Half the patients are allocated, at random, 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 the compliance is also related to 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 taken following the same procedures 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, 71 patients have been admitted to the study. The intake is continuing.

(started: 1991; expected year of completion: 1993)

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

Pulmonary tuberculosis patients who have been treated with short course regimens and who (i) show a serious clinical deterioration, or (ii) have a persistent radiographic deterioration due to tuberculosis, or (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 $3EmbHRZ_2/6HR_2$, or $3EmbHRZ_2/9HR_2$, namely ethambutol 1200 mg plus isoniazid 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.

months). Every dose is administered under supervision.

6SEmbRZ$_2$/6EmbRZ$_2$: So far, 50 patients have been admitted to this regimen. Of the 33 eligible cases for whom information up to 1 year is available, 11 are excluded (Table 2). Of the remaining, 4 have shown an unfavourable bacteriological response. Two patients required a change of chemotherapy. Of these, 1 had positive cultures persistently; his treatment was changed at the 6th month, from intermittent to daily chemotherapy. The other 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. 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 4 patients had associated clinical or radiographic deterioration.

6KEmbRZ$_2$/6EmbRZ$_2$: So far, 49 patients have been admitted to this regimen. Of the 36 eligible cases, 6 are excluded (Table 2). Of the 30 in analysis,

Table 2

	H resist.	SH resist.	RH resist.	SHR resist.
	6SEmbRZ$_2$/ 6EmbRZ$_2$	6KEmbRZ$_2$/ 6EmbRZ$_2$	3S$_3$EmbEthZ$_7$/ 9EmbEthZ$_7$	3K$_3$EmbEthZ$_7$/ 9EmbEthZ$_7$
No. with information up to 1 year	33	36	14	27
Change of Rx due to adverse drug reactions	3	1	4	3
Non TB death	1	1	0	1
< 75% chemo-therapy	7	4	4	7
No. in analysis	22	30	6	16
Favourable response	18	23	2	6
Untavourable response	4	7	4	10

23 had a favourable bacteriological response. Of the remaining 7 patients, 5 had a change of treatment between 5 and 10 months for persistent culture positivity; all 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 ethambutol 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-administration.

$3S_3EmbEthZ_7/9EmbEthZ_7$: So far, 20 patients have been admitted to this regimen. Of the 14 eligible cases, 8 are excluded (Table 2). Of the remaining 6 patients, 2 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, 31 patients have been admitted to this regimen. Of the 27 eligible cases, 11 are excluded (Table 2). Of the 16 in analysis, 6 had a favourable bacteriological response. Of the remaining 10, all except one persistently had positive cultures and had a change of treatment between 6 and 12 months. The other 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.

The intake to all the regimens is continuing.

(started: 1987)

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

Pulmonary function tests are being carried out in patients with tuberculosis of the spine who had been treated with a short-course regimen, namely, 6 months of ambulatory out-patient treatment with rifampicin and isoniazid daily, or 9

months of ambulatory out-patient treatment with rifampicin and isoniazid daily, or radical resection of the diseased vertebrae with bone grafting plus rifampicin and isoniazid daily for 6 months. Since these tests had not been undertaken on admission to treatment, comparative pretreatment values for the different groups are not available.

The aims of the study are 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 thoraco-lumbar region of the spine compromises the respiratory function to a greater extent than a lesion in the lumbar region. The following pulmonary function tests are being carried 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 are recorded in each patient every year. A total of 175 patients were initially tested 3-6 years after treatment, and are being followed up yearly.

(started: 1982; expected year of completion: 1992)

A controlled clinical trial of dapsone as continuation chemotherapy beyond 7 years

As mentioned in previous (1986-87 & 1990) 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 BI value at 57, 58, 59 and 60 months, namely, 0.5 or more and less than 0.5, and were randomly allocated to one of two regimens, namely, clofazimine 50 mg with dapsone 100 mg daily (CD group) or dapsone 100 mg daily (D group) for a further period of 2 years. At the end of 84 months, the patients were randomly allocated to either dapsone (DDS) or placebo if their BI value at 84 months was 1.0 or less; if the BI 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.) had been admitted to the trial, of whom 159 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, 29 had failed to attend for long periods and 1 patient developed toxicity to dapsone. Of the 159 patients, 27 (14 DDS, 13 placebo) are yet to complete 120 months; 10 patients (8 DDS, 2 placebo) have been excluded (1 died, 2 developed tuberculosis and were prescribed antituberculosis chemotherapy, and 7 failed to attend for over 6 months). The findings up to 120 months in the remaining 122 patients (59 DDS , 63 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 in table 1. Over 0-84 months, moderate or marked improvement was reported in 55 (96%) in the DDS group and 61 (97%) in the placebo group. The corresponding figures were 57 (100%) and 58 (98%) over 0-96 months, 48 (100%) and 53 (98%) over 0-108 months, and 48 (100%)

Table 1

Regimen (85 - 120 months)	Progress	Period (months) 0 - 84 No.	%	0 - 96 No.	%	0 - 108 No.	%	0 - 120* No.	%
Dapsone (n=59)	Improvement marked	54	**95**	56	**98**	47	**98**	48	**100**
	moderate	1	**2**	1	**2**	1	**2**	0	**0**
	slight	2	**4**	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	-	2	-	11	-	11	-
Placebo (n=63)	Improvement marked	55	**87**	56	**95**	52	**96**	50	**96**
	moderate	6	**10**	2	**3**	1	**2**	2	**4**
	slight	0	**0**	0	**0**	0	**0**	0	**0**
	No change	1	**2**	1	**2**	0	**0**	0	**0**
	Deterioration	1	**2**	0	**0**	1	**2**	0	**0**
	Absent for assessment	0	-	4	-	9	-	10	-

* Excluding one patient in the placebo group who was assessed as deteriorated at 107 months and retreated.

and 52 (100%) over 0-120 months, respectively. Thus, there was excellent clinical improvement in both series.

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 0.54 for the DDS group and 0.49 for the placebo group at 84 months, 0.30 each at 96 months, 0.21 and 0.19 at 108 months, and 0.14 and 0.07 respectively, at 120 months.

Table 2

Regimen (85-120m)	BI	Months			
		84	96	108*	120*
Dapsone (n=59)	Mean	0.54	0.30	0.21	0.14
	Range	(0.00-1.50)	(0.00-2.00)	(0.00-1.17)	(0.00-1.00)
Placebo (n=63)	Mean	0.49	0.30	0.19	0.07
	Range	(0.00-1.67)	(0.00-1.33)	(0.00-0.83)	(0.00-0.67)

* Excluding one patient in the placebo group who was assessed as detereriorated 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.

(started:1977; expected year of completion:1992).

Controlled clinical study of multi-drug therapy in multibacillary leprosy

As mentioned in the previous (1988 - 1990) annual reports, the Centre is undertaking a controlled clinical trial to assess the relative efficacies of pyrazinamide and rifampicin in combination with clofazimine and DDS in the treatment of multibacillary leprosy, at the Govt.Royapettah Hospital, Madras.

The following 4 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 first 3 months and 50 mg/kg body-weight twice-weekly for the next 9 months.

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

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

It is proposed to admit 25 patients to each regimen; 76 patients have been admitted so far to the study - 19 patients to each of the 4 regimens. In all, 37 have information up to 2 years. Of these, 3 were excluded (2 patients died due to reasons other than leprosy (carcinoma of the stomach, suicide) and treatment was changed in 1 due to jaundice); the findings in the remaining 34 patients are presented here.

The mean age on admission was 30 years (range 15-55 years). There were 28 males and 6 females; the average weight was 46.9 kg (range 25.0-69.8 kg). Regarding previous treatment it was found that 15 patients had not had any previous antileprosy treatment, 4 had less than one year of treatment, 11 had 1-5 years, and 4 had over 5 years. All the patients were tested for lepromin induration and were found to be negative for early (2nd day) and late (21st day) readings. An induration of 10 mm or more to 1TU of PPD (RT23 with tween 80) was seen in 12 patients; however, chest PA radiographs taken as a routine were found to be normal in all.

The clinical classification on admission was polar lepromatous leprosy (LLP) in 7, subpolar lepromatous leprosy (LLS) in 17, and borderline lepromatous leprosy (BL) in 10.

The mean BI was 4.26 on admission, 3.74 at 12 months, and 3.11 at 24 months. Finger smears on admission were positive for all the patients except one.

Periodic skin biopsies are taken for histopathology and mouse foot-pad inoculation, and skin, nasal and finger smears are examined. The intake is continuing; the patients are being followed up till 5 years from the start of treatment.

(started: 1988; expected year of completion of intake: 1992)

LABORATORY STUDIES

STUDIES COMPLETED

Use of cetylpyridium chloride (CPC) for storage of sputum specimens and isolation of *M.tuberculosis*

A fresh study was undertaken to assess the usefulness of CPC for storage of sputum samples and isolation of **M.tuberculosis**, after slightly modifying the procedure followed in an earlier study (see 1985-86, 1987 & 1988 annual reports) for the processing of CPC-Nacl treated sputum samples.

A total of 220 sputum samples were studied. Each sample was homogenised and divided into 3 aliquots of 4-5 ml each. One of these aliquots was taken up for culture by the conventional NaOH method, on the same day. The remaining 2 aliquots were randomly allocated to CPC and NaOH methods with storage. To the sputum aliquot allocated to the CPC method, an equal volume of CPC (1%) - Nacl (2%) reagent was added; after shaking well, it was kept along with the other aliquot in a cupboard at ambient temperature. On the 7th day, these two aliquots were processed for culture by the respective methods.

CPC method: The sputum aliquot treated with CPC-Nacl reagent was centrifuged, and a loopful of the deposit was inoculated on to each of 2 LJ slopes. Three serial ten-fold dilutions of the deposit were made in sterile water and a loopful of each of these suspensions was inoculated on to each of 2 LJ slopes. The cultures were examined weekly. The culture results obtained using 1:10 diluted deposit were considered for analysis as a high proportion (52%) was contaminated with the normal inoculum and the proportions of positive cultures were lower with the 1:100 and 1: 1000 dilutions.

After excluding the samples which were either negative or contaminated in all the three aliquots, there remained 110 samples for the analysis.

Comparison of the culture results obtained with the 110 sputum samples processed by the CPC and NaOH methods after storage, with those obtained by the NaOH method before storage are presented in table 1. The number of cultures positive before and after storage by the NaOH method were 95 (86%) and 70 (64%), respectively; the difference was highly significant (McNemar's test: $p < 0.001$). The culture positivity obtained by the CPC method after storage was 85 (77%) which is comparable to the results obtained by the NaOH method before storage; the difference was not statistically significant ($p = 0.07$).

Table 1

Culture grade before storage by NaOH method	Culture grade after storage												Total
	NaOH method						CPC method						
	3+	2+	1+	Col.*	Neg	Cont	3+	2+	1+	Col.	Neg	Cont	
3+	4	12	4	-	-	6	11	12	-	-	-	3	26
2+	1	13	7	9	7	7	9	16	11	4	3	1	44
1+	-	1	1	4	4	3	-	3	3	4	2	1	13
Col.	-	-	1	4	6	1	-	1	-	3	7	1	12
Neg	-	2	-	2	2	2	-	1	-	1	6	-	8
Cont	-	3	2	-	1	1	2	2	1	1	1	-	7
Total	5	31	15	19	20	20	22	35	15	13	19	6	110

* Col.: 1-19 colonies; Neg: Negative; Cont: contaminated.

Of 83 samples which showed more than 19 colonies by the NaOH method before storage, 56 (67%) and 73 (88%) were positive for culture by the NaOH and CPC methods, respectively, after storage. Among 12 samples which showed 1-19 colonies before storage, 5 and 4 were positive for culture by the NaOH and CPC methods, respectively, after storage.

The findings suggest that the CPC method could avoid loss of viability of tubercle bacilli when sputum samples need to be processed after storage or transit at room temperature up to 7 days. Hence, the CPC method may be of value when culture examination is undertaken in centralised laboratories.

(started: 1990; completed: 1991)

Use of selective Kirchner's liquid medium for transporting lymph node biopsy specimens

Lymph gland specimens excised from adult and paediatric patients admitted to the ongoing lymphadenitis study at Madurai were transported to Madras in selective Kirchner's liquid medium (referred to as KL-T). On receipt,

the specimens were taken out and processed for culture using multiple media, namely, Lowenstein-Jensen (LJ), LJ with pyruvate (LJP), selective 7H11 and selective Kirchner's liquid medium (KL). The KL-T was incubated as such and subcultured on to two LJ slopes at the end of 6 weeks, or as and when any growth was observed.

Out of 327 lymph node specimens, 316 were positive by histopathology and 11 were negative. Of them, 241 (74%) yielded a positive culture, including all the 11 which were negative by histopathology. By incubating KL-T alone after removing the gland, 99 (30%) of the specimens yielded a positive culture, including 3 which were positive only in this medium. Only one specimen got lost due to contamination.

The transportation time in days and the culture positivity in the 327 specimens are presented in table 1.

Table 1

Transportation time (days)	Total specimens		Culture positive		
			No.		%
1 - 2	111	252	81	183	**73**
3 - 4	141		102		
5 - 19		75		58	**77**
Total		327		241	**74**

In 252 samples with a transportation time of 4 days or less, 73% were culture positive. In 75 samples which were received after 5-19 days, the rate of positivity was 77% . Thus, the delay due to transportation did not have any effect on the culture positivity.

It is of interest that in an earlier report (Jawahar et al, **British Medical Journal**,1990, **301**, 359) where no transport medium was used and the lymph node specimens were processed the same or the next day, the culture positivity rate was 62% . In the present study, though there was delay in transit ranging from 1 to 19 days, 74% of the specimens were positive by culture.

(started: 1989; completed: 1991)

Protective response in guinea-pigs exposed to *M.avium intracellulare / M.scrofulaceum*, BCG and South Indian isolates of *M.tuberculosis*

Exposure of the immune system to NTM, BCG and virulent mycobacteria in different sequences is very likely to be occurring in the populations of endemic areas of tuberculosis, such as the South Indian BCG Trial area, with high prevalence of exposure to NTM and also covered by BCG vaccination. The protective immunity resulting from such exposure was studied in the guinea-pig model, employing strains prevalent in the region and time kinetics to observe the immuno-modulation.

Different sensitisation, immunisation and challenge schedules were followed for each group of guinea-pigs as shown in table 1. The guinea-pigs were randomized and coded; they were sacrificed 12 weeks after challenge, scores were given for the amount of visible disease in the organs, and viable counts set up from the spleen.

Table 1

Week	Group A	Group B	Group C	Group D	Group E
0	SIV	BCG	BCG	NTM	NTM
3	|	|	|	|	|
6	BCG/None	SIV	NTM	SIV	BCG
9	|	|	|	|	|
12	Sc,VC	|	SIV	|	SIV
15		|	|	|	|
18		Sc,VC	|	Sc,VC	|
21			|		|
24			Sc,VC		Sc,VC

SIV : **M.tuberculosis** South Indian variant
NTM : Non-tuberculous mycobacteria (**M.avium intracellulare / M.scrofulaceum**)
VC : Viable count in spleen
Sc : Scoring

Time kinetics experiments: Another experiment was undertaken to determine the kinetics of the early course of challenge infection, using another strain of **M.tuberculosis**. The sensitisation, immunisation and challenge

schedule followed tor each group of guinea-pigs is shown in table 2. At 2, 4, 6 and 8 weeks after challenge, two animals from each subgroup were sacrificed. Scoring and spleen viable counts were performed as before.

Table 2

Week	Group 1	Group 2	Group 3	Group 4
0			Mai	
6		BCG	BCG	SIV
12	SIV	SIV	SIV	SIV
14	Sc,VC	Sc,VC	Sc,VC	Sc,VC
16	"	"	"	"
18	"	"	"	"
20	"	"	"	"

SIV : **M.tuberculosis** South Indian low virulent strain
VC : Viable count in spleen
Mai : **M.avium intracellulare**
Sc : Scoring

The viable count in the spleen and root index ot disease at 12 weeks after challenge are given in table 3. The root index and the viable counts in spleen at 2, 4, 6 and 8 weeks after challenge are given in table 4.

In animals exposed to **M.avium intracellulare** or **M.scrofulaceum** and challenged with **M.tuberculosis** South Indian strain, the protection, though present, was not total as seen from the small number of viable organisms in the spleen 12 weeks after challenge compared to animals which were directly challenged with a similar dose of SIV (Table 3).

The animals which were immunised with BCG, whether or not followed by NTM before challenge with SIV, did not have any viable organisms in the spleen at 12 weeks. This indicated that the level of protective immune response in those animals exposed to BCG tirst was high and was not atfected by subsequent exposure to NTM. On the other hand, in animals exposed to **M.avium intracellulare** or **M.scrofulaceum** first betore they were exposed to BCG followed by challenge with SIV, small numbers of viable organisms could be detected in the spleen after 12 weeks. This suggested that some modulation of the protective immunity due to BCG was taking place in animals exposed to NTM tirst.

Table 3

Group*	Root index of disease	Log VC in spleen
SIV	0.83	5.55
SIV-BCG	0.54	0.00
BCG-SIV	0.26	0.00
Mai-SIV	0.43	2.15
M.sc-SIV	0.45	2.33
BCG-Mai-SIV	0.41	0.00
BCG-M.sc-SIV	0.56	0.00
Mai-BCG-SIV	0.34	2.15
M.sc-BCG-SIV	0.64	2.33

SIV : **M.tuberculosis** South Indian strain
Mai : **M.avium intracellulare**
M.sc : **M.scrofulaceum**
* No. of guinea-pigs in each group = 3

During the early course, both in animals immunised with BCG and in animals sensitised with **M.avium intracellulare** before immunisation (Table 4), the challenge infection was localised and confined to the site of inoculation when compared to the animals that were directly challenged, and only a few organisms reached the spleen. The guinea-pigs used in the present study appear to have

Table 4

Group*	Root index at				Log VC in spleen			
	2wk	4wk	6wk	8wk	2wk	4wk	6wk	8wk
SIV	0.51	0.69	0.55	0.59	4.22	2.43	0.00	0.00
BCG-SIV	0.59	0.88	0.51	0.57	3.10	2.61	0.00	0.00
Mai-BCG-SIV	0.80	0.32	0.29	0.53	2.98	0.00	0.00	0.00
SIV-SIV	0.55	0.57	0.59	0.18	0.00	2.44	0.00	0.00

SIV : **M.tuberculosis** South Indian low virulent strain
Mai : **M.avium intracellulare**
* No. of guinea-pigs in each group = 8

some degree of natural resistance to mycobacterial infections as seen by their ability to control the infection. This ability was further accentuated in guinea-pigs which had been sensitised/immunised with other mycobacteria.

These findings suggest that, in guinea-pigs, there is no interference to the immunity due to BCG by prior exposure to NTM in the early course of challenge infection. However, at the later stages of the infection, as seen by the spleen viable counts at 12 weeks (Table 3), it appears that the barrier at the localised site of infection may not be completely intact in the animals with prior exposure to NTM, and a few organisms disseminate to the spleen.

(started: 1986; completed: 1990)

IgG antibody levels to various mycobacterial antigens in pre- and post-BCG/tuberculin conversion serum samples of young individuals from the South Indian BCG Trial area and Britain

A study of antibody levels to various mycobacterial antigens, determined by using a sensitive assay system such as ELISA, in the population of the South Indian BCG Trial area, with respect to BCG vaccination/ tuberculin conversion status will yield valuable information on the sensitisation pattern of this population to the antigens of mycobacteria prevalent in this region. Changes, if any, in the antibody levels to various mycobacterial antigens after BCG vaccination and tuberculin conversion will depend partly on the immunomodulatory effect due to the interaction of various mycobacterial antigenic stimuli brought about by pre-existing sensitisation patterns and BCG intervention. The estimation of antibody levels against various mycobacterial antigens in this population may also be helpful in the evaluation of any serological test for mycobacterial diseases which may be employed in this area.

With these aims, IgG antibody levels to various mycobacterial antigens were estimated by ELISA in pre- and post- BCG/ tuberculin conversion serum samples from 13 young individuals (mean age 16.5 yrs) belonging to this area and compared with the levels in 20 young British subjects (mean age 14.5 yrs). The post- BCG serum samples were collected 8 weeks after vaccination after confirming tuberculin conversion. Serum samples from the young British subjects were provided by Dr.D.B. Lowrie, National Institute of Medical Research, Mill Hill, London.

The sonicate antigens were prepared by one of the Centre's staff members in the Royal Postgraduate Medical School, London. PPD (RT22) was obtained from the BCG Laboratory, Madras. All the antigens were used at a concentration of 5 µg/ml.

All the serum samples were tested in duplicate after they had been given code numbers. The pre- and post-BCG serum samples were tested simultaneously on the same plates, with all the Indian samples in one batch and the British samples in another batch. From the O.D. values for duplicate samples tested, means were calculated. Means for the pre- and post-vaccination samples were compared by the paired t-test.

Young Indians: The pre-BCG samples from the young Indians had the highest antibody levels against BCG and **M.gordonae**, and the lowest levels against PPD and **M.terrae** (Table 1). In the post-BCG serum samples also similar levels of antibodies were present against the mycobacterial antigens and the order of antibody levels to the 15 antigens were the same except that **M.flavescens** and PPD had interchanged their positions. None of the differences between the pre- and post-BCG serum samples to any of the mycobacterial antigens was statistically significant.

Table 1

Order	Young Indians (n=13) Mean O.D.				Young British subjects (n=20) Mean O.D.			
	Pre-BCG		Post-BCG		Pre-BCG		Post-BCG	
1	BCG	0.468	BCG	0.469	M.tb7219	0.483	M.tb7219	0.510
2	M.gord	0.456	M.gord	0.454	M.chel	0.447	M.gord	0.465
3	M.fort	0.408	M.fort	0.405	M.gord	0.437	M.intra	0.449
4	M.chel	0.396	M.chel	0.401	M.intra	0.434	M.chel	0.429
5	M.tbS1	0.378	M.tbS1	0.379	BCG	0.432	BCG	0.429
6	M.avium	0.368	M.avium	0.375	PPD	0.422	M.terr	0.417
7	M.bovis	0.364	M.bovis	0.374	M.terr	0.406	PPD	0.406
8	M.scrof	0.348	M.scrof	0.361	M.bovis	0.392	M.bovis	0.397
9	M.tb7219	0.346	M.tb7219	0.340	M.avium	0.374	M.flav	0.394
10	M.kans	0.342	M.kans	0.323	M.fort	0.369	M.avium	0.375
11	H37Rv	0.320	H37Rv	0.321	M.flav	0.358	M.fort	0.364
12	M.intra	0.306	M.intra	0.301	H37Rv	0.334	H37Rv	0.343
13	M.flav	0.273	PPD	0.265	M.scrof	0.296	M.scrof	0.315
14	PPD	0.260	M.flav	0.262	M.kans	0.283	M.kans	0.290
15	M.terr	0.245	M.terr	0.236	M.tbS1	0.262	M.tbS1	0.267

Young British subjects: The highest level of antibodies in the pre- and post-BCG serum samples were found against **M.tuberculosis** 7219. The lowest levels were seen against **M.kansasii** and **M.tuberculosis** S1.

The post-BCG serum samples had significantly higher levels of antibodies to **M.tuberculosis** 7219 ($p<0.05$), **M.flavescens** ($p<0.01$) and **M.gordonae** ($p<0.01$). The antibody levels to all the other antigens were similar in the pre- and post-BCG serum samples.

While BCG vaccination has not enhanced the mean antibody levels to any of the 15 antigens tested in young Indians, it has produced significant increases in the mean antibody levels to three of the antigens in young British subjects. This difference could be due to the differences in the degree of exposure to cross reactive antigens and the type of cross reactive antigens encountered by the two groups. They could also be due to the differences in the pre-existing sensitisation patterns between these two groups. Genetic differences between the two groups studied could also be involved, acting through the HLA class 2 immune response genes.

(started: 1988; completed: 1990)

IgG antibody levels to various mycobacterial antigens in tuberculin negative and positive children and in young individuals from the South Indian BCG Trial area

A study of antibody levels to various mycobacterial antigens, in tuberculin negative children and unvaccinated tuberculin negative older individuals in the BCG trial area has been conducted.

Serum samples were collected from 36 children from Koppur village. The ages of the children ranged from 2 to 6 years and the mean was 4.4 years. The tuberculin reactivity ranged from 0 to 30 mm and the mean was 7.5 mm. Serum samples collected, before BCG vaccination and tuberculin conversion, from 13 young individuals (mean age 16.5 years) from the same area were also included.

The IgG antibody levels in the serum samples were estimated by ELISA against PPD(RT22) and sonicate supernatant antigens (at a concentration of 5 µg/ml) which were the same as the ones used in the study of pre- and post-BCG serum samples (see page 66). All the serum samples (using 1/40 dilution) were tested in duplicate in one batch after they had been coded and randomised. The mean O.D. value was calculated for each serum. The means for the different groups were compared by the t-test.

The children belonging to the different tuberculin reactivity groups had the same level of IgG antibodies to the different mycobacterial antigens (Table 1). There were no significant differences between the tuberculin reactivity groups in the antibody levels to any of the mycobacterial antigens tested.

Table 1

Order	Koppur children						Young individuals	
	Tuberculin reaction							
	0-3mm (n=18)		4-10mm (n=8)		>10mm (n=10)		(n=13)	
1	M.scrof	0.488	M.scrof	0.425	M.scrof	0.478	BCG	0.468
2	M.avium	0.386	M.avium	0.370	M.avium	0.358	M.gord	0.456
3	M.tbS1	0.284	M.tbS1	0.265	M.tbS1	0.300	M.fort	0.408
4	M.terr	0.234	M.terr	0.225	M.terr	0.259	M.chel	0.396
5	PPD	0.234	PPD	0.222	M.tb7219	0.251	M.tbS1	0.378
6	M.chel	0.232	M.chel	0.222	BCG	0.227	M.avium	0.368
7	M.fort	0.230	M.tb7219	0.220	M.chel	0.223	M.bovis	0.364
8	M.tb7219	0.222	M.fort	0.218	PPD	0.218	M.scrof	0.348
9	M.intra	0.218	M.intra	0.217	M.intra	0.215	M.tb7219	0.346
10	M.gord	0.218	M.gord	0.201	M.fort	0.204	M.kans	0.342
11	BCG	0.216	BCG	0.194	M.gord	0.202	H37Rv	0.320
12	M.flav	0.200	M.kans	0.194	M.kans	0.201	M.intra	0.306
13	M.kans	0.199	M.flav	0.184	M.flav	0.189	M.flav	0.273
14	M.bovis	0.175	M.bovis	0.172	M.bovis	0.186	PPD	0.260
15	H37Rv	0.128	H37Rv	0.138	H37Rv	0.150	M.terr	0.245

In these children, the mean antibody levels were highest against **M.scrotulaceum** and **M.avium** while the lowest levels were against **M.bovis** and **M.tuberculosis** H37Rv. The mean antibody levels in the non-BCG vaccinated young individuals were highest against BCG and **M.gordonae**. The lowest levels were seen against PPD-RT22 and **M.terrae**. Many of the differences in antibody levels between the Koppur children and the non-vaccinated young individuals were statistically significant ($p<0.05$). Thus the young individuals had significantly higher levels of antibodies to **M.tuberculosis** H37Rv, **M.tuberculosis** S1, **M.tuberculosis** 7219, **M.bovis**, BCG, **M.kansasii**, **M.intracellulare**, **M.flavescens**, **M.gordonae**, **M.fortuitum** and **M.chelonei**. On the other hand, the Koppur children had significantly higher level of antibodies than the young individuals to **M.scrotulaceum**. The Koppur children and the young individuals had similar levels of antibodies to **M.avium**, **M.terrae** and PPD RT22.

In the Koppur children, there were no significant differences between the age groups in the level of IgG antibodies to any of the mycobacterial antigens tested. In these children, the differences between the males and females in the

antibody levels to the mycobacterial antigens tested were also not statistically significant.

The pattern of antibody levels, that is, the order of antibody levels to the 15 mycobacterial antigens tested were different between the Koppur children and the unvaccinated young individuals (mean age 16.5 years).

(started: 1988; completed: 1990)

In vitro experiments with compound *Centella asiatica* to elucidate its effect on the viability of *M.tuberculosis*

Centella asiatica is a herb found throughout India. In the indigenous system of medicine, this herb has been used for leprosy patients from very early times and is widely acclaimed to have medicinal properties. In 1938, a new glycoside called asiaticoside which was active against leprosy was isolated from this plant. Boiteau and Grimes considered that it acted by dissolving the waxy covering of **M.leprae** so that the bacillus became very fragile and was easily destroyed by the tissues or by some other drugs (Nature, 1945,19:601). It was also thought that the compound may have similar effect on other organisms such as **M.tuberculosis** which also have the waxy covering. The present study has been undertaken to investigate and elucidate the effect of the indigenously produced compound of **Centella asiatica** (CA) on the viability of **M.tuberculosis** H37Rv **in vitro**.

A compound (dry powder) of **Centella asiatica**, being made locally, was used for the study. Two sets of 10ml Sauton's medium with 0.05% Tween 80 and containing 1000, 500, 200, 100, 50, 25 and 0 µg/ml of compound CA were freshly prepared. Live **M.tuberculosis** H37Rv was inoculated into one set of media, and heat killed **M.tuberculosis** H37Rv into the other set of media to give 10^5 bacilli/ml. Immediately after inoculation, from each of the 14 bottles, smears were made using 10 µl of culture with 10 µl of formal milk serum, air dried, fixed with methanol and stained by ZN. From the 7 bottles inoculated with live bacilli, viable count (VC) was also set up. This procedure was repeated at 1h, 6h, 24h and at 1 week.

Viable count: The number of viable bacilli per ml at different time points with different concentrations of CA, was estimated and its log value is presented in table 1. In all the cultures, there was a slight increase in the number of viable organisms at 1 week or at different time points on the first day, indicating that the compound CA may not have any direct action on the viability of **M.tuberculosis** in **vitro**.

Table 1

Conc. of CA (μg/ml)	0	1wk
0	4.54	5.11
25	4.65	5.26
50	4.49	5.00
100	4.77	5.23
200	4.60	5.00
500	4.69	4.95
1000	4.60	4.90

(started: 1991; completed: 1991)

Functional alteration of mouse peritoneal and tissue macrophages during primary mycobacterial infections

The macrophages play an important role as antigen presenting cells. This action leads to both humoral and cellular response in the host. At the cellular level, the intracellular killing by macrophages is effected either by oxygen-dependent mechanisms mediated through hydrogen peroxide and the associated reactive forms of reduced oxygen, or by oxygen-independent mechanisms such as the production and release of lysozyme, lipase, cathepsins, glycosidases and phosphatases.

Hydrogen peroxide-dependent and lysosomal mechanisms can operate against mycobacteria in macrophages. The relative importance of these mechanisms against the different mycobacteria is still unclear, with recent studies lending little support to earlier evidence for peroxide-mediated killing. Impairment of lysosomal function, through inhibition of fusion or escape into cytoplasm, is an established phenomenon but its significance is still unclear.

An investigation was therefore undertaken to correlate the viable count of the infecting organism with the level of hydrogen peroxide (H_2O_2) and some of the hydrolytic enzymes in the macrophages of normal mice and those infected with **M.tuberculosis** H37Rv, **M.avium intracellulare** (MAI), **M.bovis** BCG and a South Indian Low Virulent strain of **M.tuberculosis** (SILV) over a period of 29 days. The hydrolytic enzymes of mononuclear phagocytes examined were acid phosphatase, β-glucuronidase (intracellular acid hydrolases), cathepsin-D

(inducible extracellular neutral protease) and lysozyme (glycosidase).

Swiss albino mice were infected intraperitoneally with 0.1 ml containing 10^6 organisms of H37Rv, SILV, MAI and BCG in 7H9 liquid medium. Six control mice (uninfected) and six mice infected with each of the different strains of mycobacteria were sacrificed on days 1, 8 and 29 after infection.

The peritoneal exudate cells (PEC) were collected after sacrifice and a cell count made after pooling the cells of the six animals in each group. A cell smear was made on a slide for non-specific esterase staining for the determination of the macrophage population. After taking the required number of cells for the assay of hydrogen peroxide (undertaken immediately after collection of cells), the rest were kept frozen at -20° C till the assay of the 4 hydrolytic enzymes. In addition, the spleen was excised, a 10% homogenate in sucrose - EDTA medium prepared, and stored at -20° C. Viable counts of the different organisms were set up with a portion of the spleen of each mouse.

Non-specific esterase staining of PEC revealed that about 50% of the cells (range: 45-55%) were macrophages and the proportions in the different species were similar. There was no appreciable difference in the proportion of macrophages between normal and infected mice, or on the different days after infection.

The mean spleen viable counts (VC) and H_2O_2 released by PEC on the different days following infection with different species of mycobacteria are presented in table 1. With all the species except SILV, infection was established on the 1st day; H37Rv and MAI multiplied in the tissue during the first week,

Table 1

Organism	Mean VC (log units/ 100 mg spleen tissue)			H_2O_2 released (n moles/10^6 PEC)		
	Days after infection			Days after infection		
	1	8	29	1	8	29
None (control)	0	0	0	0.21	0.25	0.10
M.tuberculosis H37Rv	4.07	4.53	2.86	0.75	3.16	1.11
M.tuberculosis SILV	0	0.86	2.45	1.33	2.64	4.88
M.bovis BCG	0.69	0.97	0.63	1.09	3.00	1.20
M.avium intracellulare	2.02	5.51	4.09	1.45	2.28	1.30

followed by a variable decrease over the next three weeks. BCG had a slow growth rate (from 0.69 to 0.97 log units) in the first week; there was a marginal decline in the growth thereafter. The spleen viable counts of SILV were, however, zero on day 1; there was growth thereafter, and the counts at 29 days were substantially higher than those on day 8.

The release of H_2O_2 was substantially higher in the PEC of infected mice (with all four strains of mycobacteria) on all three occasions than from normal (resident) macrophages obtained from uninfected mice. There was an increase in the release between days 1 and 8; the mean values on day 29 were, however, lower than those on day 8 in the PEC of animals infected with H37Rv, BCG and MAI. With SILV, the mean value on day 29 was substantially higher than that on day 8.

The activities of the lysosomal hydrolases, namely acid phosphatase (ACP), B-glucuronidase (BGL), cathepsin-D (CAT-D) and lysozyme (LYS), in PEC and spleen tissue are presented in the table 2.

Table 2

Organism	Days	Specific activity (units/mg protein)							
		PEC				Spleen			
		ACP	BGL	CAT-D	LYS	ACP	BGL	CAT-D	LYS
None (Control)	1	0.17	0.59	0.12	68.80	1.95	1.10	0.12	22.60
	8	0.10	0.90	0.09	37.57	1.99	1.00	0.18	21.95
	29	0.08	0.76	0.10	56.21	1.95	0.80	0.14	17.41
M.tuberculosis H37Rv	1	0.39	1.40	0.16	428.6	7.71	10.40	1.04	42.31
	8	0.27	4.67	0.22	218.6	4.24	2.40	0.34	16.77
	29	0.30	11.25	0.30	188.7	1.73	7.38	0.24	31.48
M.tuberculosis SILV	1	0.47	7.67	0.11	674.7	2.74	4.80	0.44	34.66
	8	0.21	8.31	0.13	252.3	3.39	2.00	0.30	32.08
	29	0.28	11.92	0.25	286.1	1.46	6.09	0.22	90.14
M.bovis BCG	1	0.24	8.80	0.12	484.2	7.04	12.42	1.38	26.38
	8	0.16	8.92	0.08	254.9	2.40	2.19	0.29	37.05
	29	0.16	9.59	0.39	239.6	1.26	5.78	0.26	54.52
M.avium intracellulare	1	0.40	2.22	0.07	947.2	4.28	12.38	1.25	36.30
	8	0.40	3.33	0.27	433.3	1.68	1.89	0.23	69.28
	29	0.32	12.00	0.92	243.6	2.02	4.41	0.24	37.78

The activity of acid phosphatase in the PEC was substantially lower than that in the spleen in both the infected and the uninfected groups. The values for β-glucuronidase were fairly similar in the PEC and the spleen except with H37Rv on MAI infection on day 1. With cathepsin-D, the activity was similar in the PEC and the spleen in the uninfected group. With H37Rv, SILV and BCG, the values on days 1 and 8 were higher in the spleen than in the PEC, while the values on day 29 were similar. With MAI, the value on day 1 was higher in the spleen than in the PEC, the values on day 8 were similar and the value on day 29 was higher in the PEC. The activity of lysozyme was considerably greater in the PEC than in the spleen with all four strains and on all 3 occasions.

The activities of acid phosphatase in PEC in the infected groups were higher than the respective control values on all 3 occasions. The values on days 8 and 29 were slightly lower than those on day 1 with all 4 strains. In spleen, the activities of this enzyme were higher with H37Rv, SILV and BCG than in the uninfected controls on days 1 and 8, and the values on day 29 were lower than those on day 8. With MAI, the values were higher than in the controls on day 1, and similar on days 8 and 29.

The activities of β-glucuronidase in the PEC increased between days 1 and 29 in all the infected groups and all the values were substantially higher than those in the uninfected controls. In the spleen, however, the values on day 8 were lower than on day 1 with all four strains of mycobacteria. The values on day 29 were higher than on day 8 in all the four groups; however, they were lower than the values on day 1 with H37Rv, BCG and MAI.

The activities of cathepsin-D in the infected groups were similar to the uninfected on day 1 in the PEC. A variable rise was then observed and the activity was highest on day 29 with all 4 strains. In the spleen, the activity of this enzyme was appreciably higher in all the 4 infected groups than the respective controls on day 1. A decrease in the activity, marked with H37Rv, BCG and MAI, was observed on days 8 and 29; the activities on day 29 were, however, higher than in the control.

Lysozyme activity in the PEC on day 1 was considerably higher in the infected groups than in the control. The values were lower on day 8; on day 29, the values were still less, except for SILV where a slight increase was observed. The activities on day 29 were, however, still appreciably higher than the control value. The activity of lysozyme in the spleen was higher than the control in all the 4 infected groups on day 1. On day 8, the activity of the enzyme in the group infected with H37Rv was slightly lower than the control; the activities with SILV, BCG and MAI were, however, higher. The activities on day 29 were higher than those on day 8 with respect to H37Rv, SILV and BCG; with MAI, however, the activity on day 29 was considerably lower than that on day 8.

In primary infection with different mycobacteria, there is an initial multiplication of the infecting organism. The multiplication then stops and a decline is observed in the viable counts due to the development of host resistance. Ingestion of mycobacteria by the macrophages appears to activate both the oxygen-dependent and the oxygen-independent mechanisms of the bactericidal actions of these cells. There was a suggestion that the release of hydrogen peroxide by the PEC following infection with SILV was greater than that with H37Rv. Further, the release of hydrogen peroxide appeared to be highest when the spleen viable count was also high with all four mycobacterial infections; however, the release was not low when the viable count was low. Wide variation was observed in the activities of the four lysosomal hydrolases, both between PEC and spleen cells and also between the four infecting strains of mycobacteria. It is not clear whether there is selective release of any hydrolase by the macrophage (PEC and spleen) depending upon the nature of the activating mechanism.

(started: 1989; completed: 1991)

Macrophage function in mice vaccinated against *M.tuberculosis* infection with *M.bovis* BCG and *M.avium intracellulare*

Results of trials to assess the protective effect of BCG vaccination against tuberculosis have shown wide variation, the protection ranging from 0-80 %. The main hypotheses postulated to explain this wide variation are (a) variability in the potency of various daughter strains of BCG, (b) unusual host immune response, (c) the nature of the infecting strain of **M.tuberculosis**, and (d) influence of previous sensitization with environmental non-tuberculous mycobacteria such as **M.avium intracellulare**. BCG presumably acts by enhancing cell-mediated immunity against **M.tuberculosis** with which it shares antigenic cross-reactivity. Prior sensitization with non-tuberculous mycobacteria could impart some protection against tuberculosis; the infecting strain of **M.tuberculosis** could be of low virulence and function as an immunizing agent, competing with BCG, and both these effects could mask protection due to the latter, partially or totally.

The macrophage plays a pivotal role in cell-mediated immunity against tuberculosis, its prime functions being to ingest and possibly kill the infecting bacilli, and also to process and present the antigens to T cells. The bactericidal effect of the macrophage resides in its state of activation by its capacity to release various toxic products such as hydrogen peroxide, superoxide anion and singlet oxygen, and various lysosomal hydrolases. Sensitization with non-tuberculous mycobacteria, vaccination with BCG or infection with a low virulent strain of **M.tuberculosis** could alter the bactericidal effect of the macrophage. An investigation was therefore undertaken to correlate protective efficacy of BCG

vaccination, as assessed by the colony forming units of tubercle bacilli in the spleen, with the state ot activation of macrophage as assessed by the release of hydrogen peroxide and four lysosomal hydrolases, in mice sensitized with **M.avium intracellulare** (MAI), vaccinated with BCG or both, prior to challenge with a virulent (H37Rv) or a low-virulent (SILV) strain ot **M.tuberculosis**.

Swiss albino mice were sensilized with MAI, vaccinated with BCG or challenged with H37Rv or SILV wilh 0.2 ml of 7H9 liquid medium containing 10^6 viable organisms inoculated intraperitoneally. The schedule for sensitization, vaccination and challenge is given in table 1; 6 mice each were sacrificed on days 1, 8 and 29 atter challenge with H37Rv or SILV. Spleen viable count was set up and generation of hydrogen peroxide by the peritoneal exudate cells (PEC) and the specific activities of the lysosomal hydrolases, acid phosphatase, β-glucuronidase, cathepsin-D and lysozyme in both the PEC and the spleen cells

Table 1

Days	Group							
	I	II	III	IV	V	VI	VII	VIII
0	H37Rv	SILV	BCG	BCG	MAI	MAI	MAI	MAI
1	S'fice*	S'fice	|	|	|	|	|	|
8	S'fice	S'fice	|	|	|	|	|	|
14	|	|	H37Rv	SILV	H37Rv	SILV	BCG	BCG
15	|	|	S'fice	S'fice	S'fice	S'fice	|	|
22	|	|	S'fice	S'fice	S'fice	S'fice	|	|
28	|	|	|	|	|	|	H37Rv	SILV
29	S'fice	S'fice	|	|	|	|	S'fice	S'fice
36			|	|	|	|	S'fice	S'fice
43			S'fice	S'fice	S'fice	S'fice	|	|
57							S'fice	S'fice

* S'fice = Sacrifice

were determined as described earlier (see page 71). The mean spleen viable counts following challenge with H37Rv or SILV are presented in table 2. Following infection with H37Rv, the highest viable count was observed on day 8

Table 2

Sensitizing/ immunizing organism	Mean VC (log units/100 mg spleen tissue) after challenge with:					
	H37Rv			SILV		
	Day 1	Day 8	Day 29	Day 1	Day 8	Day 29
None (control)	4.07	4.53	2.86	0	0.86	2.45
BCG	3.64	2.90	0	5.34	4.14	0
MAI	5.15	4.85	4.64	4.78	3.18	3.99
MAI --- BCG	4.92	4.92	4.01	4.14	5.02	4.87

followed by a decrease; with SILV, however, no growth was detected on day 1 and the value on day 29 was higher than that on day 8. Vaccination with BCG 14 days prior to challenge with H37Rv or SILV resulted in a pronounced drop in the spleen viable count, none being recorded in either group on day 29. In mice sensitized with MAI, the viable count was high after challenge with H37Rv as also with SILV, suggesting that MAI had little effect in promoting immune response against tubercle bacilli. In mice sensitized with MAI and then vaccinated with BCG, the spleen count was high and there was a difference of about 4 log units at 29 days between this group and those vaccinated with BCG. This suggests that MAI interfered with the BCG-induced immune response and protective effect.

The mean hydrogen peroxide (H_2O_2) generation by the peritoneal exudate cells (PEC) on different days following challenge with H37Rv or SILV is presented in table 3.

Following infection with H37Rv, the generation of hydrogen peroxide was higher on day 8 than on days 1 and 29. In contrast, hydrogen peroxide following SILV infection was highest on day 29. In mice vaccinated with BCG, the mean generation of hydrogen peroxide was higher on days 1 and 8 than the corresponding values following primary infection with the two strains of tubercle

Table 3

Sensitizing/ immunising organism	Mean H_2O_2 generation (n moles/10^6 PEC) after challenge with					
	H37Rv			SILV		
	Day 1	Day 8	Day 29	Day 1	Day 8	Day 29
None (control)	0.75	3.16	1.11	1.33	2.64	4.88
BCG	2.54	4.68	0.65	2.46	3.73	1.02
MAI	4.47	5.28	1.39	2.19	5.25	1.04
MAI --- BCG	5.53	4.07	3.75	5.42	3.64	3.30

bacilli; however, on day 29, corresponding to the nil viable count, the generation of hydrogen peroxide had also decreased considerably. In mice sensitized with MAI, high values were obtained on days 1 and 8, with the value on day 8 being higher than those on day 1, followed by a decrease on day 29. In mice which received BCG vaccination following sensitization with MAI, the values on days 8 and 29 were lower than those on day 1. These findings demonstrate that there is an increase in the release of hydrogen peroxide following the ingestion of mycobacteria. However, hydrogen peroxide appears to have very little role in so far as protection against tuberculosis is concerned, protection being measured by the decrease in the viable count.

The findings with the four lysosomal hydrolases both in the PEC and in spleen cells (not tabulated) were similar to those obtained with hydrogen peroxide. No discernible patterns of alteration of the activities were observed, and as found in previous experiments, there was a wide variation in the activities between the PEC and spleen cells and between different infecting strains of mycobacteria (see page 71). It seems unlikely that enhanced activities of the lysosomal hydrolases alone would be responsible for the mycobactericidal effect of the macrophage.

(started : 1989; completed : 1991)

Effect of treatment with anti-tuberculosis drugs on macrophage function in mice infected with tubercle bacilli

The efficiency of an antibiotic in the treatment of bacterial infections depends upon the interactions of the drug, bacteria and the phagocytes. Some drugs enhance the killing capacity of macrophages by modulating the immune mechanisms by which macrophages kill the parasites.

Very little is known about the action of anti-tuberculosis drugs on macrophage function. It has been shown in **in vitro** experiments that rifampicin has immunosuppressive effects like depression of phagocytic activity of mouse peritoneal macrophages, impairment of antibody responses and skin test reactivity to several antigens. Isoniazid when administered orally to volunteers caused a significant reduction in circulating T-lymphocytes and a significant increase in B-lymphocytes. The interaction of pyrazinamide with the immune system is unique for its bactericidal action against **M. tuberculosis**. Pyrazinamide is believed to be effective in an acid environment such as prevalent inside the macrophage, an effect similar to that of clofazimine in the treatment of leprosy.

A study was initiated to investigate the effect of pyrazinamide and rifampicin on hydrogen peroxide generation and the activities of lysosomal hydrolases of peritoneal and spleen macrophages in **M. tuberculosis** H37Rv infection in mice. Further, since it has been suggested that the mycobactericidal effect of pyrazinamide is mediated through its primary metabolite, pyrazinoic acid, the hydrolysis being catalysed by pyrazinamide deamidase, the activity of pyrazinamide deamidase in tissue and peritoneal macrophages of normal and infected mice treated with pyrazinamide and rifampicin were also assessed.

One hundred and twelve Swiss albino mice were infected intravenously with 0.1 ml of 7H9 liquid medium containing 10^6 organisms of **M. tuberculosis**, while another batch of 112 uninfected control animals received only the i.v. dose of the medium. Eight mice from each of the two groups were sacrificed 3 hours after the infection and viable counts set up with a portion of the spleen to determine the establishment of infection in the infected group. The remaining animals in each of the groups were divided into 4 subgroups; one subgroup of 32 animals did not receive any drug, while the other 3 subgroups (of 24 animals each) received pyrazinamide 160 mg/kg or rifampicin 25 mg/kg or pyrazinamide 160 mg/kg plus rifampicin 25 mg/kg daily from the 15th day after infection. Eight animals from the untreated groups (both infected and uninfected) were sacrificed on days 15, 22, 29 and 36 after the start of the experiment, while in animals that received treatment, 8 animals from each subgroup were sacrificed on days 22, 29 and 36.

The peritoneal exudate cells were collected immediately after sacrifice and cell count made after pooling the cells of 2 animals in each group. A cell smear was made on a slide for non-specific esterase staining to determine the macrophage population. After taking the required number of cells for the assay of hydrogen peroxide (undertaken immediately after collection of cells), the rest were kept frozen at -20°C till the assay of the hydrolytic enzymes and pyrazinamide deamidase. Further, the spleen was excised, a 10% homogenate in sucrose - EDTA medium prepared, and stored at -20°C till assay of the lysosomal hydrolases and pyrazinamide deamidase. Viable counts of **M. tuberculosis** were set up for each mouse with a portion of the spleen.

The mean spleen viable counts (VC) on treatment with the two drugs, either alone or in combination following infection with **M. tuberculosis** are presented in table 1.

Table 1

Days after infection	VC log units/100 mg spleen tissue (mean ± SEM)			
	Control	PZA	RMP	PZA+RMP
14	5.98 ± 0.18	-	-	-
21	5.74 ± 0.09	4.96 ± 0.21	4.53 ± 0.11	4.61 ± 0.13
28	5.18 ± 0.07	4.61 ± 0.20	3.92 ± 0.15	0.40 ± 0.24
35	4.93 ± 0.15	4.79 ± 0.09	3.92 ± 0.16	0

The infection was well established in spleen tissues at 14 days. The combined treatment of pyrazinamide and rifampicin for 14 days reduced the viable count considerably, and treatment for 21 days completely sterilized the spleen of **M. tuberculosis** H37Rv. Treatment with pyrazinamide alone had little effect on the spleen viable count whereas rifampicin alone caused a statistically significant reduction in the spleen viable counts ($P < 0.001$).

The mean values of hydrogen peroxide released by the PEC in the uninfected animals ranged from 0.05 to 0.49 n mol/10^6 cells (data not tabulated). The mean values for hydrogen peroxide, ranging from 1.66 to 2.73 n mol/10^6 cells in animals infected with **M. tuberculosis** H37Rv were significantly higher than those in uninfected animals ($P < 0.01$). Treatment with pyrazinamide, rifampicin or the combination of the 2 drugs did not appear to have any effect on

the release of hydrogen peroxide by the PEC in the uninfected or the infected groups of animals. Further, no correlation was observed between hydrogen peroxide generation and the spleen viable counts of **M. tuberculosis** H37Rv, the correlation co-efficient being 0.17.

Analysis of data (not presented) of the activities of acid phosphatase, β-glucuronidase, cathepsin-D and lysozyme in spleen tissues and PEC indicated no differences in the activities on days 7, 14 and 21 after the start of treatment with pyrazinamide and rifampicin in normal mice or those infected with **M. tuberculosis** H37Rv.

The mean specific activities of pyrazinamide deamidase released by PEC on treatment with different regimens in uninfected mice and those infected with **M. tuberculosis** H37Rv are presented in table 2.

Table 2

Days after treatment	Specific activity (mean±SEM) of PZA deamidase (unit/mg protein)			
	Control	PZA	RMP	PZA + RMP
Uninfected				
7	10.69 ± 1.98	16.60 ± 1.64	18.50 ± 7.05	11.15 ± 1.09
14	9.93 ± 1.56	9.73 ± 0.70	14.00 ± 4.64	12.74 ± 3.56
21	18.65 ± 1.42	8.90 ± 0.75	9.52 ± 1.32	13.10 ± 4.25
Infected				
7	14.66 ± 2.64	30.40 ±10.53	22.22 ± 5.77	16.20 ± 1.82
14	10.92 ± 2.01	42.54 ±15.97	15.06 ± 1.95	11.22 ± 1.19
21	16.16 ± 1.79	23.36 ±14.46	19.70 ± 1.55	13.69 ± 0.66

The mean activity of pyrazinamide deamidase in PEC of the infected group was slightly higher than that in the uninfected group. Treatment with pyrazinamide in the infected group of mice increased pyrazinamide deamidase activity at 7, 14 and 21 days ($P < 0.01$). However, treatment with rifampicin alone or in combination with pyrazinamide in the infected group did not appear to have any effect on the activity of pyrazinamide deamidase at 7, 14 or 21 days.

In spleen tissues (data not presented) no difference was observed in the mean activities of pyrazinamide deamidase on the different days (7, 14 and 21) and the two drugs (pyrazinamide and rifampicin), administered either alone or in combination, did not appear to have any effect in the infected or the uninfected groups.

The host-mycobacteria-drug (pyrazinamide or rifampicin) relationship is influenced by the changes following infection. During chemotherapy, started two weeks after infection, only the combined treatment is able to kill all the multiplying tubercle bacilli and the organisms get cleared from the spleen tissue within 3 weeks. Treatment with pyrazinamide or rifampicin alone is not able to clear the multiplying bacilli. It has been shown in this study that both pyrazinamide and rifampicin are not acting through the intracellular killing mechanisms of hydrogen peroxide generation or enhanced release of the four lysosomal hydrolases investigated.

Pyrazinamide deamidase is increased in the peritoneal exudate cells of infected mice treated with pyrazinamide monotherapy. So far no report is available on the presence of deamidase in macrophages. The increase observed in pyrazinamide deamidase activity within the macrophage may be from **M. tuberculosis** H37Rv.

(started : 1990; completed : 1991)

Analysis of immune complexes from tuberculous sera

Experiments were undertaken to characterise the mycobacterial antigens and specific antibodies in the circulating immune complexes (CIC) by ELISA and Western blotting.

Sera were obtained from the following three groups of subjects.

1. Clinically, radiologically and bacteriologically confirmed cases of pulmonary tuberculosis (smear positive, culture positive, S+ C+).

2. Clinically and radiologically confirmed, but bacteriologically negative cases of tuberculosis. (smear negative, culture negative, S- C-).

3. Normal healthy blood bank volunteers as endemic controls (NHC).

(Subjects in groups 1 and 2 did not have a history of previous anti-tuberculous chemotherapy.)

CIC levels in the 3 study groups were measured by 2 assays: C1q binding assay (C1qBA) and 3.5% polyethylene glycol precipitation followed by measurement of optical density at 280 nm (PEG OD 280).

The mean value ± S.D. are presented in Table 1. C1qBA does not show any significant difference in CIC levels between the 3 groups. But, PEG OD 280 is significantly elevated in both S+ C+ and S- C- groups as compared to NHC ($P < 0.001$).

Table 1

Assay	S+ C+	S- C-	NHC
C1q BA(µg eq/ml)	68.8 ± 32.2 (51) +	67.3 ± 17.8 (17)	62.8 ± 20.3 (66)
PEG OD 280	0.53 ± 0.35* (49)	1.01 ± 0.65* (17)	0.20 ± 0.21 (62)

\+ Number of subjects is shown in parenthesis.

* mean level significantly higher than the mean among NHC group ($P < 0.001$)

For 16 of the S+ C+ patients who were treated with short course chemotherapy, sera were collected at 0, 2 and 6 months after the start of treatment. The results are presented in Figure 1. The mean ODs at 0, 2 and 6 months are 0.456, 0.832 and 0.332 respectively. The mean OD at 2 months is significantly higher than at 0 month and at 6 months ($P < 0.001$). The antigen

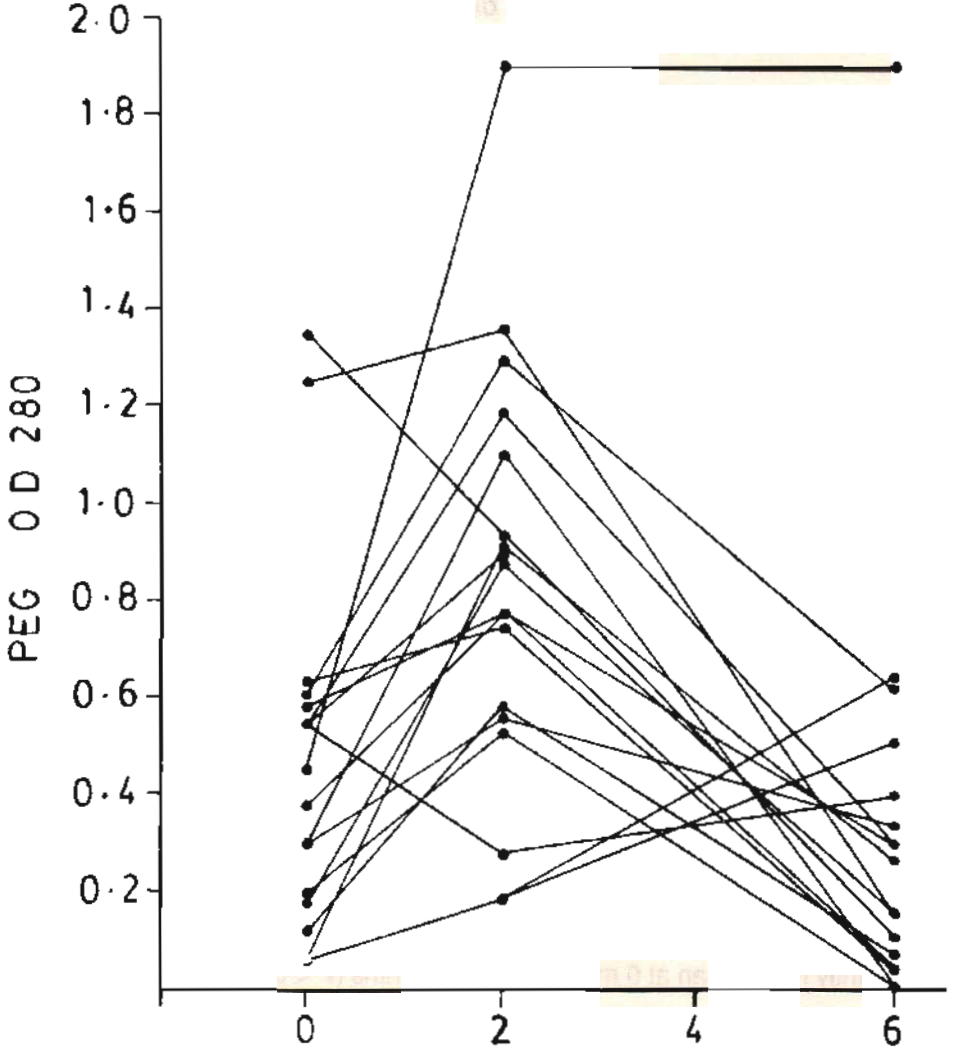

Fig. 1: Effect of anti-tuberculous treatment on circulating immune complex levels at 0, 2 and 6 months.

load in the initially sputum-positive patients increases during successful chemotherapy as the bacilli are killed and disintegrated. With short course chemotherapy, more than 90% of the patients become sputum negative by 2 months of treatment. Consequently, the antibodies and hence the CIC also would increase. When the antigens and the complexes are being cleared from circulation, the CIC levels start falling. Therefore, CIC could serve as an indicator of the effect of treatment.

Anti-PPD IgG and IgM antibodies were estimated from the three groups by ELISA. Figures 2a and 2b show the mean ODs. Both IgG and IgM mean levels are significantly higher in the S+ C+ than in NHC. But the values in S- C- were not significantly different from those in NHC. In order to test the specificity of the CIC, ELISA tests were carried out using the PEG precipitates. The results are shown in Figures 3a and 3b. In the S+ C+ as well as in S- C-, both IgG and IgM type of antibodies were significantly higher than in NHC. Even though both IgG and IgM type of antibodies were present in the complexes, IgG was more discriminatory than IgM between tuberculous cases and normal individuals, just as in the case of sera.

By employing a cut-off point of Mean + 2 S.D. of NHC, the number of positives in each of the groups has been counted and indicated in Figures 2 and 3. The proportions of positives for serum IgG are 14/51 in S+ C+, 1/17 in S- C- and 2/66 in NHC. But higher proportions, i.e., 20/49 in S+ C+, 9/16 in S- C- and 3/61 in NHC are positive for CIC IgG. A similar trend is seen in IgM also, although the increase in positivity is less pronounced.

The antigenic components in the CIC were analysed by resolving the PEG precipitates in SDS-PAGE and Western blotting with anti-BCG sera. Three batches of pooled sera from S+ C+ and NHC groups were used for precipitation. The pattern obtained with one batch of pooled serum is shown in Fig. 4. When the results obtained with the three batches were analysed, it was observed that 56, 35, 26, 23 and 18 KDa bands were specifically seen in the TB CIC alone. However, when the analysis was extended to individual TB sera, no antigenic band could be detected in a majority of them. One of the reasons for failure to demonstrate antigen could be its presence in very low concentrations. In order to characterise the complexed antibody components, the precipitates were reconstituted with PBS and used in Western blotting.

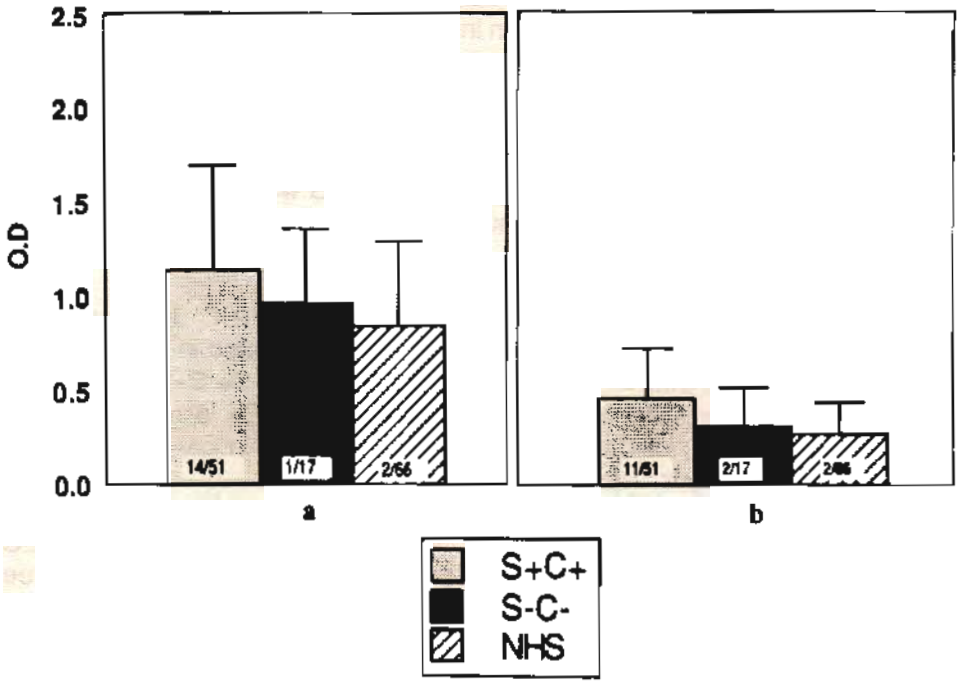

Fig. 2: Serum antibody levels in tuberculosis patients and normal subjects. a) Anti-PPD IgG b) Anti-PPD IgM

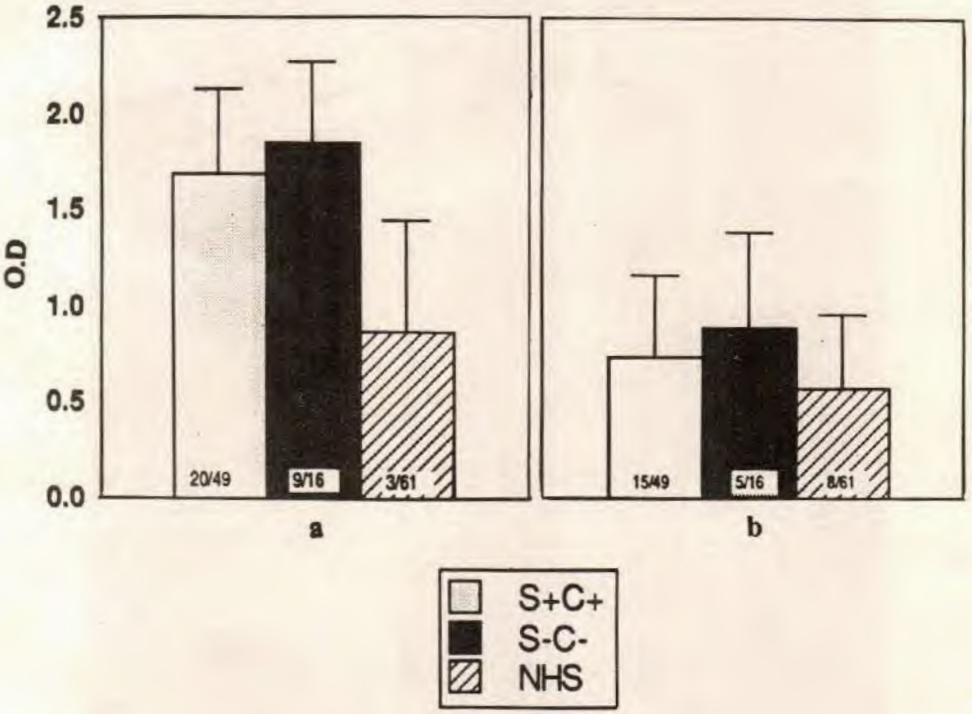

Fig. 3: CIC antibody levels in tuberculosis patients and normal subjects. a) Anti-PPD IgG b) Anti-PPD IgM

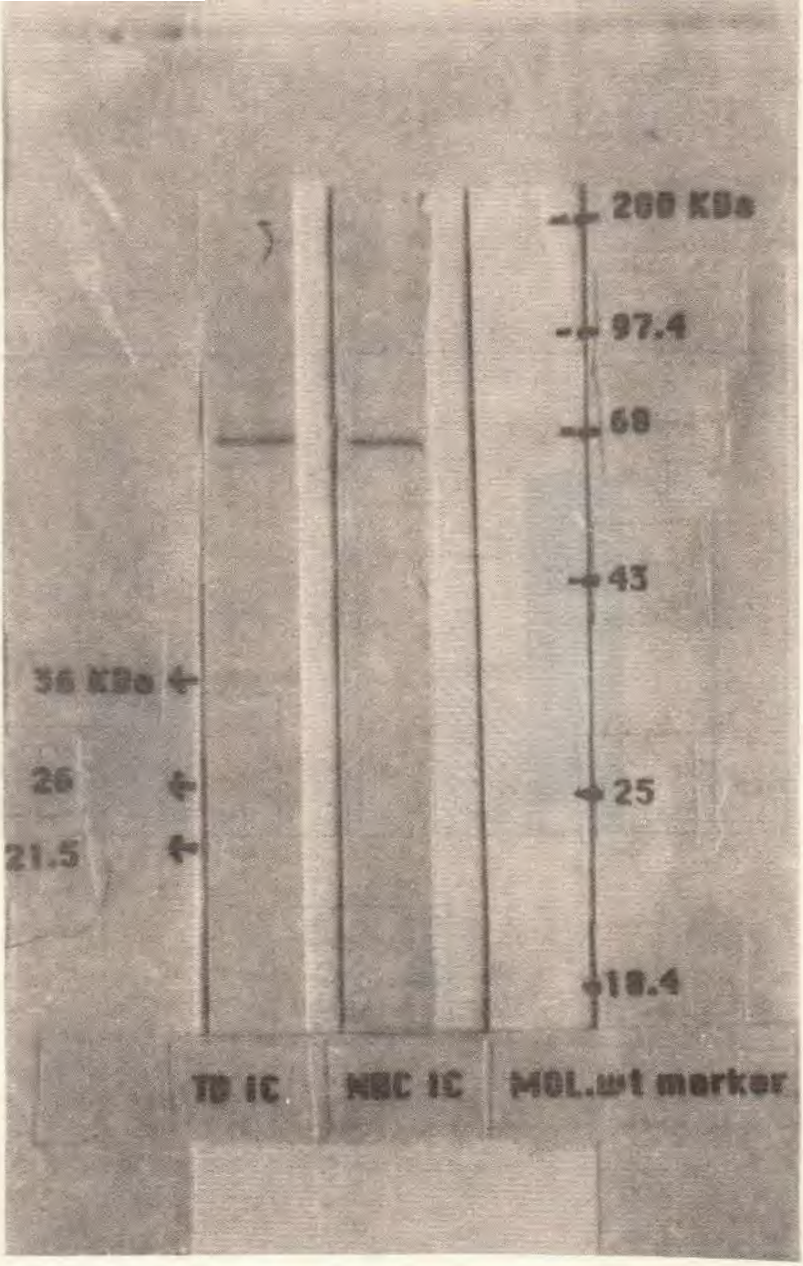

Fig. 4: Mycobacterial antigenic components in pooled tuberculous CIC and pooled normal CIC. The bands which are restricted to TB CIC alone have been indicated by arrows.

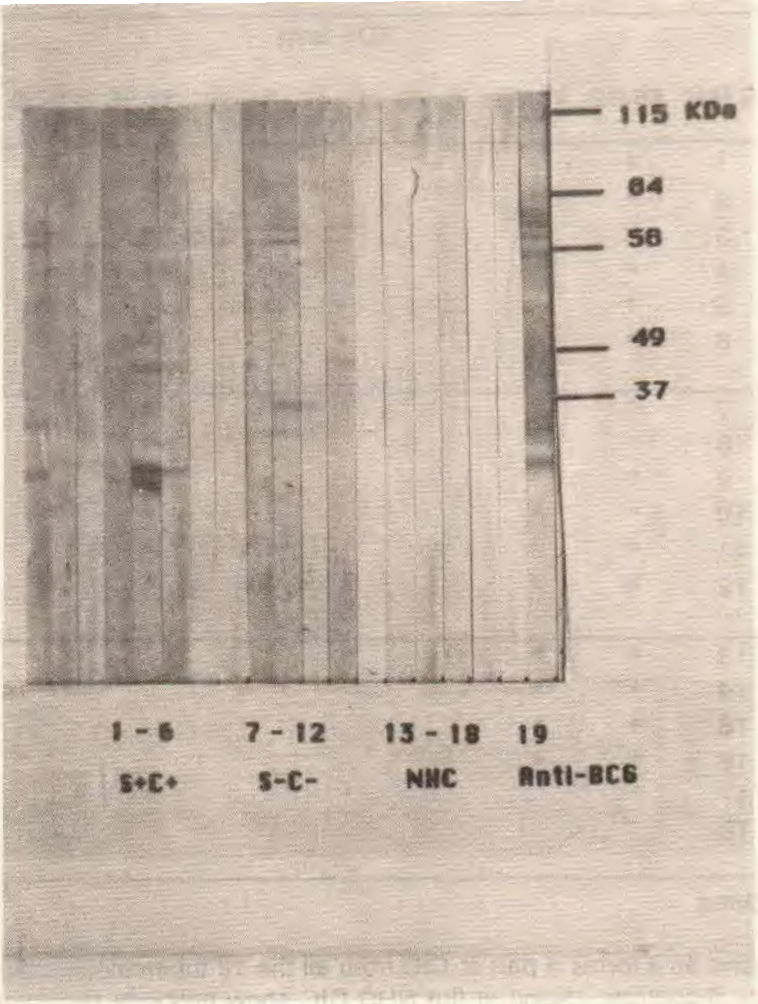

Fig. 5: Antibody profile of TB CIC and normal CIC.
Lanes 1 - 6 : S+C+ ; Lanes 7 - 12 : S-C- ; Lanes 13 - 18 : NHC ;
Lane 19 : Anti-BCG control

Fig. 5 shows the antibody pattern in CIC of 6 S+ C+, 6 S- C- and 6 NHC individuals. The antibody pattern is heterogeneous, varying from patient to patient within the same group. The antibody response to the individual components is shown in Table 2. Antigen of molecular weight 32-35 KDa is an

Table 2

		KDa level							
Group	S.No.	85-92	78-84	72-76	43-47	36-39	32-35	29-30	26.5-28
S+ C+	1	*	*	*			*		*
	2	*	*				*	*	
	3	*	*				*	*	
	4	*	*				*	*	
	5	*	*		*	*	*	*	*
	6	*	*		*		*	*	
S- C-	7	*	*				*		
	8	*	*				*		
	9	*	*				*		
	10	*	*			*	*		
	11	*	*	*		*	*		
	12	*	*		*	*	*		
NHC	13	*	*						
	14	*	*						
	15	*	*						
	16	*	*						
	17								
	18								

* Response

important one as it forms a part of CIC from all the 12 tuberculous patients and none of the 6 controls. None of the NHC CIC show antibody response below molecular weight 70 KDa. When the number of subjects in each group was increased, response to 47 KDa or less was observed in all 24 S+ C+, 11 of 16 S- C- and none of the 26 NHC CIC. The results are promising in that the measurement of immune complex IgG for mycobacterial antigens of molecular weight 47 KDa or less in ELISA and/or Dot-blot might prove to be useful in discriminating between the tuberculosis patients and endemic normals. It may be worthwhile confirming the above finding on larger numbers, with special efforts to

ensure accuracy of diagnosis in the S- C- group. Further attempts are being made to fractionate the mycobacterial antigens by molecular sieving chromatography.

(started: 1987; completed: 1991)

Development of an assay for studying the anti-mycobacterial activity of human monocyte derived macrophages

In continuation of the work carried out earlier, a few modifications were made in the assay system. Consistent results have been arrived at, and the assay is ready for application.

Phagocytosis was carried out on the third day of monocyte culture, the monocytes to bacteria ratio being 5:1. Monocytes were stimulated separately with crude lymphokine (20% v/v), autologous lymphocytes, addition of 100 ng/ml Phorbol myristate acetate and 100 units of recombinant interferon gamma. The growth of intracellular **M. tuberculosis** was compared with a cell-free control which consisted of RPMI 1640 with 10% autologous serum. The macrophages with intracellular **M.tuberculosis** were terminated at 0, 48, 96, 168 and 240 hours after phagocytosis and viable counts were estimated.

The assay was done in triplicate on the monocytes of each of 6 individuals. The data are being analysed and the results will be reported next year.

(started: 1991; completed: 1991)

Early bactericidal effect of pulsed exposure to RE and HZ in tuberculosis patients

In the ongoing study of short course chemotherapy regimens of 6 months' duration (page 43), rifampicin (R) and ethambutol (E) are given on one day and isoniazid (H) and pyrazinamide (Z) on the next day, each combination given thrice a week for the first 2 or 3 months, followed by R and H twice weekly in the remaining months. This is being compared with R, E, H and Z given together in a single dose thrice weekly in the initial 2 months, followed by R and H twice weekly in the next 4 months, in order to find out whether by giving RE and HZ on alternate days, adverse reactions could be minimised without compromising on efficacy.

The early bactericidal effect of pulsed exposure to RE and HZ in the tuberculosis patients included in the study is being examined by conducting serial sputum viable counts (as reported in the 1990 annual report). Estimation of the viable units of tubercle bacilli is being made from single overnight collection specimens of sputum. The sputum specimens are collected on days 0, 1, 2, 3, 4, 9 and 16 from patients receiving RE and HZ on alternate days (RE_3/HZ_3 and HZ_3/RE_3), and on days 0, 2, 4, 9 and 16 from patients receiving REHZ thrice a week ($REHZ_3$).

The results obtained from the 27 patients studied so far are presented in table 1. From these results, both when R, E, H and Z are given together and

Table 1

Group	Regimen	Mean log VC/ml on day						
		0	1	2	3	4	9	16
1	RE_3/HZ_3 n=8	5.67	4.81	4.75	4.97	4.55	3.34	3.15
2	HZ_3/RE_3 n=8	6.43	5.44	4.49	4.07	4.25	3.19	2.76
3	$REHZ_3$ n=11	6.14		5.24		4.50	3.28	3.01

Note: n represents the number of patients in the regimen.

when RE and HZ are given on alternate days, considerable early bactericidal effect is observed. The reduction in the mean log viable count/ml after 16 days of chemotherapy in the RE_3/HZ_3, HZ_3/RE_3 and $REHZ_3$ groups are 2.52, 3.67 and 3.13, respectively. Four days after starting chemotherapy, when each patient would have received 2 doses of RE and HZ or REHZ, the fall in the log viable counts in these three groups of patients is 1.12, 2.18 and 1.64, respectively.

The intake of patients for this investigation is continuing.

(started: 1991; expected year of completion: 1992)

Isolation of non-tuberculous mycobacteria from soil, dust and water in the environment

Non-tuberculous mycobacteria (NTM) are widely distributed in the environment. Man is constantly exposed to NTM by various means. In the BCG Trial area of South India, surveys using PPD-B have shown that prevalence of infection with NTM reaches 90% in children by the time they attain 14 years of age. The implications of such high levels of sensitisation are of considerable importance. In India, studies on NTM in the environment are confined to the work done by Kazda et al. in Bombay (1983, Biology of mycobacteria, Vol.2, Academic Press, London, p.323).

The study aims to identify the strains of NTM prevalent in the environment of the BCG Trial area of South India. The study area is situated in Chingleput district of Tamil Nadu. An area having very low non-specific sensitivity in England has been chosen for the purpose of comparison.

Three types of specimens have been included in the study, namely, soil, dust and water. The samples will be collected at two time points, namely, one immediately after the monsoon i.e., in the month of January, and the second in the summer i.e., in the months of May and June. An attempt is being made to get samples from England during the same time periods.

In order to choose suitable methods of processing for the above study, standardisation experiments were carried out in two stages for the isolation of NTM.

In the preliminary experiments, 15 samples each of soil and water were collected and processed by each of the six methods listed below and the results were compared. Each sample was set up in four aliquots. In all the methods, 10 ml of the processed samples were inoculated onto 4 slopes of LJ and 4 slopes of

Falkinham's Selective medium (FSM) (Falkinham et al., 1985, **Canadian J Microbiol, 32**, 10) and duplicate slopes of each medium were incubated at 30°C and 37°C, respectively.

Soil and dust:

1. Falkinham's method using 1% NaOH (Brooks et al., 1984, **ARRD,130**, 630).

2. Variation of Falkinham's method using 2% NaOH.

3. Variation of Falkinham's method using 4% NaOH.

4. Reznikov and Leggo's method using 4% NaOH (Reznikov and Leggo, 1974, **Pathology, 6**, 269).

5. Engbaek's method using 3% Sodium lauryl sulphate (SLS) and 1% NaOH (Engbaek et al., 1967, **Scandinavian J.Resp. Dis., 48**, 268).

6. Method using 1% cetrimide (Joseph et al., 1969, **Tubercle, 50,** 299).

Water :

1. Variation of Falkinham's method using 4% NaOH (Falkinham et al., 1980, **ARRD, 121,** 931).

2. Variation of Falkinham's method using 8% NaOH.

3. Goslee and Wolinsky's method using NaOH-NaOCl (Goslee and Wolinsky, 1976, **ARRD, 113,** 287).

4. Variation of Goslee and Wolinsky's method using 4% NaOH.

5. Variation of Goslee and Wolinsky's method using 4% H_2SO_4.

6. Engel's method using 3% SLS and 1% NaOH (Engel et al., 1980, **Tubercle, 61,** 21).

Preliminary identification of the isolates obtained was done on the basis of colony morphology, pigmentation and growth rate. The basis of comparison of the methods was the net number of positive samples and the number of strains obtained by each method.

On reviewing the results obtained in the preliminary experiments, it was decided to further evaluate three of the methods for soil (Falkinham's method using 4% NaOH on FSM at 37°C, Reznikov and Leggo's method using

4% NaOH on LJ at 30°C and Engbaek's method using 3% SLS and 1% NaOH on LJ at 30°C) and two methods for water (Falkinham's method using 8% NaOH on LJ and Engel's method using 3% SLS and 1% NaOH, both on LJ at 37°C) using 15 samples each of soil and water. The samples were coded before processing. For soil, Engbaek's method on LJ at 30°C gave more number of positive samples and more number of strains (Table 1) as compared to the other

Table 1

Method	Medium	Incubation temp.°C	No. of positive samples	No. of samples positive with contamination	Net No. of positive samples	No. of strains isolated
Soil						
Engbaek's	LJ	30	15	0	15	20
Falkinham's	FSM	37	4	8	10	12
Reznikov & Leggo's	LJ	30	5	4	8	8
Water						
Engel's	LJ	37	10	1	11	21
Falkinham's	LJ	37	10	1	11	13

two methods; so it was selected for the main study. Similarly, for water, Engel's method on LJ at 37°C was selected for the main study as it gave more number of strains as compared to the other method.

Using a method of random sampling, the first set of samples from 15 randomly selected villages in Kadambathur Panchayat Union of Trivellore taluk was subsequently collected. The samples were transported to the laboratory under sterile conditions and processed in lots over a period of three weeks. Soil and dust samples were stored in the dark at room temperature, and water samples in the dark at 4°C till they were processed. Studies on the isolates obtained from these samples are now in progress.

(started: 1991; expected year of completion: 1992)

Drug susceptibility testing of *M.tuberculosis* cultures by bioluminescence assay

M.tuberculosis H37Rv and clinical isolates of **M.tuberculosis** obtained from individual patients with various drug susceptibility patterns are being tested by bioluminescence assay (1990 annual report).

Standardisation experiments have been carried out with **M.tuberculosis** H37Rv and 3 clinical isolates, using two different methods of mycobacterial adenosine triphosphate (ATP) extraction and different periods of incubation (0, 2, 5, 7 and 10 days). Based on these results, mycobacterial ATP extraction by nucleotide-releasing agent for bacteria (NRB) supplied by Lumac, was found to give reproducible results. Similarly, incubation for 5 days and ATP assay done at the end of the 5th day was found to be optimum.

Forty cultures of **M.tuberculosis** isolated and purified on 7H11 medium have been coded and tested so far by ATP assay and by conventional method. The critical concentration of drugs to classify the strains as susceptible or resistant to antimycobacterial drugs will be ascertained after obtaining the results on 50 cultures. With these criteria, drug susceptibility test by ATP assay will be done on another batch of 50 coded strains to find out the validity of the test. The study is in progress.

(started: 1990; expected year of completion: 1992)

Susceptibility of *M.tuberculosis* to cefadroxil- a cephalosporin antibiotic.

A study was undertaken to assess the susceptibility of **M.tuberculosis** to cefadroxil (see 1990 annual report). The susceptibility pattern of 29 clinical

Table 1

Strain	Drug sensitivity	Number tested	MIC of cephadroxil (mg/l) in 7H11 Agar				
			≤ 5	10	20	40	> 40
H37Rv	Sensitive	6	4	2	0	0	0
Clinical isolates	Sensitive	10	7	0	0	1	2
Clinical isolates	Resistant	19	7	2	1	1	8

isolates of **M.tuberculosis** and **M.tuberculosis** H37Rv is presented table 1. The MIC ot cetadroxil against **M.tubercutosis** H37Rv was tound to be 10 mg/l or less when tested on 6 occasions. Ot the 29 clinical isolales, 14 had MIC ≤5 mg/l and 12 were not inhibited even by 40 mg/l of cetadroxil. Nine of 19 drug-resistant strains were not susceptibte to 40 mg/l compared to 3 of 10 drug sensitive strains. In Sauton's liquid medium, cetadroxil was bactericidal against **M.tuberculosis** H37Rv at a concentration ot 7 mg/l.

The results ot this preliminary investigation show that a high proportion of the clinical isolates ot **M.tuberculosis** were not susceptible to cetadroxil even at 40 mg/l which is much higher than the peak plasma level (28 mg/l) attained in human beings, thereby reducing the scope ot this drug tor turther studies against **M.tuberculosis**.

The study is being continued to know the susceptibility ot non-tuberculous mycobacteria (NTM) to this drug.

(started: 1990; expected year of completion: 1992)

Adrenocortical function tn chitdren with tuberculous meningitis and tubercutous lymphadenttis

Tuberculosis is betieved to be one of the important causes of adrenat insutficiency in man. Meningitis is the commonest cause of death trom tuberculosis in children and clinically it is difficult to distinguish between children with tuberculous meningitis, particularly during the later stages of the disease, and those with severe adrenocortical insutficiency (Addison's disease). Moreover, children with tuberculous meningitis receive steroids and phenobarbitone in addition to ritampicin and other anti-tuberculosis drugs. Rifampicin and phenobarbitone are inducers ot the hepatic microsomal enzyme system and have been shown to enhance the clearance of exogenously administered steroids; turther, ritampicin has been shown to exert a deleterious effect on the adrenocortical function in patients with pulmonary tuberculosis. No intormation is available on the adrenocortical tunction in children with tuberculous meningitis, particularly on response to stimulation with ACTH (synacthen), and it is theretore proposed to obtain the same on admission and during conventional treatment with regimens containing anti-tuberculosis drugs including rifampicin, in addition to steroids and phenobarbitone. It is also proposed to study the response to stimulation with synacthen in children with a less serious form of tuberculosis, namely tuberculous lymphadenilis; these children will receive the same anti-tuberculosis drugs but will not receive any steroids or phenobarbitone.

These investigations will be undertaken at the Institute of Child Health, Madras. About 40 children aged 1-12 years in whom tuberculous meningitis is diagnosed on the basis of clinical and CSF findings and about 12 children of the same age group in whom tuberculous lymphadenitis is diagnosed on the basis of histopathological examination will be investigated. Bacteriological examination of CSF in children with tuberculous meningitis and of the gland tissue in those with tuberculous lymphadenitis will also be undertaken.

Children with tuberculous meningitis will receive rifampicin (10 mg/kg) plus isoniazid (7.5 mg/kg) plus streptomycin (40 mg/kg) plus pyrazinamide (30 mg/kg) daily for 2 months, followed by rifampicin (10 mg/kg) plus isoniazid (7.5 mg/kg) daily for an additional period of 10 months. These children will also receive decadron (dexamethasone) injections (0.3 mg/kg) every 8 hours for the first 4 days, followed by oral prednisolone 2 mg/kg daily for the next 15 days and then 1 mg/kg till the end of the 6th week. Phenobarbitone 5 mg/kg will also be administered daily for the first 6 weeks. The treatment regimen will be the same in children with tuberculous lymphadenitis except that steroids and phenobarbitone will not be administered and the continuation phase with rifampicin and isoniazid will be restricted to 4 months.

The synacthen stimulation test will be performed on admission and at 2 and 6 months after the start of treatment in both groups of patients. On all 3 occasions, a sample of blood will be collected for the determination of the basal plasma cortisol level; the patients will then be given an i.m. dose of synacthen 0.25 mg and blood will be collected 1/2 hour and 1 hour later. Response to synacthen will be assessed on the basis of the increase in plasma cortisol following stimulation.

Three patients with tuberculous meningitis have been admitted to the study till 31.12.91.

(started: 1991; expected year of completion: 1992)

Use of monoclonal antibodies for antigen detection assays

In the 1990 annual report, we had reported production and partial characterization of monoclonal antibodies against the antigens of **M. tuberculosis.** During 1991, nine monoclonal antibodies were evaluated for their utility in antigen detection assay.

The assay procedure in brief is as follows. Each dilution of monoclonal antibody was mixed with three different concentrations of culture filtrate (CF) antigen of **M.tuberculosis**, with zero concentration of CF serving as control in the assay. Antigen-antibody mixture was incubated overnight at 4°C and, on the next day, it was assayed for free antibody by ELISA with culture filtrate antigen of **M.tuberculosis**. Due to antigen-antibody binding, availability of free antibody in the mixture was reduced and it was reflected in lowering of the O.D. in ELISA. Reduction in ELISA O.D. was measured as % inhibition using the formula:

$$\% \text{ inhibition} = 1 - \left\{ \frac{\text{O.D. of antibody in presence of antigen}}{\text{O.D. of antibody in control}} \right\} \times 100$$

Higher percentage values of inhibition indicate the presence of higher amounts of antigen in the sample.

Nine monoclonal antibodies (six in ascites form and three affinity purified on protein A) were evaluated using the above procedure and the results are presented in table 1.

Table 1

Monoclonal antibody	State	Dilution factor	% inhibition in antigen concentration		
			10 µg/ml	1 µg/ml	0.1 µg/ml
41 - 33	Ascites	100	3	2	0
		1000	0	0	0
5-27-F2	Ascites	100	5	13	14
		1000	2	18	6
4C2-7	Ascites	100	24	13	9
		1000	23	7	0
5-13-C3	Ascites	100	0	0	0
		1000	18	15	27
4F6	Ascites	100	42	27	16
41-10	AP	100	8	9	7
		1000	15	0	10
1H12	AP	100	15	9	0
31-3	AP	200	88	41	4
		2000	64	50	23
5-10-E4	Ascites	100	0	0	0

It is obvious from the data shown in table 1 that monoclonal antibody 31-3 is promising as it has shown good inhibition even at the lowest concentration of the antigen, i.e., 0.1 µg/ml. This competitive inhibition ELISA using monoclonal antibody 31-3 was studied further at different dilutions of antibody. The specificity of the assay was also studied using unrelated antigens, **Setaria digitata** and **E.coli**. The results are presented in table 2. The maximum

Table 2

Dilution factor of McAb (X1000)	Control O.D.	Antigen concentration (µg/ml)				
		H37Rv-CF			**S.digitata**	**E.coli**
		10	1	0.1	10	10
50	1.926	0.159 (92%)*	1.575 (18%)	1.754 (9%)	1.668 (13%)	1.725 (10%)
100	1.862	-0.035 (100%)	1.182 (37%)	1.658 (11%)	1.628 (13%)	1.692 (9%)
200	1.783	-0.127 (100%)	0.567 (68%)	1.425 (20%)	1.519 (15%)	1.611 (10%)
400	1.475	-0.136 (100%)	0.186 (87%)	1.1 (25%)	1.288 (13%)	1.414 (4%)
600	1.303	-0.112 (100%)	0.138 (89%)	0.88 (32%)	1.163 (11%)	1.253 (4%)
800	1.154	-0.059 (100%)	0.064 (94%)	0.597 (48%)	0.904 (22%)	1.038 (10%)
1600	0.641	-0.073 (100%)	0.006 (99%)	0.361 (44%)	0.507 (21%)	0.698 (0%)

* The figures in parentheses are percentage inhibition values.

inhibition with **Setaria digitata** and **E. coli** antigens at 10 µg/ml was 22% and 10%, respectively. The same concentration of tuberculosis antigen resulted in 100% inhibition at all dilutions of antibodies tested except 50 (92%). Even at 100-fold lower concentration of mycobacterial antigen, the percentage inhibition was higher at all dilutions of monoclonal antibody. Thus this assay has potential for sensitivity up to 100 ng. Considering the fact that the antigen recognised by

monoclonal antibody 31-3 may form a small fraction of total **M.tuberculosis** H37Rv culture filtrate, the actual sensitivity of the assay may be higher. Since non-mycobacterial antigens, **E.coli** and **S. digitata** did not show cross reactivity, the next step will be to study cross reactivity with other mycobacterial antigens. The assay will also be tested further on clinical samples.

(started: 1990; expected year of completion: 1995)

Characterization and purification of antigenic components of *M.tuberculosis*.

An affinity chromatography procedure, using IgG obtained from pooled tuberculous sera (Tb-IgG) and pooled sera from endemic healthy subjects (HS-IgG) was utilized for isolation of useful antigens. The rationale behind the approach and methods used have been described in detail in previous annual reports (1989, 1990).

The crude culture filtrate of **M.tuberculosis** (CF) was passed through TB-IgG column and the unbound antigens were removed by extensive washing with PBS. The bound antigens were eluted with 4.0 M guanidine hydrochloride. The set of all eluted antigens was designated as APA-A. The recovery of the bound antigens was about 10%. To circumvent the difficulties in elution, the following procedure was adopted. The CF was passed through a column of normal human IgG to absorb out the commonly reacting antigens. The material unbound to the column, which was expected to be rich in specific antigens, was actually found to contain two prominent bands of 30 and 15 KDa and designated as APA-B (Fig.1). The column-bound antigens also were eluted with guanidine hydrochloride and designated as APA-C.

ELISA was carried out using the purified antigens and the values of O.D. were obtained at a dilution of 1:100 in tuberculous and normal sera. The mean O.D. values and the standard deviations for different antigens are presented in table 1. Using a cut-off point of Mean O.D. + 2 SD of endemic healthy subjects (HS), the number of positives in the 2 groups were calculated. As shown in table 2, the sensitivity is 43%, using CF. The sensitivities using APA-A, APA-B and APA-C were 27, 74 and 29%, respectively. The specificity obtained was high and similar for all the antigens, ranging from 95 to 98%, because of the mode of selection of the cut-off point. Thus, APA-B showed much higher sensitivity than the other purified antigens as well as crude CF.

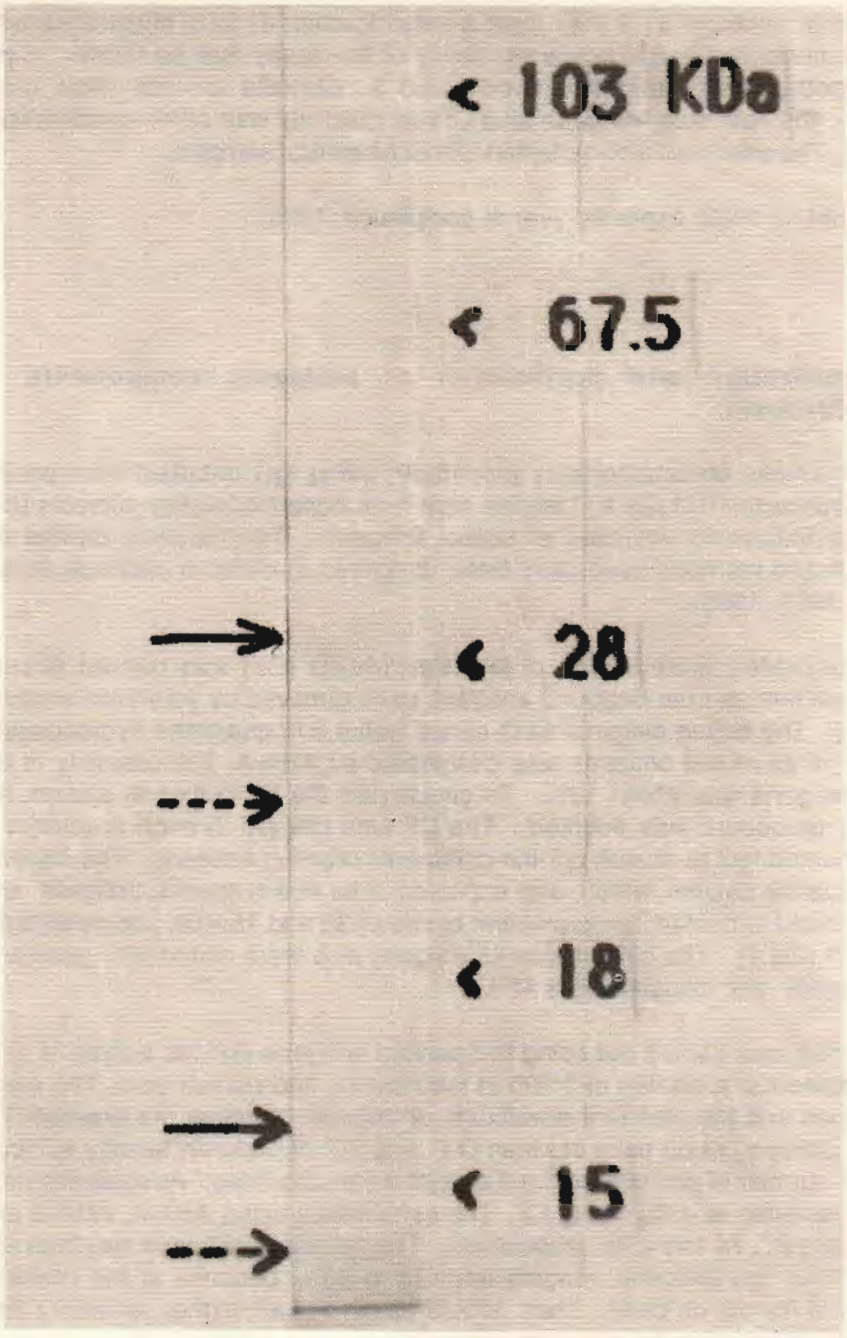

FIG.1: Characterization of the purified antigens. APA-B stained with Coomassie Blue.

Table 1

Antigen	Mean O.D. ± S.D.			
	TB		HS	
C.F.	2.282 ± 0.193	(42)*	1.459 ± 0.429	(40)
APA-A	1.026 ± 0.924	(45)	0.485 ± 0.407	(44)
APA-B	1.479 ± 0.525	(42)	0.537 ± 0.249	(42)
APA-C	0.807 ± 0.228	(42)	0.606 ± 0.164	(42)

*The figure in paranthesis indicates the number of subjects.

Table 2

Classification by ELISA	C.F.		APA-A		APA-B		APA-C	
	TB	HS	TB	HS	TB	HS	TB	HS
Positive	17	2	12	2	31	1	12	1
Negative	23	38	33	42	11	41	30	41
Total	40	40	45	44	42	42	42	42
Sensitivity	43%		27%		74%		29%	

When the affinity chromatography experiments were designed, it was expected that mycobacterial antigens which were cross-reactive and commonly recognised by both tuberculous patients and endemic normals could be absorbed out by HS-IgG. Therefore, the resulting unabsorbed components of the crude mixture were expected to be an enriched source of antigens specific for tuberculosis. This expectation is justified, as APA-B possesses a high degree of sensitivity and possibly specificity. Thus the usefulness of the affinity chromatographic technique using HS-IgG immunoabsorbent, as a single step purification method, has been established.

The sensitivity of detection of cases with APA-B was 74%. This is not sufficient to develop a diagnostic or screening test based on this antigen. Alternative approaches will have to be developed to increase the sensitivity of APA-B. One approach could be to enhance the concentration of the other

minor proteins by individually purifying them. Other approaches such as evaluating the performance of APA-B and NCP (Nitro Cellulose Paper) based assays like Dot-blot might also prove fruitful.

(started:1988; expected year of completion:1993)

Development of DNA probes for *M.tuberculosis*

The development of DNA probes is in progress. In the 1990 annual report, the construction of genomic libraries of **M.tuberculosis** in two vectors and the selection of recombinant clones for further evaluation based on the criteria of their specificity and sensitivity, were reported.

The evaluation of one of the 18 recombinant clones, namely pTRC4 was undertaken during the year. The specificity of this clone was studied using DNA from **E.coli** kk6, **Bacillus subtilis, Proteus mirabilis, Proteus vulgaris, Clostridium perfringens, S. hemolytica** and human placenta. The DNA from these species were spotted in four concentrations: 20, 2.0, 0.2 and 0.02 µg. The DNA was hybridised with ^{32}P labelled pTRC4. This probe did not recognise any of the atypical mycobacteria; however, it recognised the highest concentration of human placental DNA.

The mycobacterial fragment of pTRC4 is 2.2 kb in size. pTRC4 was subjected to restriction digestion with various enzymes to see whether the mycobacterial fragment in pTRC4 could be mapped. pTRC4 was found to have 3 sites for Sal 1. Since the mycobacterial fragment pTRC4 has been cloned in between Eco R1 and Hind III sites, pTRC4 was restriction digested with Eco R1, Hind III, and Sal 1 and run on 1% agarose gel in triplicate wells. The gel was Southern transferred to Gene Screen membrane. The individual lanes were hybridised with ^{32}P labelled **M.tuberculosis** DNA, ^{32}P labelled pTRC4 and ^{32}P labelled human placental DNA respectively. Human placental DNA did not cross-react with the individual fragments of pTRC4 (Fig. 1). The individual fragments of pTRC4 would be further sequenced and evaluated for their usefulness as a DNA probe for **M. tuberculosis.**

Another aspect of the probe is its usefulness in restriction fragment length polymorphism (RFLP) studies and, therefore, it has been evaluated. This is a useful tool for epidemiological studies. The DNA of various strains isolated from tuberculous patients were restriction digested with Pst1 and Sal 1, run on 1% agarose gel, Southern transferred on to Gene Screen membrane and probed with ^{32}P labelled pTRC4. Except **M.bovis BCG** DNA, none of the DNA from other mycobacteria reacted with the probe (Fig.2). The various atypical mycobacteria used were **M. kansasii, M. avium, M. phlei, M. simiae, M. smegmatis, M. chelonei, M. fortuitum, M. chitae, and M. xenopi.**

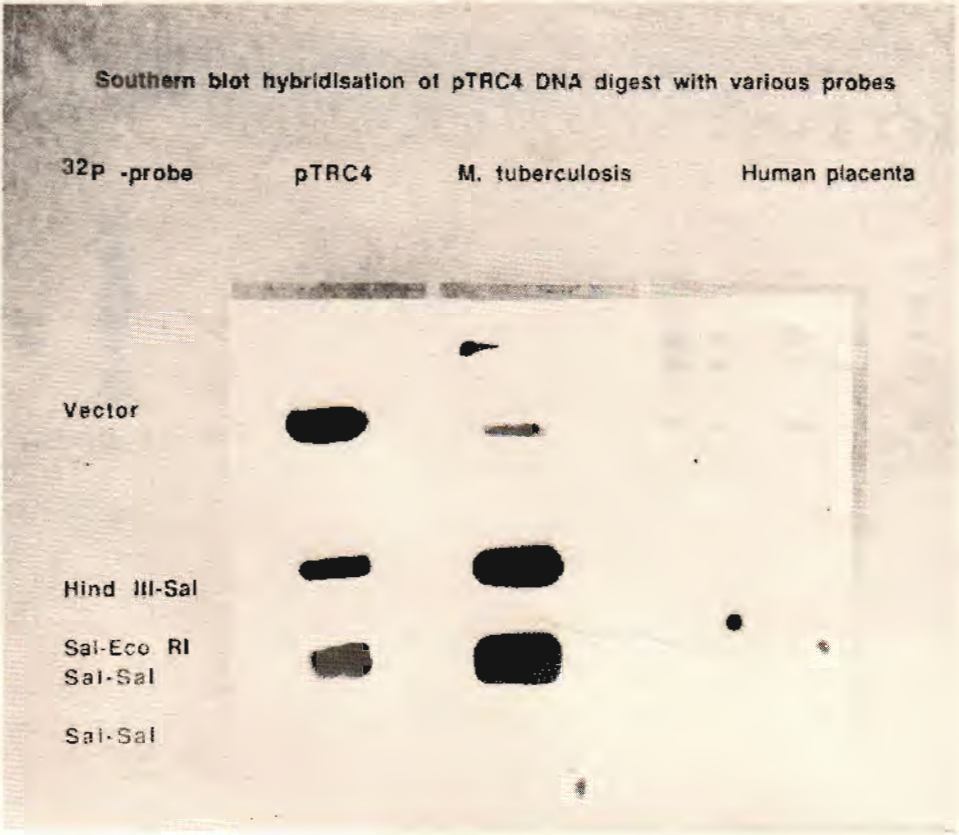

Fig. 1: The three lanes show pTRC4 clone restriction digested with Hind III, Eco R1, Sal 1 (i.e., triplicate): 1st lane is probed with ^{32}P labeled pTRC4, 2nd lane with ^{32}P labelled **M.tuberculosis** and 3rd lane with ^{32}P labelled human placental DNA.

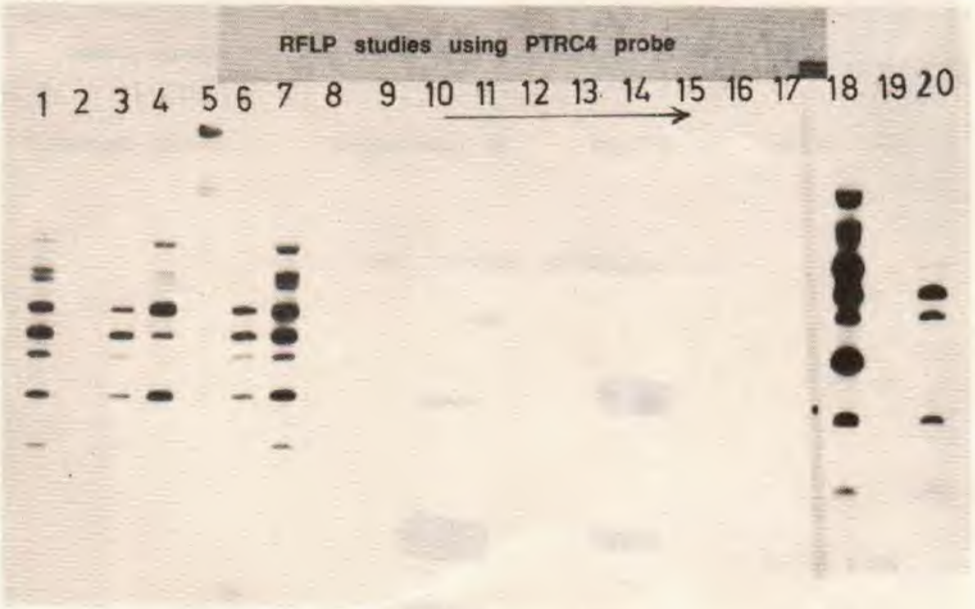

Fig. 2: DNA from clinical isolates of **M.tuberculosis** and atypical mycobacteria, restriction digested with Sal 1 and Pst 1. Lanes 1-7: various clinical isolates of **M.tuberculosis**; 8: **M.kansasii; 9: M.avium; 10: M.phlei; 11: M.simiae; 12: M.smegmatis; 13: M.gordonae; 14: M.chelonei; 15: M.fortuitum; 16: M.chitae; 17: M.xenopi; 18: M.bovis BCG; 20: Rifampicin-resistant clinical isolate.**

pTRC4 is a repetitive fragment occurring more than 10 times in the mycobacterial genome. This is found to be usetul in RFLP sludies and further work is in progress.

(started:1988; expected year ot completion:1993)

Human Leucocyte Antigen (HLA) studies in tuberculosis

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

For this investigation, blood samples will be collected from two groups of subjects, one group consisting ot present or past cases of pulmonary or extra-pulmonary tuberculosis and the other group consisting of subjects who have been tree trom tuberculosis till the time of collection of blood samples. HLA-phenotyping and genotyping will be carried out by HLA-antisera and HLA-gene probes, respectively.

Further, the role ot HLA-DR, DQ and DP genes (immune response genes) and gene products on tubercular immunity, will also be analysed.

Out ot a number ot HLA-antisera procured, 40 trom Indian sources (received trom Indian laboralories) and 68 from commercial sources (Biotest, West Germany) are being used for HLA-A and -B typing.

To ensure the quality ot the procured HLA antisera, their 'behaviour' or the 'reaction patterns' were checked against ficoll hypaque density gradient separated peripheral blood mononuclear cells of HLA-A and -B pretyped individuals (cell panel members of Tamil Nadu Forensic Science Laboratory, Madras). A minimum number of 3 cells (from 3 different individuals) were used for each antigen speciticity. The analysis ot the 'behaviour' or the serum 'reaction patterns' ot lhe HLA-A and -B antisera against the various HLA-A and -B antigens revealed that the antisera were in good condition.

During the year, HLA-A, -B, -DR and -DQ serological typing was carried out in 26 volunteers in addition to the 14 volunteers mentioned in the 1990 annual report. A total number ot 108 antisera were used tor HLA-A and -B typing. Ot these, 38 antisera tor HLA-A locus antigens and 80 antisera tor -B locus antigens (10 of them being common) were used.

For HLA-DR and -DQ antigen typing, 36 antisera from commercial sources were used. Since these are well-defined antisera, they were used directly for HLA-DR and -DQ typing. For confirmation of each antigen, at least two well-defined HLA antisera from two different human sources were used.

Attempts are being made to procure HLA-gene probes for HLA-DR, -DQ and -DP genotyping.

(started:1990; expected year of completion:1997)

Generation and characterization of T-lymphocyte clones in BCG vaccinated individuals

BCG vaccination converts tuberculin negative reactors, after a gap of 8 weeks, to tuberculin positive reactors. The implication of this conversion is that the T-lymphocytes of the individual become sensitized to the antigens of BCG and are able to mount a delayed hypersensitivity response upon subsequent challenge with antigens shared by BCG (like PPD). The nature of the T-cell response to BCG vaccination has not been understood. Therefore, a study was undertaken to characterize T-cell clones obtained from individuals before and eight weeks after BCG vaccination. The selection of the individuals is described below.

All the members in each of the households from one village in the BCG Trial area were screened for tuberculosis by identifying the symptomatics and by 70mm x-ray examination and sputum examination, if indicated. After excluding the households with one or more symptomatics and/or x-ray abnormals, attempts were made to test individuals in the 15-24 year age group in the remaining households with 1 TU RT 23. In all ,160 were tested and the reactions were read at 72 hours. Individuals with less than 6 mm induration were defined as tuberculin negatives, while those with an induration of 12 mm or more were defined as tuberculin positives. Only tuberculin negatives and positives (as controls) were considered for this investigation. Fifty ml of blood in a heparinized tube was collected from each of 8 eligible individuals. From tuberculin negatives, blood collection was made on two occasions, one before BCG vaccination and the second, 8 weeks after BCG vaccination.

T-cell clones were generated against mycobacterial antigens from six tuberculin negatives (before and after BCG vaccination). A total of 83 clones and 43 cell lines have been prepared so far and these will be characterized in the following months.

(started:1991; expected year of completion:1995)

Haematological profile of pulmonary tuberculosis patients

This study was initiated to understand the relationship of haematological parameters with the clinical and bacteriological features of patients suffering from pulmonary tuberculosis. The material is drawn from previous chemotherapy trials of the Centre.

As reported earlier (see 1990 annual report), analysis of data from one of the trials in which 920 patients received fully intermittent short course chemotherapy of 6-month duration revealed that there was leucocytosis with neutrophilia, thrombocytosis and decrease in haemoglobin in patients on admission to treatment. The levels of these parameters were associated with the radiographic extent of disease, extent of cavitation and smear grading on admission. However, the values at the end of chemotherapy were significantly different. Another trial with 604 patients receiving chemotherapy regimens of 3 or 5 months' duration confirmed these findings. The relationship of these parameters with response to treatment and relapse is being analysed.

(started: 1990; expected year of completion: 1992)

Histopathological classification of tuberculous lymphadenitis

The aims, objectives and methodology of this study have been described in the annual reports of 1989 and 1990. During 1991, a total of 180 lymphnode biopsies were received, of which 98 (54.4%) specimens showed evidence of tuberculosis. So far, of 817 biopsies included in the study, 428 (52.4%) have been found to be tuberculous. Of these, 70 (16.4%) biopsies showed definite histological evidence of tuberculosis but could not be graded because of insufficient quantity of tissue. A further 43 (10.0%) could not be clearly graded as they presented features of two adjacent groups, i.e., non-reactive and hyporeactive, hyporeactive and reactive, or reactive and hyperplastic types. Of the remaining 315 lymphnodes, 206 (65.4%) showed the reactive pattern of response, 70 (22.2%) belonged to the hyperplastic variety, 27 (8.6%) to the hyporeactive type and 12 (3.8)% to the non-reactive type.

A detailed analysis of the relationship between the histological classification and the clinical and bacteriological features of these cases is in progress.

(started: 1988; expected year of completion:1995)

EPIDEMIOLOGICAL STUDIES

STUDIES COMPLETED

Awareness of tuberculosis among the general population as compared to the awareness among patients

It is now well accepted that health education, aimed at improving community participation, is an essential component tor the improvement of the National Tuberculosis Program. In order to formulate successful health education strategies, it is necessary to have an understanding of the community's awareness and perception regarding tuberculosis.

An earlier study had collected information about awareness of TB among chest symptomatics (see 1989 annual report). In the present study, patients registered (in the Longitudinal Study of Bacteriological Quiescence and Relapse) were interviewed using a questionnaire, to find out their awareness about TB. As a sample survey tor tuberculosis in North Arcot was in progress, the information regarding awareness was also collected from the general population in three villages. Multiple responses were received for some questions from some of the respondents.

Table 1 gives the number screened and the number who were interviewed (those who had heard of TB).

Table 1

	Total number screened	Heard of TB	
		No.	%
General population	7540	2010	27
Patients	886	533	60

It is seen that only 27% of the general population had heard ot TB as compared to 60% of the tuberculosis patients. Forty percent of the patients had come to the clinic without even having heard of TB.

The source of information through which the respondents came to know about TB is given in table 2. Awareness appears to spread mostly by word of mouth--trom relatives and other TB patients. Books, magazines and mass media appear to have had only a limited role in spreading the awareness of TB.

Table 2

Source	General population		Tuberculosis patients	
	No.	%	No.	%
Health institution	537	26.7	193	36.2
Books/Magazines	95	4.7	26	4.9
Television/Radio/Cinema	160	8.0	99	18.6
Other TB patients	446	22.2	256	48.0
Relatives	1121	55.8	128	24.0
Heard of TB	2010		533	

The various reasons attributed for the disease are given in table 3.

Table 3

Reason(s) for getting tuberculosis	General population		Tuberculosis patients	
	No.	%	No.	%
Germs	283	14.1	18	3.4
Lack of proper food	129	6.4	149	28.0
Addiction to smoking/alcohol	225	11.2	152	28.5
Over-work	27	1.3	102	19.1
Worries	11	0.5	20	3.8
God's curse	2	0.1	4	0.8
Fate	15	0.7	2	0.4
No idea	1434	71.3	226	42.4
Heard of TB	2010		533	

In all, 71% in the general population and 42% among tuberculosis patients had 'No idea' about the cause of the disease. Lack of food (6%, 28%), addiction to

smoking/alcohol (11%, 28%), or over-work (1%, 19%) were given as reasons for getting TB by the population and the patients, respectively. Only 14% of the population and 3% of the patients attributed TB to germs. In the earlier study among symptomatics from a rural area, 4% attributed the disease to germs.

As regards the knowledge about the spread of tuberculosis, most patients (60%) believed the disease to be hereditary, but 53% of those from the general population said that the disease was infectious (see table 4).

Table 4

Cause of spread of tuberculosis	General population		Tuberculosis patients	
	No.	%	No.	%
Infectious	1067	**53.1**	20	**3.8**
Hereditary	148	**7.4**	321	**60.2**
Congenital	10	**0.5**	9	**1.7**
Others	6	**0.3**	2	**0.4**
No idea	850	**42.3**	200	**37.5**
Heard of TB	2010		533	

Table 5 gives the knowledge of the respondents about the methods of diagnosis. Most patients thought that blood test and/or physical examination were the means of diagnosis; only 29% of the population and 37% of the patients mentioned sputum examination as a method of diagnosis.

Table 5

Method(s) of diagnosis of tuberculosis	General population		Tuberculosis patients	
	No.	%	No.	%
X-ray	476	**23.7**	16	**3.0**
Blood test	314	**15.6**	328	**61.5**
Sputum examination	581	**28.9**	197	**37.0**
Physical examination	308	**15.3**	410	**76.9**
Others	110	**5.5**	43	**8.1**
No idea	986	**49.1**	110	**20.6**
Heard of TB	2010		533	

The patients in this study were interviewed after they had been diagnosed, started on treatment and had their initial motivation -- i.e., after exposure to a health facility. They were interviewed within 10 days of having been motivated at the start of treatment; still their knowledge about the disease was unsatisfactory. This study has also shown that most information spreads by word of mouth. Therefore, it is possible that the awareness of TB in the general population may be improved if the patients and their relatives are properly educated about tuberculosis.

(started:1989; completed:1991)

Validation of scoring system in the diagnosis of tuberculosis in children

The World Health Organisation commissioned a series of retrospective case control and contact studies to investigate the protective efficacy of BCG against tuberculosis in children. In these studies, a scoring system had been used to diagnose tuberculosis in children. This scoring system makes use of clinical and radiological findings, and does not rely on bacteriological confirmation. The scoring system was made available by the Tuberculosis Division of the WHO on request.

Earlier, an attempt was made to develop diagnostic criteria based on clinical and radiological signs for tuberculosis in children. However, when these were validated against bacteriological confirmation, the sensitivity and specificity were found to be low. An attempt to develop a mathematical model using a probabilistic approach was also not successful (see 1990 annual report). Therefore, with the objective of finding standardised criteria for the diagnosis of tuberculosis in children for use in field trials of vaccine efficacy, an analysis was undertaken to validate the WHO scoring system using the same data set.

The data set : There were 1285 case records of children with different forms of TB, of whom 301 were confirmed to have tuberculosis either by bacteriology or by histopathology. Bacteriological confirmation was available from any one of the specimens such as sputum, body fluids or tissues, gastric lavage fluid, CSF, pleural or ascitic fluid, gland and other biopsy specimens. Details of the clinical condition and radiological findings were also recorded. Investigations were not done for 456 children as the clinical and radiological findings were considered to be clearly non-tuberculous.

FREQUENCY DISTRIBUTION (%) ACCORDING TO SCORES FOR 828 CHILDREN

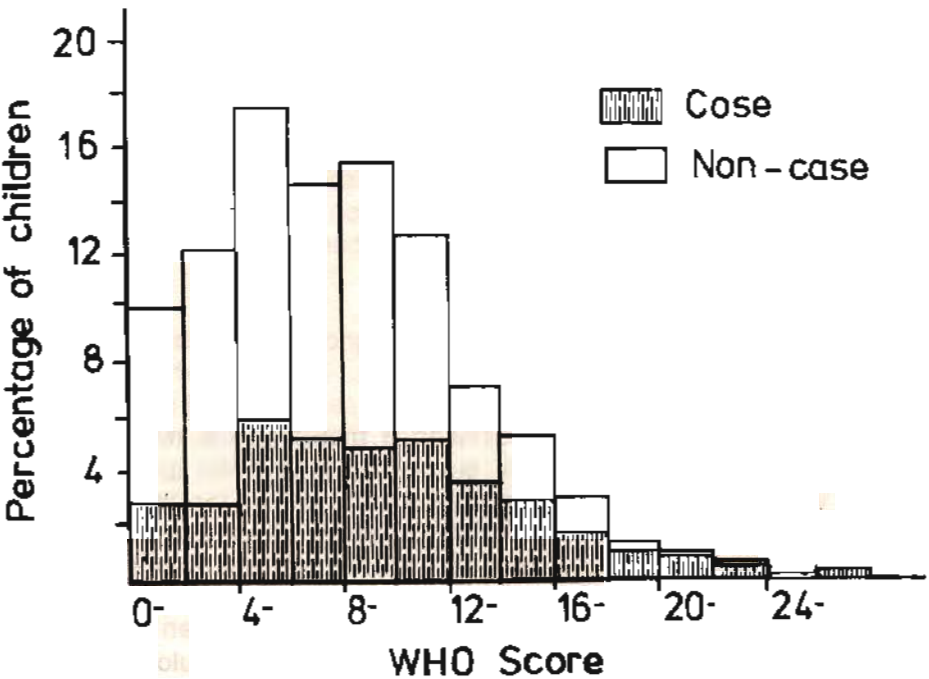

Fig 1

Methodology: A physician, who was "blinded" to the scoring system and the bacteriological findings, went through the case records and extracted information on whether the variables relevant for the scoring were present or not. Later, a statistician independently assigned scores to these variables according to the system recommended by the WHO, and calculated the total scores. The distribution of these scores were then plotted. The sensitivity, specificity and predictive values were calculated using the bacteriology and/or histopathology as the gold standard.

Results: It was found that the distribution of scores for the 828 children for whom the investigations were done was unimodal, unlike that reported by WHO (see figure 1). The classification as a case/non case based on the WHO scores was compared with the results by bacteriology/histopathology; the findings are presented in table 1. Validation analysis showed that the sensitivity

Table 1

WHO score	Bacteriology and/or histopathology		Total
	Positive	Negative	
≥ 6 (Case)	207	294	501
< 6 (Non-case)	94	234	328
Total	301	528	829

was 69% and specificity was only 44%. The positive predictive value was 41% in a situation where the prevalence was around 10%. In field studies, where the prevalence is likely to be much less, the predictive values are likely to be still lower. Considering those children for whom investigation was not done as negative by bacteriology and/or histopathology , the specificity was 66% .

This study shows the limitations of the WHO scoring system, particularly for use in field trials of vaccine efficacy, as the positive predictive value, sensitivity and specificity were unacceptably low.

(started:1991; completed:1991)

STUDIES IN PROGRESS

Development of surveillance methodology for tuberculosis

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.

Methodology: The following surveys/activities are being undertaken.

1. Tuberculin surveys in children aged 0-14 years in selected areas, to study the risk of infection over a period of about 20 years. Each survey will cover about 10,000 children, and the interval between surveys will be two years to avoid boosting effect.

2. Comprehensive survey for detecting tuberculosis disease will be undertaken in the same area as the infection surveys, and will cover a population of about 100,000. All individuals aged 10 years and above will be screened by 70 mm radiography as well as assessment of symptomatic status. Sputum will be collected from chest symptomatics and those with radiographic shadows, and examined for smear, culture and drug sensitivity status at the Centre's laboratory. This survey will also be carried out once in two years.

The methods and procedures established by the Tuberculosis Prevention Trial (Epidemiology Unit at present) will be followed for census and registration, tuberculin testing, radiography, screening for symptomatics and sputum collection.

Selective follow-up: Those found to be symptomatic or to have an abnormal chest radiograph but who are sputum negative will be treated as suspects and followed up every 6 months. Such selective follow-up rounds will be undertaken every 6 months. The suspects will be radiographed, screened for chest symptoms and sputum collected from eligibles.

Passive case finding: In addition, Passive Case Finding Centres will function at the peripheral health institutions, normally once a week. Patients reporting with symptoms will be attended to at these centres. Facilities for radiography and sputum collection will be provided.

Treatment: Those found to be sputum positive either by smear or by culture will be offered treatment with short course regimens (either daily unsupervised (2EHRZ/6EH) or supervised twice weekly ($2EHRZ_2/4RH_2$)) through the Passive Case Finding Centres. If a radiograph is read as definite or doubtful tuberculosis by two readers but the sputum is negative, another sputum examination will be done and if that is also negative, the patient will be prescribed a standard regimen. Treatment activities will be undertaken by the staff of the Epidemiology Unit. Patients prescribed treatment will be followed up every three months, and sputum collected. Those found to have resistance to isoniazid and/or rifampicin will be offered more intensive treatment. Defaulter action will be taken according to the District Tuberculosis Program procedures.

By December 1991, all the 15 panchayats selected from Kadambathur panchayat union had been covered. A population of 29,392 has been registered. Coverages of over 90% have been obtained for all examinations (Table 1).

Table 1

Examination	Age group (years)	Population eligible	Population covered	
			No.	%
Symptomatic	≥10	22777	21547	95
X-ray	≥10	22777	21108	93
Tuberculin test	0-14	9523	8844	93
Sputum	≥10	4042	3921	97

Two hundred and thirty-four sputum positive patients have been identified and referred for treatment and among these, 195 were culture positive. Drug sensitivity test results are available for 156. Of these, 22 had a history of previous chemotherapy. The resistance to streptomycin (S), isoniazid (H) and rifampicin (R) at the time of detection is shown in table 2.

Table 2

History of Rx	Total culture positives	Sensitive to S,H & R	Resistant to					
			S	H	R	SH	HR	SHR
No	134	118	3	6	0	1	3	3
Yes	22	16	0	1	1	3	1	0
Total	156	134	3	7	1	4	4	3

Eight of the 15 panchayats have been covered for selective follow-up. The coverages are shown in table 3.

Table 3

Examination	Population eligible	Population covered	
		No.	%
Symptomatic	3360	2773	83
X-ray	3360	2746	82
Sputum	1471	1413	96

The data collected in the first 5 months was reviewed by the Epidemiology Sub-Committee, which recommended the following changes in the methodology of the study.

1. All individuals up to the age of 24 and all sputum positive cases will be tested with 1 TU RT 23.
2. Surveillance interval will be 3 years alternating with 2 years. The first resurvey will be done at 3 years, the second at 5 years, the third at 8 years, and so on.
3. Tuberculin testing will be done in mutually exclusive sub-samples of villages for each survey.

The study is in progress and will be reviewed by the Sub-Committee from time to time.

(started: 1990; expected year of completion of intake: 1994)

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

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 pre-treatment 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.

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 examined in these four centres, 886 were eligible for admission to the study (Table 1).

Table 1

	No.	%
Total number of patients with a positive smear	2558	-
Self-reported old patients	985	39
Found to be old patient on home visit	70	3
New cases	1503	59
Dead	25	2
Outside area	448	30
Migrated	136	9
Sputum not collected	8	0.5
Admitted to the study	886	59

It was seen that 1055 (42%) of positive smears were from old patients, who were already on treatment. Of the new cases, 25 (2%) were dead, 448 (30%) were from outside the study area, and 136 (9%) had migrated. Sputum could not be collected from 8 (0.5%) cases. Of the 886 patients admitted to the study, 29 did not report back for initiation of treatment. Of the remaining 857, a specimen of sputum on admission was culture positive in 757 (450 from those started on SCC and 307 from standard regimens). Of these, 160 (21%) had resistance to one or more anti-tuberculosis drugs, namely, 80 (11%) to streptomycin, 112 (15%) to isoniazid and 28 (4%) to rifampicin. Of these, 88 were admitted to short course regimens and 72 to standard regimens. There was no difference in the pattern of initial resistance between the patients on SCC and on standard chemotherapy.

The coverages obtained for each of the follow-up rounds for those registered between October 1989 and October 1990 is shown in table 2.

Table 2

	Month of follow up			
	6 months		12 months*	
	No.	%	No.	%
Eligible	435		431	
Available	369	85	334	77
Dead	4	1	19	4
Migrated	62	14	78	18

* Includes migrations at 6 months also.

Eighty-five percent of those initially admitted to the study could be seen at 6 months and 77% at 12 months. The study is in progress.

(started: 1989; expected year of completion: 1993)

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

This study was undertaken to examine the feasibility of health workers using an algorithm developed for screening children at the community level, the applicability of the algorithm in referring children for investigations and the logistic problems which could arise while documenting cases of tuberculosis in children in the community. The rationale for the study, the algorithm developed for use in the study, and the methodology of the study have been described in detail in the 1990 annual report.

During the year under report, 6324 children have been registered and the coverages for the different examinations are given in table 1.

Table 1

Examination	Eligible	Examined	
		No.	%
Screening	6324	6092	96
X-ray	6262	5828	93
Tuberculin test	6195	5812	94
Clinical examination	1450	1361	94
Gastric lavage/Sputum	961	939	98
Blood collection	148	146	99
Gland biopsy	23	20	87

Children who were clinically abnormal or whose radiograph at intake was assessed as abnormal by one of two independent readers, were screened again by health workers at 3 months. Those whose X-ray at intake was abnormal were re-X-rayed. Those abnormal on either of these as well as those clinically considered as tuberculous (definite or doubtful) at intake were clinically re-examined.

At 6 months, all children were screened by the health workers and referred for investigations or clinical examination according to the algorithm. A few children who were normal were also referred for clinical examination as controls.

The coverages obtained at each follow-up were high.

In all, 12 children have been referred for treatment for bacteriologically proven pulmonary TB, 4 for glandular TB (positive on histopathology or on culture) and 1 for miliary TB on the basis of X-ray. Other forms of tuberculosis have not been encountered so far.

The study was reviewed by the Sub-Committee and a decision was taken to limit the intake to Kadambathur Panchayat Union. The study is in progress. Three more complete re-surveys, at 12, 18 and 24 months are planned to identify new cases that may develop.

(started: 1990; expected year of completion: 1993)

Surveillance of individuals infected with the human immunodeficiency virus for the development of tuberculosis

The existence of a relationship between infection with the Human Immunodeficiency Virus (HIV) and the development of tuberculosis is now well established. There is evidence in the literature of the occurrence of different forms of tuberculosis in HIV-infected individuals, especially in countries with a high prevalence of tuberculosis. An intensive surveillance of HIV infected individuals has been undertaken since June 1989, by the Epidemiology Unit of the Centre, to monitor the occurrence and form of tuberculosis in these individuals and also to study the pattern of transmission of HIV infection in family contacts. The methodology for this study, which is essentially a longitudinal cohort study for prognosis, has been described in the 1990 annual report.

Up to December 1991, addresses of 437 individuals had been obtained in both Madras and Pondicherry centres. It has been possible to trace 220 (50%) of these individuals and register them along with their contacts. Of these, 116 individuals are from Madras city, 47 from Pondicherry and the rest from other places in Tamil Nadu state. The age ranged from 15 years to 65 years. There were 78 (35%) males and 142 (65%) females; 113 (51%) were prostitutes. There were 16 couples, both of whom were infected. Thirteen individuals had died.

The profile of the HIV infected individuals is given in table 1.

Table 1

	Madras centre		Pondicherry centre		Total	
	Male	Female	Male	Female	Male	Female
Registered	65	108	13	34	78	142
Abnormal radiograph	38	62	5	10	43	72
Sputum positive M.TB*	19	11	2	2	21	13
Sputum positive NTM	12	19	0	3	12	22
Mantoux (1TU RT23) positive (≥12mm)	38	58	3	14	41	72

* M.TB - M.tuberculosis; NTM - Non-tuberculous mycobacteria

Out of the 220 individuals registered, 115 had an abnormal chest radiograph. Thirty-four of these had produced **M.tuberculosis** on culture. Another 34 had grown non-tuberculous mycobacteria, 9 of them on more than one occasion. Tuberculin reaction of 12 mm or more was seen in 113 patients (51%).

Up to December 1991, 42 HIV infected individuals (34 with positive bacteriology and 8 patients with persistent abnormalities on x-ray), had been started on anti-tuberculosis chemotherapy. Thirty-three of them showed a positive reaction (12mm or more) to 1TU (RT23). Two of the 42 also had cervical lymphadenopathy. The profile of the 42 patients with HIV infection and tuberculosis is given in table 2.

Six patients were labelled as full-blown AIDS, and 8 had AIDS-related complex. Of the 13 who died, 3 had tuberculosis with AIDS, 3 had tuberculosis without evidence of AIDS, 1 was labelled as AIDS without tuberculosis, 1 had committed suicide, 1 died in a road accident and the cause of death was unknown in the other 4.

Table 2

Characteristic		No. of patients
Sex	Male	26
	Female	16
Age	15 - 24	15
	25 - 34	15
	35 - 44	9
	45 - 54	1
	≥ 55	2
Mantoux	< 12mm	5
	≥ 12mm	33
	Not done	4
Culture	Positive	34
	Negative	6
	Not done	2

All the registered individuals are to be followed up every 6 months. Table 3 gives the coverages obtained at each follow-up round at both centres.

Table 3

Month of follow-up	No. eligible	No. examined	Absent	Left	Fate unknown	Refused	Dead
6	180	143	18	6	2	2	9
12	107	70	23	-	13	1	-
18	43	31	1	-	9	1	1
24	19	16	2	-	-	-	1
30	3	1	-	-	-	-	2

It is seen that, although this is a highly unstable population, only 4 patiente refused to be followed up. The study is in progreee.

(started: 1989; expected year of completion: 1995)

Surveillance of tuberculosis patients for human immuno-deficiency virus infection

In order to study the trend of HIV infection in tuberculosis patients, screening of all cases of tuberculosis who reported to the Centre (TRC), the DTC, Vellore or the TB Sanatorium, Vellore (GTBS) has been undertaken, irrespective of whether they were spulum positive caees or X-ray cases or extra-pulmonary cases. Each patient gave a blood specimen which was tested by ELISA for HIV antibody. Specimens found positive by ELISA were tested by ELISA again; if the repeat test result was also positive, they were eent for Weetem Blol confirmation.

Table 1

Centre	No. screened	ELISA positive		Western Blot		
		Single	Double	Positive	Equivocal	Negative
TRC, Madras	2165	39	16	3	12	1
DTC, Vellore	66	4	8	2	2	2
GTBS, Vellore	840	15	21	7	5	9

In TRC, 2165 patients were screened, of whom 3 were poeitive by ELISA and WB (Table 1). Another 12 were equivocal on WB. Thirty-nine were positive on one occasion by ELISA but were negative on the eecond occaeion. From Vellore, 906 patients were screened and nine were confirmed by WB. Another 7 were found lo be equivocal on WB. Nineteen were poeitive by ELISA on one occasion, but were negative on the repeat test.

During the period under report, it can be seen that HIV infection ie preeent in a proportion of patients with luberculosis. However, it is too early to comment about the magnitude of the problem or the trend.

(started:1991; expected year of completion:1993)

A collaborative survey of tuberculosis in Karhal block, a remote area inhabited by tribal population in Madhya Pradesh

The Regional Medical Research Centre (RMRC) for Tribal Health, Jabalpur had proposed a survey for tuberculosis among the Saharias, a most backward tribal population in Karhal block, Morena district, with the objective of studying the prevalence of both infection and sputum positive tuberculosis. Karhal block is spread in a plateau over 4000 feet above sea level. The terrain is barren and sandy with almost no approach roads. Our Centre (TRC) was actively involved in the planning of the survey, and also in providing technical expertise and consultancy.

Support was provided to the RMRC in the following areas.

1. **Preparation of the protocol:** The protocol for the survey in Karhal block was prepared, based on the experience gained in the tribal survey (Jawadhu Hills) of North Arcot (see 1990 annual report). X-ray examination was not done as this was not feasible. The protocol included a treatment program to suit the conditions in Morena district, where SCC is not in operation. The tribal population, constituting 47% of the total population, was mixed up with non-tribals in Karhal block. The total population in the block was surveyed without any sampling. The forms for data collection and work instructions were also developed at our Centre.

2. **Training for the RMRC staff:** Training was given for the RMRC staff in census taking, tuberculin testing, reading and in sputum microscopy. Team leaders were also trained in the organisation and supervision of the field work, packing and transport of sputum specimens and maintaining the accuracy and completeness of data.

The testers and readers were assessed after training, using the standard protocols that had been used in the Tuberculosis Prevention Trial. On the basis of the assessment, two testers and two readers qualified as standard testers and readers. The whole exercise took 8-12 weeks.

Training was given in computer programming for checking the data for completeness, detecting discrepancies and also for preparing simple tables. Support is being continued for data management and analysis.

Senior staff from the Epidemiology Unit were present for the initial 15 days and assisted in the organisation and smooth running of the survey work. Sputum specimens are being transported by messenger to the TRC Laboratory for culture and sensitivity testing. Smears are read in the field by the trained technicians. Cases detected are being referred to the Tehsil Hospital at Sheopur for management.

So far, in 37 villages, 22, 257 persons have been registered and examined. In all, 162 persons were found positive by sputum smear and referred for treatment. The study is in progress.

(started:1990; expected year of completion:1992)

STATISTICAL STUDY

Study completed

Confidence Interval for the difference between proportions in 2 x 2 tables with the use of Multinomial Distribution and the role of McNemar's Test

In medical research, 2x2 tables for statistical analysis occur frequently. Of such tables, one particular group in which the classification (presence or absence of a phenomenon) is identical in rows and columns can be distinguished from those in which it is different. This group consists of application of the same method by two different investigators or application of two different methods on the same or equivalent specimens (for example, two aliquots of the same specimen). The following two examples will illustrate the common nature of such a group.

Example 1 : Aliquots of 220 sputum samples were processed by each of two culture methods (NaOH and CPC) and the culture result was classified as having growth or not having growth.

Example 2 : Smears for a total of 9422 sputum specimens, one aliquot each, were independently examined for Acid Fast Bacilli by the same method at two different laboratories.

In such tables, the results tend to be associated because the examinations are done on the same or equivalent specimens, even though the two methods/examinations were applied independently. Two questions arise in the statistical analysis of such tables :

1. Is there any evidence that the proportions by the two methods/laboratories differ significantly?
2. What is the magnitude of the difference in the proportions by the two methods/laboratories and how precise is the difference?

The answer to the first question (yes or no type) is usually obtained by applying McNemar's test. But an answer to the second question provides the answer to the first question, and therefore it will be more useful to find a solution to the second question. The objective of this report is to suggest the use of the Multinomial Distribution to answer the second question and to illustrates its application.

The Multinomial Distribution for 2 x 2 tables : Let a, b, c and d be the observed frequencies of 4 mutually exclusive categories as shown in table 1

Table 1

Method A	Method B		
	Positive	Negative	Total
Positive	a (T1)	b (T2)	a+b
Negative	c (T3)	d (T4)	c+d
Total	a+c	b+d	N

Let T1, T2, T3 and T4 represent the theoretical probabilities (proportions) of the four categories. The observed proportions t1 (=a/N), t2(=b/N), t3 (=c/N), and t4 (=d/N) provide the estimates for T1, T2, T3 and T4 respectively. It is also known from mathematical theory that the estimates t1, t2, t3 and t4 are associated. Further, if L1t1 + L2t2 + L3t3 + L4t4 and M1t1 + M2t2 + M3t3 + M4t4 are any two linear functions of the estimates, where Li and Mi (i=1 to 4) are constants, formulae for their variances and co-variance are given in statistical text-books (see Chapter 2, Advanced Statistical Methods in Biometric Research by C.R.Rao, 1952).
For example,

$$\text{Variance of } (L1t1 + L2t2 + L3t3 + L4t4) = \frac{1}{N}\{ Li^2Ti - (\Sigma LiTi)^2\}$$

Application of the model : By giving suitable values to the coefficients in the general linear function, the estimate for any linear function of proportions of interest can be obtained. Then the above formula can be used to estimate its variance. If N is sufficiently large, Normal approximation can be made and the confidence interval calculated.

For comparing the proportions of positives by the two methods/laboratories, their difference and 95% confidence interval for the difference, the estimate for the proportion of positives is (t1 + t2) by method A and (t1 + t3) by method B. Therefore, the estimate for the difference in the proportions of positives reduces to (t2-t3) and its standard error is estimated by Square Root of $\frac{1}{N}\{(t2+t3) - (t2-t3)^2\}$

If N is large, (t2-t3) divided by its standard error is distributed as a Standard Normal Deviate under the Null Hypothesis of no difference between the two proportions. So the confidence limits can be calculated.

The above formula can be used for comparing the proportions of positives in the two examples described earlier. The data for first example is given in table 2.

Table 2

CPC method	NaOH method Positive	Negative	Total
Positive	61	24	85
Negative	9	126	135
Total	70	150	220

Estimated difference between the two proportions ot positives is
t2−t3 = 85/220 − 70/220 = 0.068

Standard error of the estimated difference = 0.0257.

The 95% confidence limits are given by 0.068 ± (1.96) 0.0257, where 1.96 is the value ot Standard Normal Deviate corresponding to 95% probability. The 95% confidence limits reduce to 0.018 and 0.118.

The data for the second example is given in table 3.

Table 3

Laboratory A	Laboratory B Positive	Negative	Total
Positive	3376	384	3760
Negative	200	5462	5662
Total	3576	5846	9422

Estimated difference in the proportion of positives = t2−t3= 0.019
Its standard error = 0.002556
The 95% contidence limits tor the difference are 0.014 and 0.024, or 1.4% and 2.4% .

As the confidence intervals for the above two examples do not contain zero in their ranges, it is concluded that the difference in the proportions in each example is statistically significant at the 5% level. The differences were also found to be statistically significant by McNemar's test ($P < 0.05$ for the first example and $P < 0.01$ tor the second example).

Comments and Conclusions: Basically, McNemar's test consists of testing that the two discrepant sample observations (b and c) in the 2x2 table are distributed in 1:1 ratio. The test is carried out as a chi-square (one degree of freedom) or as an exact Binomial test. One disadvantage is that the test uses only the conditional distribution, i.e., the distribution of b and c when (b+c) is kept constant. In other words, the total number of observations, N, is not utilized. Another disadvantage is that the significance of the test result might sometimes be misleading; the second example highlights this possibility. When N is very large, b and c could also be large, and so even a small deviation from the 1:1 ratio could become statistically significant, perhaps giving undue importance to the small difference in the proportions. In the second example, the maximum difference is unlikely to exceed the upper 95% confidence limit of 2.4% . If differences of less than, say, 5% are not of practical importance, statistical significance by the McNemar's test will be of no interest.

In view of the above considerations, it is always advantageous to use the confidence interval obtained by applying the Multinomial Distribution, provided N is sufficiently large.

(started: 1991; completed: 1991)

ELECTRONIC DATA PROCESSING

The Electronic Data Processing (EDP) Unit is continuing the computerisation activities in general, apart from routine computer operations on the mainframe VAX-11/750 computer. The data of major studies such as Surveillance Study in Tuberculosis, District Tuberculosis Program monitoring and HIV Studies have been organised on the VAX computer and regular data processing jobs are undertaken.

The data structures, data base organisation and management techniques, have been designed to meet the specific needs of the study that is being computerised. Data Entry and Verification activities are normally carried out on IBM-PCs using a suitable data entry package and then transferred to the VAX computer through an IBM-PC connected to it. The EDP Unit has developed several tailor-made computer programmes for data processing. The statistical software packages such as BMDP and SPSS are used for statistical analysis.

Development of Surveillance Methodology for Tuberculosis (see page 116): System analysis and system design for this study was finalised during the year. Computer programmes, in languages such as FORTRAN, BASIC and C, were developed on the VAX computer system, to meet every specific need of the study.

Objectives :

1. To create and update a data base, for long-term study of tuberculosis;
2. to provide computer-printed lists of persons who are eligible for sputum collection based on X-ray results, or who are to be referred for treatment based on the X-ray, smear or culture results;
3. to support field teams with printed lists to undertake selective follow-up examination in villages;
4. to provide listing of individuals in alphabetical order or household number order for use by the field teams;
5. to provide list of patients and several other useful lists for reference by field teams; and
6. to study and analyse the data.

Record formats have been designed to accommodate the identity of the study, the serial number of the subject, the category of record and the round of examination, apart from the data collected on various other aspects. The system

has been divided into the following tive sub_systems tor the convenience ot data management and operations :

(i) Organisation ot data entry, verilication and tile transter to VAX. (ii) Organisation ol data for printing cards tor persons found eligible for regular sputum collection. (iii) Identitication ot smear, culture and drug sensitivity results received trom the laboratory. (iv) Checking tor consistency ot data and reterral of persons for treatment based on X-ray, smear or culture results. (v) Organising the basic data and checking tor completeness, integrity and consistency betore closure ol file lor tuture reterence and analysis.

With the completion of system implementation, procedures have been established to supply computer-printed lists or teedbacks to field teams ot the Epidemiology Unit on rouline basis, tor tacilitating the smooth operation of the survey work.

Pilot study in Childhood Tuberculosis (see page 121): Computer programmes were developed and procedures were established to supply computer-printed name cards on pre-printed computer stationery on routine basis, according to time schedule, for use by the tield teams. Development ot turther data processing techniques is underway.

District Tuberculosis Programme: A methodology has been developed to process and tabulate the data collected trom the 18 districts which are being monitored. Data for the years 1990 and 1991 have been processed. In-house computer programmes were developed, to provide the statt monitoring the DTP work with the relevant computer teedbacks and tabulations, for speedy review ot the on-going work and for taking future course ot action in the dislricts. The tabulated reports contain various items of intormation, some ot which are (1) Listing of PHIs with poor performance and those not sending reports; (2) Reports giving details on new outpalients and supervision visits by DTC team; and (3) Analysis ot PHIs regarding SCC and DTP implementation, and performance by sputum examination and sputum positivity.

Surveillance ot TB patients for HIV infection (see page 125): Record formats have been designed, and data entry and verification is underway. Computer programmes have been developed and the required print outputs and tabulations are being provided to those monitoring the study.

Surveillance of individuals infected with HIV for the development of Tuberculosis (see page 122): The data processing support is similar to the one mentioned in the previous study.

Routine Data Processing: Approximately 19,000 records corresponding to DTP study were entered into computer files and veritied. Nearly 1,00,000 records belonging to other studies were entered and veritied.

A total of about 950 files were transferred from IBM-PC to the VAX computer during the year, while 10 files were transferred from the VAX to IBM-PC using communication software.

Editing and printing of Annual Report: Computers have been increasingly used for editing and printing of the annual Report of Research Activities of the Centre. The matter was stored in floppy diskettes of IBM-PCs and printouts taken by the individual Divisions. Then they were scrutinized, edited and revised in consultation with the staff concerned. As a further development for the printing of the present annual report, the matter was then fed into an Apple Macintosh PC and Laser prints obtained, which were then handed over to the printers for obtaining the necessary number of copies and binding. Thus it was possible to have close control over the lay out of tables and textual matter.

APPENDICES

TRAINING PROGRAMMES

WHO Fellow

Dr. Abdul Wassay Habibi, Afghanistan, from 27.9.91 to 11.10.91.

Trainees

The following underwent training in different departments as follows:

Bacteriology

Nineteen students of Diploma Course in Medical Laboratory Technology, Voluntary Health Services, Adyar, Madras, from 6.5.91 to 17.5.91.

Miss.Anuja, Miss.Y.Sheeba Praveena, Mr.S.Aravindh Pratap and Mr.Arul Selvan, M.Sc. (Microbiology) students from Christian Medical College, Vellore, from 18.9.91 to 19.9.91.

Fourteen M.Sc. (Microbiology) and 3 MD (Microbiology) students from Dr.A.L.Mudaliar Post Graduate Institute of Basic Medical Sciences, Taramani, Madras, from 16.12.91 to 21.12.91.

Cardio-Pulmonary Medicine

Dr.P.Sukumaran, Assistant Professor, Department of Chest Diseases, Govt. Medical College, Allappuzha, from 15.4.91 to 25.4.91.

Statistics

Mr.Deepak Jain, Junior Programmer, RMRC for Tribals, Jabalpur, from 1.4.91 to 8.4.91.

General

Dr.Elango, Arogya Agam, Aundipatty, Madurai District, from 25.2.91 to 2.3.91.

Dr.G.Srinivasan, Leprosy and TB Control Unit, Nellore, Andhra Pradesh, from 25.2.91 to 2.3.91.

Miss.Sharmila Sarang, Medical Social Worker, TB Control Project, Maharashtra Lokahita Seva Mandal, Bombay, from 2.9.91 to 14.9.91.

Mr. Daniel Fox, medical student, Bristol University, U.K., for 10 days from 24.9.91.

Dr. Anita and Dr.Sheela Rangan, Foundation for Research in Community Health, Bombay, from 16.10.91 to 18.10.91.

Others

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

Medical students

Kilpauk Medical College, Madras - 1 batch.

Sri Ramachandra Medical College, Porur - 4 batches.

C.S.I. Kalyani General Hospital, Madras - 2 batches.

Andhra Medical College, Vishakapatinam - 1 batch.

Post-graduate students

Ten PG Research students from Biomedical Engineering Division, Indian Institute of Technology, Madras.

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

Fifteen M.Sc. (Zoology) students from Presidency College, Madras.

Nine DTRD students from the Institute of Thoracic Medicine, Madras.

Dr.Bhargava Prasad and Dr.Ayyappa, M.D.(TB) students, from Andhra Medical College, Vishakapatinam, Andhra Pradesh.

Nursing and para-medical students

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

Multi-purpose Health Workers from Durgabai Deshmuk Hospital, Madras - 1 batch.

B.Sc.(Nursing) students from Madras Medical College, Madras - 1 batch.

STAFF DEVELOPMENT PROGRAMME

1. Dr.Sulochana Das was awarded a 1-year TCTP Fellowship under British DDA programme to work on "RFLP analysis of **M.tuberculosis** strains from Hong Kong patients" at the National Institute of Medical Research, Mill Hill, London, U.K., from October, 1990.

2. Dr.K.Thilakavathy was awarded Ph.D. in Social Work by the University of Madras, Madras, during 1991.

3. Dr.P.Venkatesan was awarded Ph.D. in Mathematics - Statistics (Inter-disciplinary) by the University of Madras, Madras, during 1991.

4. Dr.Geetha Ramani Shanmugam was awarded Ph.D. in Social Work by the University of Madras, Madras, during 1991.

5. Mr.P.G.Gopi was awarded a 3-month WHO Fellowship for training in Management Information Systems under Biomedical Research at the Medical College of Wisconsin, Milwaukee, U.S.A., from April, 1991.

6. Mrs.Geetha Ramani Shanmugam was awarded a 3-month WHO Fellowship for training in Sociological and Behavioral Aspects of Tuberculosis at the Centre for Disease Control, Atlanta, U.S.A., from April, 1991.

7. Dr.C.N.Paramasivan was awarded a 3-month WHO Fellowship for advanced training in the mycobacteriology division at the Centre for Disease Control, Atlanta, U.S.A. and in the mycobacteriology section of the Virginia State University, U.S.A., from April, 1991.

8. Dr.C.N.Paramasivan underwent training for 10 days in the Molecular Biology Laboratory, Antonie Van Leeuwenhoeklaan, Bithoven, Netherlands, in June, 1991.

9. Dr.A.Thomas was awarded a 2-month WHO Fellowship for training in Research Methodology in Clinical Leprosy at the Gills W.Long Hansen's Disease Centre, Carville, Louisiana, U.S.A., from July, 1991.

10. Dr.K.Sadacharam was awarded a 2-week WHO Fellowship for training in "Laboratory Techniques" at London and Netherlands, during June, 1991.

11. Dr.K.Sadacharam is undergoing a 1-year diploma course in Public Health in the All India Institute of Hygiene and Public Health, Calcutta, from July, 1991.

PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

Name of conference, venue and date	Title of paper	Name of staff member
45th National Conference on Tuberculosis and Chest Diseases, Rohtak (Haryana), 6-9 January, 1991	Controlled clinical trial of fully oral short course chemotherapy in the treatment of smear positive pulmonary tuberculosis	Dr.K.Rajaram
-do-	Fully intermittent six-month regimens for pulmonary TB in South India	Dr.Rani Balasubramanian
-do-	Effect of administration of rifampicin on the adrenocortical function in patients with pulmonary tuberculosis	Dr.G.Raghupati Sarma
-do-	Pharmacokinetics of isoniazid and rifampicin in patients with chronic renal failure	Mr.K.Jayasankar
-do-	Adenosine dearninase levels in CSF ot tuberculous meningitis	Dr.N.Selvakumar
-do-	Influence of initial and repeated motivation on case-holding in North Arcot district	Mrs.Niruparani Charles
-do-	Compliance and quiescence in smear positive pulmonary TB under programme conditions	Dr.Manjula Datta

Name of conference, venue and date	Title of paper	Name of staff member
4th Annual Conference of Digestive Disease Research Forum of India (Indian Academy of Gastroenterology), India International Centre, New Delhi, 30-31 January, 1991	Short term therapy of abdominal tuberculosis	Dr.R.Prabhakar
Conference on Progress towards Development of Vaccine for Onchocerciasis, Woods Hole, Massachusetts, U.S.A., 7-11 April, 1991	Current studies of ivermectin trials in India	Dr.V.Kumaraswami
IX Annual Conference of Indian Society for Medical Statistics and National Seminar on Statistics in Maternal and Child Health, New Delhi, 26-28 September, 1991	A model for testing the proportional hazards and the accelerated failure time hypothesis in the analysis of survival data	Dr.P.Venkatesan
-do-	Applications of simultaneous test procedures to tuberculosis survival data	-do-
-do-	An application of generalised additive model to survival data	-do-
-do-	Sample size for serial serum estimation of drug concentrations	Mr.P.R.Somasundaram
-do-	Anthropometry in children aged 0-12 years	Mrs.M.P.Radhamani

Name of conference, venue and date	Title of paper	Name of staff member
IX Annual Conference of Indian Society for Medical Statistics and National Seminar on Statistics in Maternal and Child Health, New Delhi, 26-28 September,1991	Regression-based test towards the paired sample t-test	Mr.P.G.Gopi
46th National Conference on Tuberculosis and Chest Diseases, New Delhi, 22-24 November,1991	Under-estimation of tuberculosis prevalence under two separate screening methods*	- do -
- do -	Prevalence of tuberculosis in North Arcot district of Tamil Nadu	Dr.C.Kolappan
- do -	Short course chemotherapy in the treatment of brain tuberculoma - A controlled clinical trial	Dr.R.Balambal
- do -	Controlled clinical trial in tuberculous lymphadenitis	Dr.K.C.Umapathy
- do -	Strategies to improve case-finding in tuberculosis programme	Ms.Theresa Xavier
-do-	Development of DNA probes for **M.tuberculosis**	Dr.Sujatha Narayanan

* Paper read by Dr.Manjula Datta

Name of conference, venue and date	Title of paper	Name of staff member
40th Annual Conference of Indian Association of Pathologists and Microbiologists, Madras, 18-22 December, 1991	Advantage of using selective Kirchner's liquid medium (SKLM) to transport lymph-node biopsy specimens	Dr.Vanaja Kumar
- do -	Susceptibility of South Indian strains of **M.tuberculosis** to tuberactinomycin	Dr.N.Selvakumar
- do -	Immunopathology of tuberculous lymphadenitis	Dr.V.D.Ramanathan
XXXV All India Obstetric and Gynecological Congress,Madras, 27-30 December, 1991	Tuberculosis in pregnancy	Dr.R.Prabhakar

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

Name of the event, venue and date	Name of staff member	Title of paper
CME Programme, Apollo Hospital, 6 January, 1991	Dr. V.K. Vijayan (Faculty member)	Basic pulmonary function tests
Seminar on National Health Programme on Tuberculosis, Gandhigram Institute of Rural Health and Family Welfare Trust, Ambathurai, Tamil Nadu, 19 January, 1991	Dr. R. Prabhakar	Chemotherapy of tuberculosis in the District Tuberculosis Programme, including SCC
-do-	Dr.N.M.Sudarsanam	District Tuberculosis Programme
-do-	Mrs.Sudha Ganapathy	Social aspects with reference to DTP
Meeting of Field Research Investigations Committee on Lymphatic Filariasis in India, Alleppey, 28-31 January, 1991	Dr.V.Kumaraswami	-
Workshop on Research Methodology and Critical Appraisal, Clinical Epidemiology Unit, MMC and ACCERT (ICMR), 7-9 March, 1991	Dr.R.Prabhakar	Key-note address
-do-	Dr. Manjula Datta (Resource person)	-
-do-	Mr.P.V.Krishnamurthy (Resource person)	-

Name of the event, venue and date	Name of staff member	Title of paper
Workshop on Common Laboratory Equipment, their Theory, Practice and Maintenance, Dr.A.L.M. Post-graduate Institute of Basic Medical Sciences, Taramani, Madras, 12-21 March, 1991	Dr.K.V.Kuppu Rao	-
Steering Committee Meeting of Filariasis, TDR/WHO, Geneva, 19-22 March, 1991	Dr.V.Kumaraswami	-
Expert Committee Meeting for establishing a Centre of pulmonary medicine at Bhopal conducted by Chief Medical Officer, Gas Relief, Bhopal, 27 March 1991	Dr.V.K.Vijayan	-
National Update in Pulmonology, Kovai Medical Centre and Hospital Ltd., Coimbatore, 7 April,1991	Dr.V.K.Vijayan	Exercise testing in pulmonology techniques and usefulness
- do -	- do -	Aerosol therapy
91st General Meeting of the American Society for Microbiology, Dallas, Texas, 5-9 May, 1991	Dr.C.N.Paramasivan	-
International Conference of American Lung Association and American Thoracic Society, Anaheim,California, 12-15 May, 1991	Mrs.Geetha Ramani Shanmugam	-

Name of the event, venue and date	Name of staff member	Title of paper
Seminar on TB, A.V. Jesani TB. Hospital, Rajkot, 10 August, 1991	Dr. N.M.Sudarsanam	DTP rationale and SCC in Rajkot District Tuberculosis Programme
-do-	Mr.A.S.L. Narayana	Recording, reporting and monitoring aspects of Rajkot District Tuberculosis Programme
Project Advisory Committee on Clinical and Bronchoalveolar Lavage Studies in MIC-Exposed Subjects at Bhopal, Bhopal Gas Disaster Research Centre (ICMR), Bhopal, 13 August, 1991	Dr.V.K.Vijayan	Bronchoalveolar lavage in MIC-exposed subjects
Acid Fast Club, Bath University, London, August, 1991	Dr.Sulochana Das	Application of RFLP analysis to the origins of relapses and isolated positive cultures from tuberculosis patients in Hong Kong
Tuberculosis Research and Development Working Group, WHO, Geneva, 9-11 September, 1991	Dr.R.Prabhakar	-
Steering Committee Meeting of Filariasis, TDR/WHO, Geneva, 23-27 September,1991	Dr.V.Kumaraswami	-
Ethical Committee, Bhopal Gas Disaster Research Centre (ICMR), Bhopal, 24 September, 1991	Dr.V.K.Vijayan	Findings of BAL studies in MIC-exposed subjects

Name of the event, venue and date	Name of staff member	Title of paper
Meeting of Expert Committee on Lymphatic Filariasis, WHO, Geneva, 1-8 October, 1991.	Dr.V.Kumaraswami	-
Annual Conference of State Leprosy Officers, Directorate General of Health Services, Madras, 3 October, 1991	Dr.R.Prabhakar	-
Indian Medical Association, Eluru, 6 October, 1991	Dr. Rajeswari Ramachandran	Evolution of chemotherapy of TB
-do-	Mr.A.S.L.Narayana	Overview of the District Tuberculosis Programme
Workshop on Research Methodology and Critical Appraisal, Clinical Epidemiology Unit and ACCERT (ICMR), Madras, 21-23 and 28-30 October, 1991	Dr.Manjula Datta (Resource person)	-
-do-	Mr.P.V.Krishnamurthy (Resource person)	-
Workshop on Health Economics, Department of Health Service Studies, Tata Institute of Social Sciences,Bombay, 28 October -2 November, 1991	Dr.M.S.Jawahar	-
Symposium on Social and Operational Constraints on Tuberculosis Control in Maharashtra, Foundation for Research in Community Health, Pune, 2-3 November, 1991	Dr.R.Prabhakar	-

Name of the event, venue and date	Name of staff member	Title of paper
Association of Physicians of India, Madras,13 November, 1991	Dr.V.K.Vijayan	Pulmonary function tests (Guest lecture)
Meeting of Task Force for "Criteria for Diagnosis of Childhood Tuberculosis", ICMR Headquarters, New Delhi, 21 November, 1991	Dr.R.Prabhakar	-
-do-	Dr.Manjula Datta	Diagnostic criteria on childhood tuberculosis
Neuro-Epidemiology Symposium, Neuro Epidemiology Research Centre, Bombay Hospital, Bombay, 22-23 November, 1991	Dr.Rajeswari Ramachandran	Tuberculosis treatment trial
Indo-US Vaccine Action Programme Workshop on Acute Respiratory Infections, National Institute of Immunology, New Delhi, 22-24 November, 1991	Dr.R.Prabhakar	Control of tuberculosis: An Indian experience
-do-	Dr.C.N.Paramasivan	Bacteriological investigation in children with acute respiratory illness *
-do-	Dr.Manjula Datta	-

* Submitted for publication in the proceedings; the paper could not be presented for want of time.

Name of the event, venue and date	Name of staff member	Title of paper
Workshop on Reversal Reaction and Relapses with Multi-Drug Therapy Treatment in Leprosy, Dhoolpel Leprosy Research Centre, Hyderabad, 9-10 December, 1991	Dr.A.Thomas	Reactions in patients with fixed duration of MDT regimens - an observation
Indo-American Symposium on Pulmonary Medicine, American Association of Physicians of Indian Origin and Medical Council of India, Gandhi Medical College, Bhopal, 9-11 December, 1991	Dr.V.K.Vijayan (Faculty member and Chair person for a scientific session)	Pleural effusions
-do-	Dr.T.Santha Devi	Complicated TB
Workshop cum Symposium on "Genes and Expression", Indian Institute of Science, Bangalore, 12-14 December, 1991	Dr.Prema Gurumurthy	
CME Programme, Tamil Nadu Hospital, Madras, 13 December, 1991	Dr.M.S.Jawahar	Short-course chemotherapy in tuberculosis
Silver Jubilee Convention, Indian College of Allergy and Applied Immunology, VP Chest Institute, Delhi, 21 December, 1991	Dr.V.K.Vijayan	Immunology of tuberculosis (Guest lecture)

LIST OF PUBLICATIONS

Papers published

1. Paramasivan, C.N. Quality control in isolation and identification of mycobacteria from clinical specimens. In: **Proceedings of WHO Workshop on Quality Control in Clinical Microbiology (1990), Department of Microbiology, JIPMER, 1990, 27-38.**

2. Rajeswari , R., Parthasarathy, R., Sivasubramanian, S., Somasundaram, P.R., Venkatesan, P. and Prabhakar, R. Isoniazid-induced peripheral neuropathy in the treatment of pulmonary tuberculosis. **Neurology India**, 1990, **38**.

3. Vijayan, V.K., Kuppu Rao, K.V., Sankaran, K., Venkatesan, P. and Prabhakar, R. Tropical eosinophilia: clinical and physiological response to diethylcarbamazine. **Respiratory Medicine**, 1991, **85**, 17-20.

4. Hong Kong Chest Service/Tuberculosis Research Centre, Madras/British Medical Research Council. A controlled clinical comparison of 6 and 8 months of antituberculosis chemotherapy in the treatment of patients with silicotuberculosis in Hong Kong. **American Review of Respiratory Diseases,** 1991, **143**, 262-267.

5. Mayurnath, S., Vallishayee, R.S., Radhamani, M.P. and Prabhakar, R. Prevalence study of tuberculous infection, over fifteen years, in a rural population in Chingleput district (South India). **Indian Journal of Medical Research (A),** 1991, **93**, 74-80.

6. Sulochana Das, Sujatha Narayanan, Paramasivan, C.N., Lowrie, D.B. and Narayanan, P.R. Human tuberculosis sera show prominent antibody responses to particulate fractions of **Mycobacterium tuberculosis. Journal of Clinical Immunology,** 1991, **11,** 74-77.

7. Raghupati Sarma, G. Iron-sequestering mechanisms of mycobacteria. In: **Proceedings of Indo-US Workshop on Major Advances in Tuberculosis Research, 1989, Tuberculosis Research Centre (ICMR), Madras,** 1991, 43-44.

8. Paramasivan, C.N. Phage-typing of mycobacteria. In: **Proceedings of Indo-US Workshop on Major Advances in Tuberculosis Research, 1989, Tuberculosis Research Centre (ICMR), Madras,** 1991, 45-51.

9. Santha Devi, T. Status of case-finding and case-holding. In: **Proceedings of Indo-US Workshop on Major Advances in Tuberculosis Research, 1989, Tuberculosis Research Centre (ICMR), Madras,** 1991, 107-109.

10. Rajajee, S. and Sundareswar. Maternal fetal immunological relationship particularly mycobacterial immunity. **Indian Pediatrics,** 1991, **28**, 363-366.

11. Rani Balasubramanian. Fully intermittent six month regimens for pulmonary tuberculosis in South India. **Indian Journal of Tuberculosis,** 1991, **38**, 51-53.

12. Manjula Datta. Status of smear positive pulmonary tuberculosis patients after chemotherapy under the District Tuberculosis Programme. **Indian Journal of Tuberculosis,** 1991, **38**, 63-68.

13. Niruparani Charles. Influence of initial and repeated motivation on case holding in North Arcot district. **Indian Journal of Tuberculosis,** 1991, **38**, 69-71.

14. Tuberculosis Research Centre, Madras. Controlled clinical trial of fully oral short-course chemotherapy in the treatment of smear positive pulmonary tuberculosis (Summary). **Indian Journal of Tuberculosis,** 1991, **38**, 104.

15. Raghupati Sarma, G. Effect of administration of rifampicin on the adrenocortical function in patients with pulmonary tuberculosis (Summary). **Indian Journal of Tuberculosis,** 1991, **38**, 105.

16. Jayasankar, K. Pharmacokinetics of isoniazid and rifampicin in patients with chronic renal failure (Summary). **Indian Journal of Tuberculosis,** 1991, **38**, 105.

17. Selvakumar, N. Adenosine deaminase (ADA) levels in CSF of tuberculous meningitis (TBM) (Summary). **Indian Journal of Tuberculosis,** 1991, **38**, 106.

18. Brahmajothi, V., Pitchappan, R.M., Kakkanaiah, V.N., Sashidhar, M., Rajaram, K., Ramu, S., Palanimurugan, K., Paramasivan, C.N. and Prabhakar, R. Association of pulmonary tuberculosis and HLA in South India. **Tubercle,** 1991, **72**, 123-132.

19. Pitchappan, R.M., Brahmajothi, V., Rajaram, K., Thirumalaikolundu Subramanyam, P., Balakrishnan, K. and Muthuveeralakshmi, R. Spectrum of immune reactivity to mycobacterial (BCG) antigens in healthy hospital contacts in South India. **Tubercle,** 1991, **72,** 133-139.

20. Vijayan, V.K., Sankaran, K., Venkatesan, P. and Kuppu Rao, K.V. Correlation of lower respiratory tract inflammation with changes in lung function and chest roentgenograms in patients with untreated tropical pulmonary eosinophilia. **Singapore Medical Journal, 1991, 32,** 122-125.

21. Rajeswari Ramachandran, Balambal, R., Ramanathan, V.D. and Prabhakar, R. Cutaneous vasculitis during anti-tuberculosis treatment in tuberculoma brain - a report on 2 cases. **Indian Journal of Tuberculosis,** 1991, **38**, 155-157.

22. Sharma, S., Narayanan, P.S., Sriramachari, S., Vijayan, V.K., Kamath, S.R. and Chandra, H. Objective thoracic CT scan findings in a Bhopal Gas disaster victim. **Respiratory Medicine,** 1991, **85,** 539-541.

23. Selvakumar, N., Vanaja Kumar, Duraipandian, M., Thilothammal, N. and Prabhakar, R. Cerebrospinal fluid adenosine deaminase and lysozyme levels in the diagnosis of tuberculous meningitis. **Indian Journal of Tuberculosis,** 1991, **38,** 217-220.

24. Ramanathan, V.D. The pathophysiology of the complement system in leprosy. **Indian Journal of Leprosy**, 1991, **63**, 418-434.

25. Reetha, A.M., Vijayan, V.K. and Prabhakar, R. Primary tuberculosis of skin (Tuberculous chancre) in an infant of tuberculous mother - a case report. **Lung India**, 1991, **9**, 153-155.

26. Vijayan, V.K., Sankaran, K., Venkatesan, P. and Prabhakar, R. Effect of diethylcarbamazine on the alveolitis of Tropical Eosinophilia. **Respiration,** 1991, **58,** 255-259.

27. Vijayan, V.K. Allergic broncho-pulmonary helminthiasis (ABPH). **Lung India**, 1991, **9**, 145-148.

28. Padma Ramachandran. Tuberculous meningitis in children. **In: Selected Topics in Paediatrics, Ed: A.Parthasarathy, Tamil Nadu State Branch of the Indian Academy of Paediatrics,** Madras, 1991, 185-187.

Papers accepted for publication

1. Selvakumar, N., Vanaja Kumar, Acharyulu, G.S., Fathima Rahman, Paramasivan, C.N. and Prabhakar, R. Susceptibility of South Indian strains of **M.tuberculosis** to Tuberactinomycin. **Indian Journal of Medical Research.**

2. Rajeswari Ramachandran, Ranjani Ramachandran and Reginald, J. Brain tuberculoma presenting as Epilepsia Partialis Continua - Case report. **Neurology India.**

3. 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.**

4. Rajajee, S. and Narayanan, P.R. Immunological spectrum of childhood tuberculosis. **Journal of Tropical Paediatrics.**

5. Rajajee, S. and Alamelu Raja. Immunodiagnosis of tuberculous meningitis. **Journal of Tropical Paediatrics.**

6. Sanjeevi, C.B., Vivekanandan, S. and Narayanan, P.R. Fetal response to maternal ascariasis as evidenced by anti-ascariasis lumbricoides IgM antibodies in the cord blood. **Acta Paediatrica Scandinavica.**

7. 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.**

8. Prema Gurumurthy, Raghupati Sarma, G., Jayasankar, K., Thyagarajan, 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.**

9. 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.**

10. Padma Ramachandran and Prabhakar, R. Defaults, defaulter actions and retrieval of patients in tuberculous meningitis studies in children. **Tubercle and Lung Disease.**

11. Sujatha Narayanan, Sahadevan, R., Ramanujam, S., Prabhakar, R. and Narayanan, P.R. Development of DNA probes for **M.tuberculosis**. **Indian Journal of Tuberculosis**.

12. Vijayan, V.K., Reetha, A.M., Jawahar, M.S., Sankaran, K. and Prabhakar, R. Pulmonary eosinophilia in pulmonary tuberculosis. **Chest**.

13. Vijayan, V.K. Diffusion capacity in tropical eosinophilia. **Chest**.

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

15. Venkataraman, P., Paramasivan, C.N. and Prabhakar, R. **In vitro** activity of capreomycin and ciprofloxacin against South Indian isolates of **M.tuberculosis**. **Indian Journal of Tuberculosis**.

16. Ramanathan, V.D. Current concepts of behavioural immunology. In: **Proceedings of the National Symposium on Behavioural Sciences and XIX Annual Meeting of the Ethological Society of India, American College, Madurai.**

JOURNAL CLUB

Journal club meetings were held each week, at which published scientific articles covering different areas of research were reviewed by staff members of various departments in turn. A synopsis of the paper(s) to be presented and the reference details were circulated in advance, to facilitate better participation by the audience in the discussion that followed the presentation. In all, 33 such meetings were conducted during the year.

Discussion on the current activities of the Statistics Department was held, after presentations by the statisticians.

LECTURE BY VISITING SCIENTIST

Subject	Speaker
DNA finger-printing of mycobacteria	Dr.J.D.A.Van Embden, Molecular Biologist, Bithoven, Netherlands.

DISTINGUISHED VISITORS

1. Dr.Morris T.Jones, Chief, Special Foreign Currency Program, Fogarty International Centre, National Institutes of Health, Bethesda, U.S.A.

2. Dr.G.K.Crompton, Northern General Hospital, Edinburgh, U.K.

3. Dr.D.R.G. Barr, Royal Hospital for Sick Children, Edinburgh, U.K.

4. Dr.N.C.Allan, Western General Hospital, Edinburgh, U.K.

5. Dr.Welsby, City Hospital, Edinburgh, U.K.

6. Prof.A.M.Selvaraj, Consultant Physician, Madras.

7. Prof.M.Eshankhanov, Prof.D.Alimov and Prof.G.Kuzmin - Russian scientists.

8. Dr.E.A.Ottesen, Dr.Chris King and Dr.Siddhartha Mohanty, National Institutes of Health, Bethesda, U.S.A.

9. Dr.D.P.Nag, Deputy Director, Regional Occupational Health Centre, Bangalore.

10. Dr.D.S.Aggarwal, Dean, Maulana Azad Medical College, New Delhi.

11. Dr. S. P. Tripathy, Director-General, ICMR, 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 Chest Physicians, Illinois, USA.
- do -	Editorial Board, **Ceylon Medical Journal**, Colombo, Sri Lanka.
- do -	Project Review Committee, Indo-US Science and Technology Initiative, Department of Science and Technology, Government of India, New Delhi.
- do -	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.
- do -	Scientific Advisory Committee, Regional Medical Research Centre, ICMR, Port Blair, Andamans.
- do -	Planning and Research - Medical Research Committee of the University of Health Sciences, Vijayawada.
- do -	Research Advisory Panel, Schieffelin Leprosy Research and Training Centre, Karigiri.

Staff member	Name of committee
Dr.R.Prabhakar	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 Research 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**, New Delhi.
- do -	Editorial Board, **Indian Journal of Tuberculosis**, New Delhi.
- do -	Research Committee, Drug Addiction Research Centre, Madras.
Dr.P.R.Narayanan	Editorial Board, **Indian Journal of Tuberculosis**, New Delhi
Dr.C.N.Paramasivan	Editorial Board, **Indian Journal of Tuberculosis**, New Delhi
Dr.V.K.Vijayan	Central Crisis Group for Chemical Disasters, Ministry of Environment and Forest, Government of India, New Delhi.

Staff member	Name of committee
Dr.V.K.Vijayan	Fellow, International Academy of Chest Physicians and Surgeons of the American College of Chest Physicians, Illinois, USA.
- do -	Fellow, National College of Chest Physicians (India), New Delhi.
- do -	Fellow, Indian College of Allergy and Applied Immunology, New Delhi.
- do -	Project Advisory Committee on Clinical studies and Broncho-alveolar Lavage studies on MIC-exposed people at Bhopal, Bhopal Gas Disaster Research Centre, ICMR, Bhopal.
- do -	Expert member, Centre for Pulmonary Medicine, Madhya Pradesh Government, Bhopal.
- do -	Treasurer, International Academy of Chest Physicians and Surgeons, South India Chapter.
- do -	Assistant Editor, **Lung India,** Madras.
- do -	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 -	Special invitee, 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. University of Medical Sciences, Madras.

Staff member	Name of committee
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.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.V.K.Vijayan was awarded the "Prof. B.K.Aikat Oration Award" of the ICMR for research in Tropical Diseases for the year 1991.

2. Dr.Manjula Datta was awarded the "Dr.R.Krishna Memorial Award" for the best paper presented at the 45th National Conference on Tuberculosis and Chest Diseases held in Rohtak in January 1991, entitled "Compliance and quiescence in smear positive pulmonary TB under programme conditions".

3. Dr.Prema Gurumurthy was awarded the "R.C.Garg Memorial Award" for the best article published in 1990 in the **Indian Journal of Tuberculosis,** for the paper entitled "Gastro-intestinal absorption of isoniazid and rifampicin in patients with intestinal tuberculosis".

OBITUARY

Dr. C. G. Pandit

We express our heart-felt condolences at the demise of Dr. C. G. Pandit, the Founder-Director of the council, who passed away on the 10th July , 1991. Dr. Pandit, who was the Director from 1948 to 1964, played a key role in the establishment of this Centre on a firm footing in Madras. In his passing away, the nation has lost an eminent medical scientist. It is an irrepairable loss to the Council which he had nurtured and put in the world map of medical research.

We pay our respectful homage to the departed soul and pray for it to rest in eternal peace.

Prof. K.V. Krishnaswamy

We express our deep condolences on the demise of Prof. K.V. Krishnaswamy, former Director, Institute of Tuberculosis and Chest Diseases, Madras, on 17th November 1991. Prof. Krishnaswamy had been undertaking the independent assessment of radiographs for the Centre's controlled clinical trials for over two decades. During his tenure, many collaborative Operational Research studies have been initiated and conducted successfully.

As a member of the Scientific Advisory Committee, he evinced keen interest in the Centre's activities and gave valuable suggestions and guidelines. With his vast experience in the field of tuberculosis, he actively participated in helping the Centre to plan the implementation and monitoring of Short Course Chemotherapy in the National TB Programme in 18 districts spread over India

His passing away is a great loss to the Centre.

Mr. R.S.V.Appa Rao

We record with sorrow the demise of Mr.R.S.V.Appa Rao, former Administrative Officer of the Centre, on 13th December 1991. Mr. Appa Rao, who was the Administrative Officer of the Centre from 1966 to 1976, was closely involved with the expansion of the Centre and in obtaining land on lease from the Government of Tamil Nadu for building a multi-storeyed building to house the Centre's laboratories. He had taken a keen interest in improving the efficiency of the administration, which was of great help to the medical officers and research scientists in conducting clinical trials and undertaking scientific research programmes.

We deeply mourn the passing away of this former senior member of our staff.

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