TUBERCULOSIS RESEARCH CENTRE

CHETPUT MADRAS - 600 031

REPORT ON RESEARCH ACTIVITIES DURING 1985-86

INDIAN COUNCIL OF MEDICAL RESEARCH
NEW DELHI

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PREFACE

The main aim of the research activities of the Centre is to develop regimens of chemotherapy for tuberculosis which are suitable for application under the National Tuberculosis Programme. With this in view, controlled clinical studies, in pulmonary and extra-pulmonary forms of tuberculosis, of short duration of treatment are being continued, using combinations of highly bactericidal drugs.

The monitoring of the pilot study of Short Course Chemotherapy for pulmonary tuberculosis under the District Tuberculosis Programme in 18 districts is continuing. A sociological study is in progress in the district of North Arcot, to investigate the awareness of tuberculosis in the general population and the utilisation of available medical facilities for treatment of the disease. Laboratories have been set up at North Arcot and Pondicherry to carry out culture examination at the district level.

A team from the Centre is assisting the Hamidia Hospital, Bhopal, in the long-term assessment of the effect of exposure to MIC. Detailed pulmonary function tests and broncho-alveolar lavage technique are used for this purpose.

Dr. H.T. Mahler, Director-General, WHO, during his visit to the Centre in November, 1985, acclaimed the research findings of the Centre as 'monumental'. He also appreciated the research work turned out by the Centre in the field of chemotherapy of tuberculosis and its contributions to the National Tuberculosis Programme. He urged the Centre to take up research in operational aspects of the programme and study the socio-psychological problems, in order to improve the functional efficiency of the programme.

The expertise gained in the conduct of controlled clinical trials in tuberculosis has been utilised for investigating the efficacy of new antifilarial drugs which are claimed to be micro and macrofilaricidal. A collaborative study with the compound CGP 20376 is in progress as part of a collaborative programme of the Working Group of Filariasis of the Tropical Diseases Research Unit of the WHO.

Preliminary studies are being carried out to try and develop specific immuno-diagnostic agents that can be used for the diagnosis of pauci-bacillary and extra-pulmonary tuberculosis where bacteriological confirmation is not easy, and for early diagnosis of filariasis to enable application of effective intervention measures. Considering the important role of molecular biology in medicine, a beginning has been made to establish a unit for molecular biology for conducting research in recombinant technology and genetic engineering.

Considerable progress has been made with regard to the installation of a main-frame computer system and it is hoped to commission it by the end of the year. This system will enable very speedy and accurate data processing and analyses.

Prof. K. Jagannath, Director, Institute of Tuberculosis and Chest Diseases, Madras, Prof. K.V. Krishnaswami, former Director, Institute of Tuberculosis and Chest Diseases, Dr. K.V. Thiruvengadam, former Professor of Medicine, Madras Medical College and Dr. S. Radhakrishna, Director, Institute for Research in Medical Statistics (Madras Chapter), continued to act as consultants. In addition, Prof. N.S. Venugopal and Dr. S. Thyagarajan were appointed as consultants in ophthalmology.

The Scientific Advisory Committee met on 26-2-1986 and gave valuable guidelines for future research activities.

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CLINICAL STUDIES

STUDIES COMPLETED

Relapse rates up to 60 months in Madras patients who received a 3- or 5- month regimen

A controlled clinical trial of a 3-month and two 5-month short-course regimens in the treatment of sputum-positive pulmonary tuberculosis had been conducted at the Centre. The 3 regimens investigated were as follows:

1. *R3:* Rifampicin 12mg/kg body-weight plus streptomycin 0.75 g plus isoniazid 400 mg (incorporating pyridoxine 10 mg) plus pyrazinamide 35 mg/kg body-weight, given together daily under supervision for 3 months.

2. *R5:* As in (1) but followed by streptomycin 0.75 g plus isoniazid 15 mg/kg body-weight (incorporating pyridoxine 10 mg) plus pyrazinamide 70mg/kg body-weight, given together twice-weekly under supervision for 2 months (total duration 5 months).

3. *Z5:* As in (2) but without rifampicin.

In all, 538 patients were admitted to the study from October, 1977 to April, 1980. Of these, 69 patients were excluded from the main analyses for various reasons. Of the remaining 469 patients, there were 405 who had initially drug-sensitive organisms and 64 with organisms resistant to streptomycin, isoniazid or both. Considering the patients who had initially drug-sensitive organisms, 6 R3 and 4 Z5 patients have been excluded from the relapse analysis due to (a) unfavourable bacteriological response at the end of chemotherapy (2 R3), (b) chemotherapy continued beyond the prescribed period (2 R3), (c) non-tuberculous death (1 R3 and 4 Z5) and (d) discharge against medical advice (1 R3). Thus there remain 123 R3, 133 R5 and 139 Z5 patients in the relapse analysis. The relapse rates up to 48 months from start have been presented earlier (1984 annual report). All patients have now completed 5 years from the start. The bacteriological relapses requiring treatment in patients with initially drug-sensitive bacilli up to 60 months from the start (i.e. 55-57 months after stopping chemotherapy) are presented in the table below.

Regimen	No. of patients assessed	Bacteriological relapse requiring treatment						
		Total		Year of relapse (after stopping chemotherapy)				
		No.	%	1st	2nd	3rd	4th	5th
R3	123	20	*16*	17	1	0	1	1
R5	133	5	*4*	3	1	0	0	1
Z5	139	24	*17*	14	4	2	3	1

A bacteriological relapse requiring treatment occurred in 16% of 123 R3, 4% of 133 R5 and 17% of 139 Z5 patients. The differences between the R5 and each of the other two regimens are highly significant ($P < 0.001$ for both). Of the total 49 relapses, 34 (69%) occurred within one year after stopping chemotherapy, 6 (12%) in the 2nd year, 2 (4%) in the 3rd year, 4 (8%) in the 4th year and 3 (6%) in the 5th year. The bacilli at the time of relapse were fully drug-sensitive in the majority of patients. They were prescribed streptomycin plus isoniazid plus pyrazinamide (see page 28) for one year.

A 5-month regimen containing daily rifampicin for the first 3 months thus appears to be highly effective, whereas a 3-month regimen, even though containing rifampicin, and a 5-month non-rifampicin regimen had high relapse rates.

(started: 1977; completed:1985).

Collaborative controlled clinical trial in Madurai

In order to extend the benefits of the methodology of controlled clinical trials in pulmonary tuberculosis to the southern districts of the State of Tamil Nadu, the Centre established a unit at the Government Rajaji Hospital, Madurai, which is attached to the Madurai Medical College (Dean: Dr. K.R. Jacob).

With a view to assess the organisational problems, to streamline the various procedures and to assess the domiciliary stability and long term compliance of patients, a pilot study was carried out. Adult sputum-positive patients were treated with a 6-month fully supervised intermittent regimen consisting of streptomycin plus rifampicin plus isoniazid plus pyrazinamide given thrice a week for 2 months, followed by streptomycin plus rifampicin plus isoniazid given twice a week for the next 4 months ($2SRHZ_3/4SRH_2$). The smear and culture examination of sputum specimens were carried out at the Microbiology Department, Madurai Medical College (Prof. S. Ramajayam), under the technical guidance of the Centre; positive cultures were sent periodically to the Centre for drug sensitivity tests.

In all, 200 patients were admitted to the study from December, 1983 to March, 1985. Of these, 72% were males. Twenty-five per cent were aged less than 25 years, 52% were between 25-44 years and 24% were aged 45 years or more. The duration of stay in Madurai was less than 1 year in 10%, 1 to 4 years in 28%, 5 to 9 years in 17% and 10 years or more in 45%. The distance between the patient's home and the clinic was 4 km or less in 65%, 5 to 9 km in 32% and 10 km or more in 4%.

Patients in analyses: Of the 200 patients admitted to the study, 3 had all cultures negative for *M. tuberculosis* initially, 6 had no positive culture (1) or only one (5), 2 patients died of non-tuberculous causes during treatment, and 22 became uncooperative and either did not complete their treatment (11) or received less than 75% of the prescribed doses (11). The remaining 168 patients are considered for the main analyses. Of these, 135 (80%) had bacilli initially sensitive to streptomycin, isoniazid and rifampicin, 10 (6%) were resistant to streptomycin alone, 15 (9%) to isoniazid alone and 8 (5%) to both drugs; none had bacilli initially resistant to rifampicin

Patients with drug sensitive bacilli initially: Of the 135 patients, 112 (83%) received 90% or more of the scheduled chemotherapy, 18 (13%) received 80 89%, and the remaining 5 (4%) 70-79%.

Comparing the culture negativity based on multiple specimens (3 each month), 32% were negative at one month, 84% at 2 months and 93% to 99% at 3 to 6 months. All the 135 patients had a favourable response to treatment. Considering 130 who had completed 6 months of follow up, 3 (2%) relapsed bacteriologically.

Patients with drug resistant bacilli initially: Of the 33 patients, 3 had active disease at the end of treatment, having been persistently sputum positive. All 3 had been resistant to streptomycin and isoniazid initially. Of the other 30, 29 (97%) completed 6 months of follow-up and none had a bacteriological relapse.

The patients are being followed up till 18 months after stopping chemotherapy.

(started: 1983; intake completed: 1985: 18 months follow-up expected to be completed : 1987).

Collaborative clinical trial of tuberculous lymphadenitis in children

The Centre in collaboration with the Institute of Child Health, Egmore and the Paediatric Surgery Department of the Government Stanley Hospital, Madras, has conducted a clinical trial to assess the efficacy of short-course chemotherapy in the treatment of tuberculous lymphadenitis in children. The study was started in February 1980 and the intake was completed in September 1985.

Children under 12 years of age, in whom a clinical diagnosis of tuberculous lymphadenitis was made, were assessed by the paediatric surgeons at the respective referral hospitals, provided they had received not more than one month of previous anti-tuberculous treatment. A lymphnode biopsy was done under general anaesthesia and the specimen was sent for histopathological

and bacteriological examinations. Patients in whom the diagnosis of tuberculosis was confirmed by either or both of these means were eligible for admission to the study.

The patients were treated with a fully intermittent, supervised 6-month regimen consisting of an initial intensive phase of 2 months followed by a continuation phase of 4 months. In the initial 2 months, rifampicin 10mg/kg body-weight, streptomycin 40mg/kg, isoniazid 15mg/kg and pyrazinamide 45mg/kg body-weight were administered thrice a week; in the continuation phase, streptomycin and isoniazid were given twice a week in the same dosages. All the doses were given under the supervision of a clinic nurse at the Centre or at one of the peripheral treatment centres.

The patients were assessed clinically at the Centre and also at the collaborating hospitals at monthly intervals. At the end of the scheduled period of chemotherapy, if lymphnodes of significant size were still palpable, then the lymphnode biopsy was repeated, and the specimen subjected to histopathological and bacteriological examinations.

Results: During the period of intake to the study, a total of 396 patients were registered and 197 of these were admitted to the study. Twenty patients were excluded from analysis; in 7, the diagnosis was not confirmed by biopsy or bacteriology, 3 were diagnosed as lymphoma, 6 had had more than 1 month of previous chemotherapy and 4 did not receive adequate chemotherapy.

Pre-treatment characteristics: Of the 177 patients available for analysis, 88 (50%) were males (see table on page 12); 12 (7%) were aged less than 2 years and 11 (6%) were aged 12 years.

All patients were tested with 1 TU of PPD (RT 23 with Tween 80) on admission to the study; 113 of the 177 patients (64%) had a reaction of 20mm or more and a further 42 patients (24%) had a reaction of 15-19mm. Only 15 patients (8%) had a reaction of less than 10mm. Thus a majority of patients in the study had a very strong tuberculin reaction.

Lymphnode histopathology was done on 175 patients; of these, 4 were negative. Lymphnode culture was done using 4 different media; 99 of the 177 specimens (56%) were culture positive and 60 specimens (34%) were culture negative. Of the 99 culture positive specimens, 33 (33%) were also smear positive and of the 60 culture negative specimens, 10 (17%) were smear positive. In 6 patients, the lymphnode culture yielded non-tuberculous mycobacteria.

Factor	Patients	
	No.	%
Sex:		
Male	88	*50*
Female	89	*50*
Age (years):		
< 2	12	*7*
2-	47	*27*
4-	32	*18*
6-	22	*12*
8-	23	*13*
10-	30	*17*
12	11	*6*
Induration to 1 TU of PPD (mm):		
0-9	15	*8*
10-14	7	*4*
15-19	42	*24*
20 or more	113	*64*
Lymphnode histopathology:		
Positive	171	*97*
Negative	4	*2*
Not tested	2	*1*
Lymphnode bacteriology:		
Culture positive (99)		
—Smear positive	33	*19*
—Smear negative	66	*37*
Culture negative (60)		
—Smear positive	10	*6*
—Smear negative	50	*28*
Culture non-tuberculous mycobacteria (6)		
—Smear positive	4	*2*
—Smear negative	2	*1*
Not tested	12	*7*
Total patients	177	*100*

Response to treatment: Clinical response was judged on the basis of regression or disappearance of the lymphnode, healing of sinuses, subsidence of constitutional symptoms and gain in weight. If residual lymphnodes were palpable, and the surgeon felt it was warranted, a repeat lymphnode biopsy was done at 6 months and the specimen was subjected to histopathological and bacteriological examinations.

Response to chemotherapy at the end of 6 months was assessed on the basis of clinical improvement and the results of post-treatment repeat lymphnode biopsy, if it was considered necessary. All the 177 patients showed good clinical improvement.

Retreatment: Retreatment was considered necessary under the following circumstances:

(1) Positive culture of *M. tuberculosis* from the repeat lymphnode specimen at the end of chemotherapy.

(2) Relapse of lymphadenitis, proved by biopsy.

(3) Development of extra-lymphnode tuberculosis during follow-up.

Retreatment consisted of chemotherapy for one year with streptomycin, rifampicin, isoniazid and pyrazinamide thrice-weekly for 2 months followed by rifampicin and isoniazid twice-weekly for 10 months.

The histopathology and bacteriology results of the repeat lymphnode biopsy specimen are given in the table below:

Histopathology	Culture			Total
	Positive	Negative	NTM	
Positive	1	6	0	7
Negative	1	46	1	48
Total	2	52*	1	55

*Including 7 patients with a possitive smear.

Two of the lymphnode specimens yielded *M. tuberculosis* on culture, including one which was also positive by histopathology. Both these patients were retreated. Of the 55 patients for whom histopathology results are available, in 7 patients it was suggestive of tuberculosis. Of these, apart from the patient whose lymphnode specimen also yielded *M.tuberculosis* on culture, another patient had a clinical relapse in the form of cold abscess at the 8th month and was also retreated. The other 5 patients have been closely followed up

and have shown no clinical signs of reactivation of the disease. One other patient developed abdominal tuberculosis and was retreated in the 12th month.

Thus the findings at the end of chemotherapy and after a limited period of follow-up are good. However, the final assessment of the efficacy of the regimen must be based on long-term follow-up results. The patients are being followed up till 60 months from admission; the findings will be reported in due course.

(started: 1980; expected year of completion: 1990).

Treatment of tuberculosis of the spine without paraplegia—a 3-year report

The aim of this study is to assess the efficacy of short-course chemotherapy, when given alone or when combined with radical surgery, in the treatment of tuberculosis of the spine without paraplegia.

This is a collaborative investigation in which 6 orthopaedic surgeons of Madras (Prof. T.K. Shanmugasundaram, Principal Investigator, Dr. S. Soundarapandian, Dr. S.T. Sundar Raj, Dr. S. Basheer Ahmed, Dr. P.V.A. Mohan Das and Dr. S. Rajagopal), the Indian Council of Medical Research (Tuberculosis Research Centre, Madras) and the British Medical Research Council (Tuberculosis and Chest Diseases Unit) participate.

Patients with active spinal tuberculosis involving the bodies of the first thoracic to the first sacral vertebrae and without paraplegia were allocated at random to one of the following 3 regimens.

(a) *Rad 6HR:* Chemotherapy with isoniazid (6mg/kg) plus rifampicin (10-15mg/kg) daily for 6 months plus radical surgery. The radical surgery consisted of excision of diseased vertebrae and bridging of the resultant gap with autologous bone graft.

(b) *Amb 6HR:* As in (a) but without surgery.

(c) *Amb 9HR:* As in (b) but with chemotherapy for 9 months.

In all, 304 patients were admitted to the study (100 Rad 6, 101 Amb 6, 103 Amb 9) between May 1975 and December 1978. After 44 exclusions, there remain 260 (85 Rad 6, 83 Amb 6, 92 Amb 9) patients for analysis. All the patients have completed the treatment and have been followed up for 7-10 years. The 3-year findings are presented in this report.

Condition on admission: Of the 260 patients, 33% were aged less than 15 years and 25% were aged 35 years or more. All except one patient had involvement of 2 or more vertebrae, 96% had radiographically active or probably active disease, 95% had limitation of spinal movement, 84% had

kyphosis, 20% had a clinically evident abscess and/or sinus, and 57% had radiographic evidence of mediastinal or psoas abscess.

Management: All patients allocated to the Rad 6 regimen were hospitalised and underwent the radical operation, usually within one month after admission to the study. In the ambulatory series also, the majority of patients were hospitalised, namely 60% in Amb 6 and 52% in Amb 9. The reason for this was that almost all these patients had no home in Madras and would not have been able to attend the clinic as out-patients for their chemotherapy. For all in-patients and for out-patients aged less than 5 years, every dose of drugs was administered under the direct supervision of a staff member. Out-patients aged 5 years or more attended the clinic twice-weekly; at each attendance, that day's dose was administered under direct supervision and 2-3 days' drugs were supplied for self administration at home. The regularity of attendance for drugs was high (mean in Rad 6: 96.2%; Amb 6: 95.8%, Amb 9: 96.3%) and similar in the 3 series.

Follow-up: The progress of the patients was assessed monthly until the end of chemotherapy and then every 3 months; this was based on a clinical (including detailed neurological) examination, A.P. and lateral radiographs of the vertebrae involved and bacteriological examination of pus from any sinus or abscess. For patients suspected to have pulmonary tuberculosis, a chest x-ray and sputum examination by smear and culture was also done.

The coverage for these examinations, including the follow-up examinations after completion of treatment, was very high (see table below). Motivation of the patients by the physicians, nursing and health visiting staff and social workers, and the detailed procedures for defaulter retrieval probably contributed to a large extent to this high coverage.

Month of examination	Amb 6/9			Rad 6		
	Due	Examined		Due	Examined	
		No.	%		No.	%
6/9	202	201	*99.5*	88	87	*98.9*
12	201	197	*98.0*	88	87	*98.9*
24	199	198	*99.5*	88	88	*100.0*
36	197	194	*98.5*	86	85	*98.8*

Status at 36 months: The status at 36 months for the 260 patients is given on page 16. The status was classified as favourable, still not favourable or unfavourable.

Status at 3 years	Rad 6		Amb 6		Amb 9	
	No.	%	No.	%	No.	%
Favourable	68	*80*	72	*87*	88	*96*
Still not favourable	8	*9*	6	*7*	3	*3*
Unfavourable	9	*11*	5	*6*	1	*1*
Patients assessed	85	*100*	83	*100*	92	*100*

The status was considered to be favourable if the patient had full physical activity, had radiological quiescence of the disease, had no central nervous system involvement and had no sinus or clinical abscess at the end of treatment with the allocated regimen. At 3 years, 80% of 85 Rad 6, 87% of 83 Amb 6, and 96% of 92 Amb 9 patients had a favourable status. The contrast between the Rad 6 and the Amb 9 series was significant (P=0.003) while that between the Amb 9 and Amb 6 series was not significant (P=0.07).

The status was classified as still not favourable if there was no radiological quiescence of the spinal lesion. The proportion of patients classified as still not favourable was 9% in the Rad 6 series, 7% in the Amb 6 series and 3% in the Amb 9 series.

The status was classified as unfavourable if there was death due to or associated with spinal tuberculosis, or if the patient had a persistent sinus or clinical abscess, or if there was involvement of the central nervous system, or if the patient needed additional surgery and/or chemotherapy or if the radical operation was abandoned due to technical difficulties. The proportion of patients with an unfavourable status was 11% in the Rad 6 series, 6% in the Amb 6 series and 1% in the Amb 9 series.

Conclusion: Ambulatory chemotherapy for 9 or even 6 months with daily isoniazid and rifampicin is highly satisfactory in the treatment of spinal tuberculosis without paraplegia. The result is not enhanced by additional surgery. This is an important breakthrough in the management of spinal tuberculosis, which is still being treated in many parts of the world with standard chemotherapy of 12 to 18 months' duration plus prolonged immobilisation in a hospital.

It is planned to follow-up the patients for 10 years or more.

(started: 1975; intake completed: 1978; expected year of completion of 10-year follow-up: 1988).

A double-blind controlled clinical trial to assess the role of anti-histamines in the treatment of multi-bacillary leprosy

It had been reported that anti-histamines, particularly pheniramine maleate, produce marked improvement in patients treated for leprosy. Hence the Centre conducted a double-blind controlled clinical trial to assess the effect of a supplement of pheniramine maleate on the therapeutic efficacy of a regimen of clofazimine and dapsone in the treatment of multi-bacillary leprosy. The study was conducted in the leprosy unit at the Government Royapettah Hospital, Madras (GRH) and in villages covering an area of 400 sq.km. near Tambaram in collaboration with the Central Leprosy Teaching and Research Institute (CLTRI), Thirumani, Chengalpattu (Director: Dr. R. G. Roy).

A patient was eligible for the study if he/she was aged 15 years or more, had disease classified clinically as lepromatous or near lepromatous and the mean bacterial index (BI) was at least 2.5 on Ridley's scale. Patients with evidence of renal, cardiovascular or hepatic damage, diabetes mellitus, active tuberculosis or in reactional state were not eligible.

All patients were prescribed clofazimine (C) 100mg and dapsone (D) 100mg daily for 12 months. For the first 3 months, the above regimen was supplemented by pheniramine maleate 50mg daily in half of the patients, selected at random; the other half received a placebo that was indistinguishable from pheniramine maleate in all respects.

The patients treated at the Royapettah Hospital attended the clinic daily for the first 3 months for supervised administration of drugs, and twice a week thereafter till 12 months for the collection of drugs for self-administration. For patients in the villages, a paramedical worker visited the home of each patient daily in the first 3 months to administer the drugs under supervision, and weekly during the following 9 months to supply drugs for daily self-administration. For those patients, all the clinical and bacteriological investigations including skin biopsy and clinical photography were undertaken either at the patient's home or at a central place in the village.

Assessments: Before the start of treatment, the patients underwent a general clinical examination, a clinical examination for leprosy, examination of skin smears from 6 (or more) sites for lepra bacilli with assessment of bacterial index and morphological index, and lepromin skin test. A skin biopsy was also obtained and examined histopathologically. Mouse foot-pad inoculations were done to assess the bactericidal activity of the regimens. Further, a clinical examination for leprosy was undertaken on admission and periodically thereafter by an independent assessor who was unaware of the treatment and the bacteriological and histopathological findings of individual patients. Colour slides of the lesions were also provided to the assessor.

Patients in analysis: In all, 120 patients were admitted to the trial. Of these, 17 patients were excluded; 2 patients died and 15 patients became uncooperative and discharged themselves against medical advice. Analyses have been undertaken on the remaining 103 patients (52 antihistamine (AHCD), 51 non-anti-histamine (CD)).

All but 14 of the 103 patients were males; 38 patients were under 30 years of age; 29 were aged 30-39 years, and the remaining 36 were aged 40 years or more; the mean age was 34 years (range 15-65 years) and the mean weight 44.5 kg (range 29.2—83.3 kg). Of the 103 patients, 40 had had less than 1 year of previous chemotherapy for leprosy, 29 had had 1-5 years of chemotherapy and 34 had had over 5 years of chemotherapy. The distributions in the 2 groups were broadly similar.

In all, 88 patients had histopathology findings; 62 (70%) were classified as lepromatous, 13 (15%) as borderline lepromatous and 13 (15%) as indeterminate.

Drug regularity: The regularity of drug intake is presented in the following table.

		% of treatment received						
Months	Regimen	100	95–99	90–94	80–89	70–79	69 or less	Total
0-3	AHCD	27	18	4	2	1	0	52
	CD	23	18	2	6	1	1	51
0-12	AHCD	13	27	6	4	2	0	52
	CD	12	29	4	2	4	0	51

A total of 50 patients (49%) in the two regimens did not miss a single dose in the first 3 months, 36 (35%) received between 95 and 99%, 16 (16%) received between 70 and 94%, and only one patient received less than 70% of the scheduled chemotherapy (67%). A total of 97 (94%) in the two regimens received 80% or more of their scheduled chemotherapy during the 12 months.

Clinical progress: The top table on page 19 presents the independent assessor's classification of clinical progress.

Progress	Months					
	0–3		0–6		0–12	
	AHCD	CD	AHCD	CD	AHCD	CD
Improvement :						
Marked	7	8	8	12	25	22
Moderate	29	25	34	33	23	21
Slight	10	10	4	2	3	5
No change	6	7	6	4	1	2
Deterioration	0	1	0	0	0	0
Patients assessed	52	51	52	51	52	50*

*1 patient could not be assessed due to ENL reactions at the time of assessment.

At 3 months, moderate or marked clinical improvement was reported in 36 (69%) in the anti-histamine and 33 (65%) in the non-anti-histamine regimen. At 6 months, the proportions were 81% and 88% rspectively, and at 12 months, 92% and 86% respectively. Thus, the clinical improvement was similar in the 2 regimens.

Bacterial indices: The mean bacterial indices (BI) of the 2 regimens at 0, 3, 6 and 12 months are shown in the following table.

Regimen	Months			
	0	3	6	12
AHCD (N=52)	4.1	3.9	3.8	3.4
CD (N=51)	4.2	4.0	3.8	3.3

In both the groups the mean BI was high and similar at the time of admission, (viz), 4.1 for AHCD and 4.2 for CD patients. There was a steady fall in the BI, the fall being 0.2 at 3 months, 0.3 at 6 months and 0.7 at 12 months in the anti-histamine patients. The corresponding figures for the

non-anti-histamine regimen were 0.2, 0.4 and 0.9. Thus the reduction was similar in the 2 regimens.

Morphological indices: The mean morphological indices (MI) of the 2 regimens at 0 and 12 months are shown in the table below.

MI	0m				12m			
	AHCD		CD		AHCD		CD	
	No.	%	No.	%	No.	%	No.	%
Neg	8	*30*	8	*30*	24	*89*	23	*85*
<0.5	9		13		3	*11*	4	*15*
0.5—	7		2		0	*0*	0	*0*
1.0—	1	19 *70*	3	19 *70*	0	*0*	0	*0*
1.5—	1		0		0	*0*	0	*0*
2.0—2.4	1		1		0	*0*	0	*0*
Total pts.	27	*100*	27	*100*	27	*100*	27	*100*

Of 54 patients for whom MI was done, 19 (70%) of 27 AHCD patients and 19 (70%) of 27 CD patients had positive values on admission; 3(11%) patients in AHCD and 4 (15%) patients in CD had MI of $\geqslant$ 1.0. At 12 months 3 (11%) of AHCD and 4 (15%) of CD patients were positive and all of them had an MI less than 0.5.

Reactions: Of the 103 patients, 42 had reactions and 1 had neuritis during the 12 month period. The table below presents the severity of the reactions.

Regimen	Reactions			
	Mild	Moderate	Severe	Total
AHCD	10	5	4	19
CD	12	5	6	23

The incidence of reactions was similar in the 2 regimens. The reactions were controlled with chloroquine, anti-histamines, analgesics, prednisolone and/or thalidomide.

In summary, the findings at 12 months showed that the clinical and bacteriological improvement was similar in the 2 regimens. Thus, the addition of an anti-histamine supplement for the first 3 months did not result in any additional benefit either clinically or bacteriologically.

(started: 1983; completed: 1986).

A pilot study to assess the variation between two medical officers in the diagnosis of pauci-bacillary leprosy

The clinical diagnosis of pauci-bacillary leprosy is very difficult and likely to be subjective. Hence a pilot study was undertaken to assess the extent of variation between 2 experienced medical officers in the clinical classification of pauci-bacillary leprosy.

A total of 21 patients were independently examined by each of 2 medical officers (assessors), mostly on the same day, otherwise within 7 days. A complete clinical assessment and charting of lesions on a body out-line chart were undertaken. The table below gives the classifications by the 2 assessors.

		Assessor I			
		TT	BT	I	Total
Assessor II	TT	7	1	1	9
	BT	3	8	0	11
	I	1	0	0	1
Total		11	9	1	21

Of 11 patients classified as tuberculoid type (TT) by Assessor I, 7 were also classified as TT by Assessor II, while 3 were diagnosed as borderline tuberculoid (BT) and 1 as indeterminate (I) by Assessor II; 9 patients were diagnosed as BT by Assessor I, of whom 8 were diagnosed as BT by Assessor II also. In all, there was disagreement between the 2 medical officers in 6 of the 21 cases. The Kappa value, which is a measure to assess the degree of agreement after allowing for chance, is 0.58.

Thirteen of the 21 patients were admitted to treatment and 8 referred back. Treatment consisted of daily DDS and one dose of rifampicin every month, for 6 months. The dosages employed for DDS were 25mg for patients weighing 19kg or less; 50mg for 20-29 kg; 75mg for 30-44 kg and 100mg for 45kg or more. The dose of rifampicin was 450mg for those aged 12 years or less and 600mg for the others.

The patients are clinically examined every month and the lesions marked on a body out-line chart. Skin smears are examined at 3 and 6 months and a lepromin test done at 6 months. At 3 and 6 months, estimations of haemoglobin, total and differential counts are also undertaken.

So far, 8 patients have completed the prescribed course of treatment.

(started: 1985; expected year of completion: 1987).

Treatment of Pott's paraplegia

A study of the treatment of Pott's paraplegia was started in collaboration with the orthopaedic surgeons of the Government General Hospital (Prof. T. K. Shanmugasundaram and Prof. S. Soundarapandian—see annual report, 1984). The aim of the study was to assess the efficacy of a multi-drug 9-month regimen with or without surgery in the treatment of Pott's paraplegia. Patients with clinical or radiological evidence of active tuberculosis of vertebral bodies below D3 and paraplegia of recent onset were eligible for admission to this study.

Ten patients were treated with a 9-month regimen (2RSHZEmb daily/7RHtw), combined with radical surgery within a week of starting treatment (pilot study). The chemotherapy consisted of streptomycin 0.75g plus isoniazid 300mg plus rifampicin 12mg/kg body-weight plus pyrazinamide 35mg/kg plus ethambutol 25mg/kg daily for 2 months, followed by rifampicin 12mg/kg plus isoniazid 15mg/kg twice-weekly for the next 7 months.

The main study was started in March 1983 and 25 patients were admitted up to April 1986, and the patients were treated with one of the following three regimens:

1. *CHEM Series:* Streptomycin 0.75g plus isoniazid 300mg plus ethambutol 25mg/kg daily plus rifampicin 12mg/kg twice-weekly for 2 months, followed by rifampicin 12mg/kg plus isoniazid 15 mg/kg twice-weekly for the next 7 months (2RtwSHEmb daily/7RHtw), or

2. *RAD Series:* As in (1) but with radical excision of the diseased vertebrae, or

3. *CT Series:* As in (1) but with costotransversectomy

Combining the pilot study and the main study, 35 patients (17 RAD, 4 CT, 14 CHEM) were considered for the study. Of the total of 35 patients, 2 (1 RAD, 1 CHEM) were excluded (1 (CHEM) died early in treatment; 1 (RAD) had no bony lesion on admission). There remained 33 patients (16 RAD, 4 CT, 13 CHEM) for the analysis.

Condition on admission: Of the 33 patients, 15 were males; 6 patients were aged 14 years or less and 16 were aged 35 years or more. The bladder was involved in 11 of the 33 (33%). Sixteen (48%) patients had a chest X-ray lesion suggestive of pulmonary tuberculosis but none had positive sputum by culture.

Investigations: Routine investigations included:

1. X-ray: Chest and whole spine (AP & lateral) on admission and X-rays of diseased area at monthly intervals till 9 months, at 12 months and 6 monthly intervals thereafter.

2. Bacteriological examination for *M.tuberculosis:* Examination of operation specimens, abscess/sinus pus, sputum and urine by smear and culture for tubercle bacilli and drug sensitivity tests on positive cultures.

3. Blood: Investigations on blood consisted of liver function tests, blood urea and uric acid estimation and haemogram on admission, at 2 months and at 9 months.

4. Urine: Sugar, albumin and deposits on admission.

Neurological examinations: Neurological assessment was complete and intensive. *All* patients were assessed at start, then daily for 3 days, and thereafter, on alternate days till 2 weeks, weekly till 3 months, monthly till 9 months, 3-monthly till 3 years and 6-monthly thereafter. The surgery group patients were assessed, in addition, before the operation and at 8 hours, 24 hours, and on the 2nd, 3rd, 5th and 7th days after the operation.

Management: Initially, all patients were hospitalised in the Govt. General Hospital, Madras and the drugs were given under the direct supervision of a staff member. After discharge from the hospital, the patients collected the drugs once in 15 days as ambulant out-patients. The mean time taken to become ambulant i.e. able to walk unaided was 128 days in the CHEM series (range 25-367) and 85 days in the surgery series (range 9-122). The drug

regularity has been very high. The intensity of follow-up at the various time points has been very high, ranging from 90-100%.

Status	Patients	
	No.	%
Paraplegia resolved	28	*90*
Unfavourable response*	3	*10*
Patients with assessable response	31	*100*
Non-tuberculous death	2	—
Total patients	33	—

*Rx extended beyond 9 months — 1
Death associated with spinal TB — 2

The table above gives the status at the end of 9 months of chemotherapy. Of the 31 with an assessable response, 28 had resolution of paraplegia. One patient had to have chemotherapy beyond 9 months, 1 died 6 days after surgery because of post-operative complication and 1 other patient died within a week after surgery without any improvement in the paraplegia. Thus, in all, there were 3 with an unfavourable response.

Two patients died of non-tuberculous causes, one after 6 weeks due to myocardial infarction (who had paraplegia with some improvement), and the other after 7 weeks due to aspiration pneumonia leading to respiratory failure.

Speed of resolution: The speed of resolution with respect to motor power, spasticity and bladder involvement, amalgamating the findings in the 3 regimens, is presented in the table below. Considering motor power, 18 of 29 (62%) resolved by 3 months, 26 (90%) by 6 months and 28 (97%) by 9 months. Regarding spasticity, 14 (48%) resolved by 6 months, 17 (59%) by 9 months and 21 (72%) by 12 months. Bladder recovery was noted in 6 of 9 by 3 months, and in all by 6 months.

	No. of pts.	Resolved by months			
		3	6	9	12
Motor power	29	18	26	28	28
Spasticity	29	3	14	17	21
Bladder	9	6	9	9	9

Adverse reaction: Jaundice was the only important adverse reaction encountered, and it occurred in 4 of the 33 patients, 2 each in RAD and CT series. The onset was 7th day, 9th day, 4th week and 8th week after surgery. Rifampicin was terminated and ethambutol or streptomycin was substituted, and the jaundice cleared subsequently.

Conclusion: Of 29 patients alive at the end of treatment, all had paraplegia resolved completely with or without surgery. Thirteen patients had completed 24 months of follow up. One died 11 months after Rx due to myocardial infarction. The remaining 12 patients are doing well. It appears that short-course chemotherapy of 9 months' duration is highly effective in the treatment of Pott's paraplegia.

(started: 1982; completed: 1986).

STUDIES IN PROGRESS

Controlled clinical trial of 6-month intermittent regimens in the treatment of sputum-positive pulmonary tuberculosis

Two studies at this Centre showed that short-course regimens containing daily rifampicin, streptomycin, isoniazid and pyrazinamide for 2 or 3 months had a high bactericidal activity in patients with initially drug sensitive cultures, approximately 92% of patients becoming culture-negative by 2 months and 96% by 3 months. A study in Hong Kong suggested that the bactericidal activity would be high even if these drugs are given thrice or twice a week. Because of the considerable reduction in the bacterial population by 2 months, less intensive continuation therapy is likely to be adequate. Based on these considerations, the Centre is currently carrying out a randomised controlled study of 6-month intermittent regimens which are fully supervised. Intermittent regimens are less expensive than daily regimens, and are likely to be less toxic.

The regimens under study are as follows:—

1. 2RSHZthr/4RHtw—Rifampicin 15 mg/kg body-weight plus streptomycin 0.75 g plus isoniazid 15 mg/kg plus pyrazinamide 50 mg/kg administered thrice a week for the first 2 months, followed by rifampicin 15 mg/kg plus isoniazid 15 mg/kg twice a week for the next 4 months.

2. 2RSHZthr/4RHow—same as regimen 1 except that in the continuation phase, rifampicin and isoniazid in the same dosages are given once a week.

3. 2RSHZthr/4SHtw—same as regimen 1 except that in the continuation phase, rifampicin is replaced by streptomycin 0.75 g.

4. 2RSHZtw/4RHtw
5. 2RSHZtw/4RHow
6. 2RSHZtw/4SHtw

Correspond to regimens 1, 2 and 3 respectively except that RSHZ is administered twice a week during the first 2 months (instead of thrice a week) and the dosage of pyrazinamide is 70 mg/kg (instead of 50 mg/kg.)

Further, half the patients in regimens 1, 2, 4 and 5, selected at random, receive streptomycin 0.75 g with each dose of rifampicin plus isoniazid in the continuation phase.

Pyridoxine 10mg is incorporated in every dose of isoniazid throughout the 6-month period of chemotherapy. For rifampicin, isoniazid and pyrazinamide, a dosage schedule is used, the body-weight categories being less than 30.0 kg, 30.0 – 44.9 kg and 45.0 kg or more. All the anti-tuberculosis drugs are administered in the clinic under the close supervision of a clinic nurse.

The above mentioned regimens are being investigated concurrently at Madras (Tuberculosis Research Centre), Bangalore in collaboration with the National Tuberculosis Institute (Director: Dr. G.V.J. Baily) and the Lady Willingdon State TB Centre (Director: Dr. (Mrs.) Iqbal Begum) and in a semi-urban area around Tambaram, in collaboration with the Government TB Sanatorium, Tambaram (Superintendent: Dr. K. Jagannath).

The study was started in May, 1980. The intake to the study was over by January, 1983 at Bangalore and by April, 1985 at Madras and Tambaram. The study at Bangalore was terminated in July, 1983 after a follow-up of 18 months while it is expected to last till April, 1990 at Madras and Tambaram, that is, for a follow-up period of 54 months.

The findings up to 12 months from the start had been presented earlier (1984 annual report). The findings in 1595 patients up to 24 months from the start are presented in this report.

Of 1595 patients, 145 have been excluded for various reasons. Thus, there remained 1450 patients for analyses of efficacy; 1198 had bacilli initially sensitive to streptomycin and isoniazid, and 252 had bacilli initially resistant to streptomycin or isoniazid or both.

Of the 1198 patients with initially sensitive bacilli, 5 (0.4%) had an unfavourable response during chemotherapy. One died of spontaneous pneumothorax, 2 had a change of chemotherapy (1 for spontaneous pneumothorax, and the other for miliary tuberculosis), and 2 had positive sputum cultures at 5 and 6 months.

Thus, 1193 patients with initially sensitive bacilli had a favourable response at the end of chemotherapy. Of these, 1181 were assessed for relapse.

The bacteriological relapses requiring treatment up to 24 months from start (i.e. 18 months of follow-up) are presented in the table below.

Initial Rx. (2 months)	Continuation Rx. (4 months)	No. assessed	Relapses requiring treatment							
			Total		Month of relapse after stopping drugs					
			No.	%	1–3	4–6	7–9	10–12	13–15	16–18
RSHZ thrice-weekly	SRHow	111	5	*5*	1	2	0	0	0	2
	RHow	116	2	*2*	1	0	1	0	0	0
	SRHtw	111	2	*2*	1	1	0	0	0	0
	RHtw	101	3	*3*	0	2	0	0	0	1
	SHtw	151	5	*3*	3	0	1	0	1	0
	Any	590	17	*3*	6	5	2	0	1	3
RSHZ twice-weekly	SRHow	117	5	*4*	1	3	1	0	0	0
	RHow	109	8	*7*	6	2	0	0	0	0
	SRHtw	108	3	*3*	1	0	0	1	1	0
	RHtw	102	6	*6*	5	1	0	0	0	0
	SHtw	155	15	*10*	7	5	0	1	1	1
	Any	591	37	*6*	20	11	1	2	2	1

Seventeen (3%) of 590 patients belonging to the thrice-weekly series relapsed; the rates were similar and low (range 2—5%) for the various continuation regimens. In contrast, 37 (6%) of 591 patients belonging to the twice-weekly series relapsed during the same period (P=0.008). Considering the different continuation regimens in the twice-weekly series, the relapse rates were 8 (4%) of 225 patients who received streptomycin, isoniazid and rifampicin once or twice a week, 14 (7%) of 211 patients who received isoniazid and rifampicin once or twice a week and 15 (10%) of 155 patients who received streptomycin and isoniazid twice a week. The difference in relapse rates between those who received streptomycin, isoniazid and rifampicin in the continuation regimen and those who received only streptomycin and isoniazid was highly significant (P=0.02). None of the other differences were statistically significant. As seen in the table, a total of 42 (78%) of 54 relapses (11 RSHZthr and

31 RSHZtw) occurred in the first six months of follow-up, the vast majority with drug sensitive organisms.

There were 252 patients with initially drug resistant bacilli. Of 62 patients with resistance to streptomycin alone, one (2%) had an unfavourable response and 5 relapsed. Of 82 patients with resistance to isoniazid alone, 13 (16%) had an unfavourable response and 5 relapsed. Of 108 patients with resistance to both drugs, 22 (28%) of 76 who received rifampicin in the continuation phase and 23 (72%) of 32 who did not receive rifampicin had an unfavourable response ($P=0.0001$).

In summary, a 6-month intermittent regimen with 4 drugs given thrice-weekly in the first 2 months was very effective, in contrast to a 6 month regimen containing the same 4 drugs given only twice-weekly in the first 2 months. The proportion of relapses also seems to be related to the number of drugs in the continuation phase of 4 months, and to the fact whether rifampicin was given or not beyond the first 2 months.

(started: 1980; intake completed: 1985; expected year of completion of 5-year follow-up: 1990).

Retreatment of patients who relapse with drug-sensitive organisms following short-course chemotherapy

Short-course chemotherapeutic regimens are highly effective, virtually all patients with organisms sensitive to streptomycin and isoniazid on admission responding favourably. However, a small proportion of patients relapse after completion of the course of chemotherapy; of these, the great majority relapse with drug-sensitive organisms. In order to assess the feasibility of treating these patients successfully with the primary drugs, patients who relapse after any short-course regimen at the Centre are being retreated with a regimen of streptomycin 0.75 g plus isoniazid 400 mg plus pyrazinamide 30mg/kg body-weight daily for 1 month, followed by streptomycin 0.75 g plus isoniazid 15mg/kg plus pyrazinamide 70 mg/kg twice a week for 11 months, all the drugs being given under supervision in the clinic.

Of the 117 eligible patients admitted so far, 113 have completed their scheduled chemotherapy, of whom 24 have been excluded from analyses—22 for non-cooperation and 2 for change of treatment due to toxicity.

Of the remaining 89 patients, 78 (88%) had bacteriologically quiescent disease at the end of one year of treatment; 5 patients were classified as having disease of doubtful status, i.e., sputum conversion followed by 1 positive culture in the last 3 months of treatment. However, none of the 5 had positive cultures beyond 12 months though the treatment had been stopped at one year. The remaining 6 patients (7%) had active disease, of whom 2 had their treatment changed at 10 months, 3 after 12 months and the other was discharged for non-cooperation. Of the 22 patients excluded for non-cooperation, 11 patients had sputum examination beyond 6 months, of whom 6 had positive cultures; 8 had culture examination up to 6 months of whom 3 had positive

cultures; 2 had culture examination up to 5 months of whom 1 had positive cultures and the remaining patient died of tuberculosis at 9 months receiving only 2 months of his scheduled chemotherapy and absenting for the other 7 months.

Of the 22 patients excluded for non-cooperation, 1 patient did not turn up after 3 months, 5 patients after 6 months, 9 patients after 7 months, 2 patients after 8 months and 1 patient after 9 months. Of the remaining 4 patients, 2 patients had their chemotherapy changed at 5 and 8 months as they could not attend for twice-weekly treatment; the other 2 patients were very irregular for their chemotherapy, missing more than half of their scheduled doses.

(started: 1975; expected year of completion: 1987).

Short-course chemotherapy under District Tuberculosis Programme

The efficacy of several short-course chemotherapeutic (SCC) regimens has been well established in controlled clinical trials. To study their applicability and efficiency under programme conditions, three short-course regimens of 6 or 8 months' duration were introduced in the District Tuberculosis Programme (DTP) of 8 districts during 1983 and extended to 10 other districts in 1984. The main aim of this pilot scheme is to provide information on the applicability of SCC regimens in both rural and urban parts of the country. The scheme is part of the routine DTP in these districts and is run by the local staff.

The three regimens are:

1. *$2RHZ_2/4RH_2$:* Rifampicin 600mg plus isoniazid 600mg plus pyrazinamide 2.0g twice-weekly for 2 months, followed by rifampicin 600mg plus isoniazid 600mg twice-weekly for 4 months; all the doses are to be administered in the clinic under supervision.
2. *2RHZ/6TH :* Rifampicin 450mg plus isoniazid 300 mg plus pyrazinamide 1.5g daily for 2 months, followed by thioacetazone 150mg plus isoniazid 300 mg daily for 6 months, the drugs being collected by the patients once in 15 days for self-administration.
3. *$2RHZ/4RH_2$:* Rifampicin 450mg plus isoniazid 300mg plus pyrazinamide 1.5g daily for 2 months, followed by rifampicin 600mg plus isoniazid 600mg twice-weekly for 4 months; in the first two months the drugs are to be collected once in 15 days for self-administration, and in the next 4 months, all the doses are to be administered in the clinic under supervision.

Three policies of treatment are being 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.

All patients aged 15 years or more who have smear-positive pulmonary tuberculosis are to be admitted to the scheme, provided they have not had previous specific anti-tuberculous treatment for more than 2 months.

The following table gives details regarding sputum examination and admission to the scheme, up to March '86.

Sl. No.	Policy	District	State	Total new smears examined	Sputum positive		Eligible for SCC	Put on SCC	
					No.	%		No.	%
1.	A	North Arcot	Tamil Nadu	100004	6372	*6*	6372	3606	*57*
2.	A	Puri	Orissa	24041	965	*4*	855	721	*84*
3.	A	Baroda	Gujarat	27779	4620	*17*	4244	2000	*47*
4.	A	Thane	Maha-rashtra	18243	1777	*10*	1177	804	*68*
5.	A	Ujjain	M.P.	12074	932	*8*	914	417	*46*
6.	A	Dehra Dun	U.P.	6119	585	*10*	486	210	*43*
7.	B	Karnal	Haryana	17697	1424	*8*	1421	445	*31*
8.	B	Kanpur	U.P.	20284	2632	*13*	1663	566	*34*
9.	B	Nagpur	Maha-rashtra	41949	3385	*8*	3385	838	*25*
10.	B	Rajkot	Gujarat	8061	858	*11*	553	413	*75*
11.	B	Raichur	Karnataka	16204	726	*4*	586	160	*27*
12.	B	Sagar	M.P.	4473	577	*13*	316	140	*44*
13.	C	Pondicherry	U.Terri-tory	33298	1504	*5*	1492	573	*38*
14.	C	Vidisha	M.P.	6602	650	*10*	587	453	*77*
15.	C	Aurangabad	Maha-rashtra	8783	1284	*15*	1284	501	*39*
16.	C	Varanasi	U.P.	9491	452	*5*	429	230	*54*
17.	C	Sabarkantha	Gujarat	11837	1233	*10*	1211	727	*60*
18.	C	West Goda-vari	A. P.	24190	1064	*4*	1064	256	*24*

As can be seen from the table the smear positivity rate ranged from 4–9% in 10 districts, 10-14% in 6 districts and in the remaining 2 districts it was 15% and 17% respectively. Considering the percentage of eligible patients who were put on SCC, there were 11 districts with an admission rate of 24-49%, 4 districts with 50-74% and in the remaining 3 districts 75% or more had been admitted.

Teams from the Centre have made visits to all the districts for on the spot observations, to address groups of Medical Officers of PHIs and to give guidance to the District TB Centre personnel regarding SCC in particular and DTP in general.

(started: 1983).

Field studies in North Arcot

In North Arcot District, where SCC under programme conditions was started in March '83, in-depth sociological and field studies are being undertaken. Since the district is relatively near Madras, frequent visits are made to guide the district personnel in the implementation of the programme. District level laboratories have been set up in North Arcot and Pondicherry and sputum specimens from patients are collected in duplicate, for examination by smear and culture at the district laboratory and in addition by smear, culture, sensitivity and identification tests at the Centre's laboratory.

Home visits of 'lost' cases are being continued and the findings in a sample of 305 cases are presented below:

Socio-economic reasons	...	23%
Abatement of symptoms	...	12%
Adverse reactions	...	6%
Died	...	15%
Migrated	...	11%
Rx changed/completed	...	20%
Inadequate address (not traceable)	...	16%
Total patients	...	305

Special efforts are being made to contact patients who are known to have drug-resistant strains on admission, in order to obtain sputum samples at the end of chemotherapy.

In Februray 1986, a pilot study on the prevalence of symptoms, action taken and awareness of tuberculosis in the community was initiated in a semi-urban setting in Tiruvannamalai town. For this study, a detailed two-stage questionnaire is used to elicit information. Thirty clusters, representative of the area, have been selected. Trained census takers are used to enumerate the

members of every household in the selected clusters and obtain details regarding any episode of illness during the previous four weeks, using Part-A of the questionnaire. Chest symptomatics with cough of 2 weeks' duration or more, are later interviewed by medical social workers, to obtain information regarding their knowledge of the symptoms of tuberculosis, the action taken by them for relief of symptoms and their knowledge about tuberculosis, using Part-B of the questionnaire. From every symptomatic, 2 sputum specimens (1 supervised spot and 1 overnight collection) are being obtained for processing by smear, culture, sensitivity and identification tests at the Centre's laboratory at Madras. Based on the experience gained in this pilot study, it is proposed to extend it to a rural area.

During sputum camps held by the District Tuberculosis Officer, the Centre's medical social workers conducted a sociological interview of the chest symptomatics. The data are being processed.

(started: 1983).

Collaborative study of abdominal tuberculosis

Encouraged by the results of short-course chemotherapy in the treatment of sputum-positive pulmonary tuberculosis and spinal tuberculosis, the Centre is currently carrying out a collaborative study of abdominal tuberculosis.

The objectives of this study are:

1. To assess the efficacy of a 6-month daily short-course regimen and that of a standard 12-month regimen in the treatment of abdominal tuberculosis, and
2. To evolve objective criteria for diagnosis, for assessment of progress and for relapse.

The study is conducted in collaboration with the Government General Hospital, Madras (Prof. K. V. Thiruvengadam, succeeded by Prof. S. Balakrishnan, Principal Investigator, Prof. N. Madanagopalan, Prof. N. Rangabashyam) and the Institute of Tuberculosis and Chest Diseases (Director: Dr. V. Rangaswamy succeeded by Dr. R. Kosalram and subsequently by Dr. K. Jagannath).

Adult patients with clinical evidence of tuberculosis of the abdomen are subjected to appropriate diagnostic procedures such as laparoscopy, colonoscopy, liver biopsy and in cases with ascites, percutaneous peritoneal biopsy, for obtaining material for histopathological and bacteriological examinations. Ascitic fluid when available is subjected to cytological examination, biochemical investigations and bacteriological examinations. A plain radiograph of the abdomen, barium meal and barium enema series and a chest radiograph are taken. A complete haemogram is done and 3 early morning urine specimens are examined by culture for *M. tuberculosis*. In addition, 2 sputum specimens are examined by smear and culture in patients with pulmonary tuberculosis.

Patients with histopathological, radiological or bacteriological confirmation as well as those with a clinical condition highly suggestive of abdominal tuberculosis are admitted to the study.

In order to streamline the procedures and standardise the techniques, a pilot study was carried out in the first instance. In all, 23 patients were treated with a 12-month daily regimen consisting of streptomycin 0.75 g plus ethambutol 25mg/kg plus isoniazid 300mg for 2 weeks followed by ethambutol 15 mg/kg plus isoniazid 300mg for the next 50 weeks (SEH/EH regimen).

Based on the experience gained, a full-fledged controlled study was started in September, 1983. Patients are randomly allocated to either a 6-month regimen or a standard 12-month daily regimen.

1. 2RHZ/4RH: Rifampicin 10mg/kg plus isoniazid 300mg plus pyrazinamide 30 mg/kg, daily for 2 months, followed by rifampicin 10mg/kg plus isoniazid 300mg, daily for the next 4 months.

2. SEH/EH: Streptomycin 0.75 g plus ethambutol 25mg/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.

So far, 94 patients have been admitted to the study; the intake is continuing. The aim is to admit about 120 patients and to follow them up for 5 years.

(started: 1983; expected year of completion of intake: 1987; expected year of completion of follow up: 1992).

Comparison of 2 intensive short-course regimens for the treatment of tuberculous meningitis in children

As mentioned in the previous (1984) annual report, the detailed findings of the first 3 studies showed that the mortality was high, ranging from 20-24%. While this could have been due to patients reporting late (87% had disease classified as Stage II or III on admission), a high prevalence of initial drug resistance contributing towards failure of chemotherapy could not be ruled out. It was therefore decided to study more intensive regimens, with 5 drugs in the initial 2-month phase followed by 2 drugs for 7 months.

The criteria for selection of cases and the eligibility for admission to the study are the same as for the earlier studies. Patients found suitable for admission are randomly allocated, after stratification according to clinical severity, in equal proportions to 2 regimens. The first regimen consists of initial treatment with streptomycin, ethambutol and isoniazid daily plus rifampicin and pyrazinamide thrice a week for 2 months, followed by rifampicin and isoniazid twice a week

for 7 months (2 $S_7H_7E_7R_3Z_3/7\ R_2H_2$). The second regimen consists of streptomycin, ethambutol and isoniazid daily plus rifampicin and pyrazinamide twice a week for 2 months, followed by rifampicin and isoniazid twice a week for 7 months (2 $S_7H_7E_7R_2Z_2/7\ R_2H_2$).

So far, 169 patients have been admitted (85 to the first regimen and 84 to the second); 13 patients were excluded from the study after admission, for various reasons. Of the remaining 156 patients, 42 died, 20 were discharged against medical advice, 74 completed 9 months of treatment and 20 are still on treatment.

The intake to the study is continuing.

(started: 1982; expected year of completion of intake:1987).

Pulmonary function studies in healthy South Indian subjects

Pulmonary function studies are being carried out in healthy South Indian subjects in order to establish norms for pulmonary function. Contacts of patients, aged 10 years or more and not suffering from any illness are admitted to the study. The following clinical evaluation and investigations are carried out before admitting the contacts to the study.

1. Details of smoking habit, if a smoker
2. Clinical examination, with special reference to the cardio - respiratory system
3. Haemoglobin, total and differential leucocyte counts
4. Stools examination
5. X-ray chest PA view
6. A 12-lead electrocardiogram

The following pulmonary function tests are carried out in those contacts who are found suitable for the study (normal chest X-ray and ECG).

1. Spirometry

   (i) F.V.C. (Forced Vital Capacity)
   (ii) $F.E.V_1$ (Forced Expiratory Volume in 1 Sec)
   (iii) $\frac{F.E.V_1}{F.V.C.}$ %
   (iv) P.E.F.R. (Peak Expiratory Flow Rate)
   (v) M.V.V. (Maximum Voluntary Ventilation)
   (vi) Flow 25%
   (vii) Flow 50%
   (viii) Flow 75%

2. Lung volumes by gas dilution method

   (i) T.L.C. (Total Lung Capacity)
   (ii) F.R.C. (Functional Residual Capacity)
   (iii) R.V. (Residual Volume)
   (iv) V.A. (Effective Alveolar Volume)
   (v) $\frac{\text{R.V.}}{\text{T.L.C.}}\%$

3. Single breath diffusing capacity

   (i) T.L.C.O. (Transfer Factor for CO)
   (ii) K.C.O. (Transfer Co-efficient for CO)

So far 200 contacts have been admitted to the study and the study is being continued.

(started: 1985; expected year of completion: 1990).

Clinical and pulmonary function studies in tropical pulmonary eosinophilia

Tropical Pulmonary Eosinophilia (T.P.E.) patients are conventionally treated with Diethyl Carbamazine (D.E.C.) 6 mg/kg body-weight daily for 3 weeks. Even though there is symptomatic improvement after 3 weeks of treatment in the majority of patients, a proportion of patients may develop chronic lung disease. Therefore T.P.E. patients being treated with D.E.C. for 3 weeks are being assessed for pulmonary function before treatment and at 1, 3, 6, 12, 24, 36, 48 and 60 months of follow-up, in order to understand the patho-physiology.

The following pulmonary functions tests are being done in these patients.

1. Spirometry
   (i) F.V.C.
   (ii) $F.E.V_1$.
   (iii) $\frac{F.E.V_1}{F.V.C.}\%$

2. Lung volumes
   (i) T.L.C.
   (ii) F.R.C.
   (iii) R.V.
   (iv) $\frac{\text{R.V.}}{\text{T.L.C.}}\%$

3. Single breath diffusing capacity
   (i) T.L.C.O.
   (ii) K.C.O.

In all, 66 patients have been admitted to the study so far; the intake to the study is being continued to have a total of 75 patients.

(started: 1984, expected year of completion: 1991).

Broncho-alveolar lavage studies in tropical pulmonary eosinophilia

The current concept of the pathogenesis of Tropical Pulmonary Eosinophilia suggests that it begins with a lung parenchymal inflammation in persons highly sensitised immunologically to filariasis parasites. It is hypothesised that the abnormalities in the lower respiratory tract in TPE are mediated by the inflammatory cells that accumulate in the lung parenchyma. Fibreoptic bronchoscopy and broncho-alveolar lavage are utilised to sample the inflammatory cells present in lower respiratory tract of patients with TPE and to define the inflammatory process.

The technology of fibreoptic bronchoscopy and broncho-alveolar lavage was transferred to the Centre under the Indo-US Science and Technology initiative (annual report, 1984) and is being carried out under local expertise since April 1985.

Broncho-alveolar lavage is performed under local anaesthesia using xylocain. The patients have an intravenous line in place and emergency drugs and equipment are available in the bronchoscopy suite. ECG monitoring is carried out during the entire procedure. The fluid obtained by lavage is pooled and filtered through 2 layers of gauze. The total count is done using hemocytometer and differential count after staining with hemotoxylin and eosin. The cytological examination is done in the Cardio Pulmonary Medicine Unit and the rest of the lavage fluid is analysed in the Immunology Department.

Lavages are done before treatment and at 1, 6, 12, 24, 36, 48 and 60 months of follow-up. So far 15 patients have been admitted to this study; the intake to the study is being continued. It is aimed to admit about 30 patients in all.

(started: 1985: expected year of completion: 1992).

Controlled clinical trial of two regimens in bacteriologically positive cases of leprosy

As mentioned in the previous (1984) annual report, the Centre is undertaking a controlled clinical trial in the treatment of leprosy at the Government Royapettah Hospital, Madras. Interim findings up to five years on a larger number of patients are presented here.

Patients were referred from the Government Royapettah Hospital, the Government General Hospital, the Stanley Hospital, the Madhavaram Rehabilitation Colony of Beatitude Social Welfare Centre, the Greater Madras Leprosy Relief Association and the Government Hospital, Saidapet, Madras. The criteria for eligibility to the study and the routine assessments have been presented earlier (1983 annual report). In brief, the patients were aged 12 years or more, had a bacterial index (BI) of 2.5 or more on Ridley's scale and had disease classified histopathologically as LL or LI.

Patients were randomly allocated to a standard regimen of dapsone plus clofazimine daily for 60 months (non-rifampicin regimen) or a 4-drug regimen of rifampicin, isoniazid, dapsone and clofazimine daily for 3 months, followed by dapsone and clofazimine daily up to 60 months (rifampicin regimen). The dosages were rifampicin 300, 450 or 600mg and dapsone 50, 75 or 100mg according to the body-weight, isoniazid 300mg and clofazimine 100 mg.

Patients in interim analysis: A total of 210 patients has been admitted to the trial. Of 147 patients who were considered for the present analyses, 18 were excluded. Six patients had died, 3 absconded (in the 1st, 3rd and 39th month), three patients discharged themselves against medical advice and four patients migrated at 6 months; 2 patients developed tuberculosis, one at 12 months and the other at 17 months, and were treated with anti-tuberculosis drugs. Interim analyses have been undertaken on the remaining 129 patients (66 rifampicin, 63 non-rifampicin).

All but 18 of the 129 patients were males; 70 patients were under 30 years of age, 35 were aged 30-39 years, and the remaining 24 aged 40 years or more; the mean age was 29 years (range 12-58) and the mean weight 44.2kg (range 21.3—75.8 kg). Of the 129 patients, 70 had had less than 1 year of previous chemotherapy for leprosy, 39 had had 1-5 years of chemotherapy and 20 had had over 5 years of chemotherapy. The distributions in the 2 regimens were broadly similar.

Of 116 patients who had histopathology findings, 83 (72%) were classified as lepromatous, 12 (10%) as lepromatous indeterminate and 21 (18%) as borderline lepromatous.

Drug regularity: The regularity of drug intake is presented in the following table regimen-wise.

% of Rx received	Months					
	0–3		0–12		0–60	
	Rif	Non-Rif	Rif	Non-Rif	Rif	Non-Rif
100	31	29	30	30	20	25
95—99	32	29	32	31	38	29
90—94	3	4	2	0	3	5
80—89	0	1	2	2	2	2
70—79	0	0	0	0	2	1
69 or less	0	0	0	0	1**	1*
Total	66	63	66	63	66	63

**63% Rx received; did not attend after 48m.

* 57% Rx received; did not attend after 48m.

The regularity in the 2 regimens was similar. A total of 60 patients (47%) in the two regimens did not miss a single dose in the first 3 months, 61 (47%) received between 95 and 99% of the scheduled chemotherapy and the others received between 80 and 94%. The regularity continued to be high in subsequent periods also. However, 2 patients became uncooperative in the 5th year and stopped attending for drugs.

Clinical progress: The table below presents the independent assessor's classification of clinical progress:

Progress	Period									
	0–12		0–24		0–36		0–48		0–60	
	Rif.	Non-Rif.	Rif.	Non-Rif.	Rif.	Non-Rif.	Rif.	Non-Rif.	Rif.	Non-Rif.
Improvement:										
Marked	19	20	47	43	54	54	52	53	55	55
Moderate	27	29	14	13	6	6	11	10	5	5
Slight	18	12	4	5	4	2	2	0	1	1
No change	2	2	1	1	0	0	0	0	0	0
Deterioration	0	0	0	0	0	0	0	0	0	0

At 12 months, moderate or marked improvement was reported in 46 (70%) in the rifampicin regimen and 49 (78%) in the non-rifampicin regimen. The proportions were 92% and 90% respectively, at 24 months, 94% and 97% respectively, at 36 months, 97% and 100% respectively, at 48 months and 98% and 98% respectively, at 60 months. Thus, there was excellent clinical improvement in both series.

Bacterial indices: The mean bacterial indices (BI) for the 2 groups at 0, 12, 24, 36, 48 and 60 months are shown in the following table.

Regimen	BI	Months					
		0	12	24	36	48	60
Rif. (N=66)	Mean	4.3	3.6	2.5	1.8	1.3	1.0
	Range	2.5-5.7	0.5-5.1	0.0-4.2	0.0-3.7	0.0-3.0	0.0-2.5
Non-Rif. (N=63)	Mean	4.3	3.4	2.5	1.7	1.3	1.0
	Range	2.7-5.3	0.3-4.8	0.2-4.5	0.0-3.8	0.2-3.5	0.0-2.8

In both groups there was a steady fall in the BI values. The reduction over the 60-month period was similar in the 2 regimens, namely 3.3.

It can be seen from the distribution presented below that 3 patients from the rifampicin group and 4 patients from the non-rifampicin group had BI of 0.0 at 60 months (but none had BI values of 0.0 at 3 consecutive months) and 26 other patients in each of the groups had BI less than 1.0; 30 patients (48%) from the rifampicin group and 23 (38%) from the non-rifampicin group had BI between 1.0 and 1.9; none had BI greater than 2.9.

BI	0.0	<0.5	0.5-	1.0-	1.5-	2.0-	2.5-2.9
Rif. (N=63)	3	7	19	18	12	3	1
Non-Rif. (N=61)	4	5	21	15	8	5	3

Reactions: Of the 129 patients, 5 (4 rifampicin, 1 non-rifampicin) had reactions at the time of admission, and 56 did not have any reaction either on admission or at any time during the 60-month period. Of the remaining 68 patients, 16 (10 rifampicin, 6 non-rifampicin) had neuritis and the remaining 52 (see table below) had mild to severe type of reactions during the 60-month period.

Regimen	Reactions			Total
	Mild	Moderate	Severe	
Rif.	7	9	8	24
Non-Rif.	10	3	15	28

The reactions were controlled with chloroquine, anti-histamines, analgesics, prednisolone and/or thalidomide.

In summary, the interim findings at 60 months show that patients on both regimens had improved clinically and bacteriologically, and that the improvement was similar.

(started:1977; intake completed: 1983; expected year of completion of 5-year follow up: 1988).

Evaluation of the efficacy of new anti-filarial drugs

The Centre in conjunction with the Department of Pharmacology, Madras Medical College has been recognised by the WHO as a Centre for carrying out controlled clinical trials in filariasis. Currently two studies to investigate two new drugs are being carried out.

The first study is to evaluate the efficacy of Ivermectin as an anti-microfilarial compound. Forty male microfilaremics were given Ivermectin in a single oral dose ranging from 25mcg to 200mcg/kg body weight. After an initial 21 days in the hospital, these individuals are being followed up for a period of 6 months. Preliminary analysis of the results shows that the drug is a potent anti-microfilarial compound. The drug is being currently compared against the standard drug, diethyl carbamazine (DEC) in a double-blind trial.

The second is a study using CGP 20376, a new antifilarial compound. This drug was administered in doses ranging from .01mg to 1mg to 14 normal healthy volunteers. No clinical or laboratory abnormalities were noticed at these doses. In the second phase of this study, which is now in progress, the drug is to be given in doses ranging from 2mg to 15mg. After assessing the safety of this drug at these levels, it is proposed to conduct clinical trials in microfilaremic individuals.

(started: 1986; expected year of completion: 1987).

LABORATORY STUDIES

STUDIES COMPLETED

Role of multiple media in the culture of specimens other than sputum for mycobacteria

In extra-pulmonary tuberculosis, where the number of mycobacteria is scanty, the procedures used for processing sputum specimens are often detrimental to the viability of the organisms. Also, specimens collected during surgery cannot be repeated. Hence maximum efforts have to be made to isolate the few organisms present in these samples. Hence, a study was carried out on the isolation of tubercle bacilli using multiple media from specimens other than sputum, collected during the period January, 1980 to December, 1984.

Material and methods: In all, 3807 specimens consisting of urine (818), cerebro-spinal fluid (CSF) (1428), lymphnode (567), pus (94), operation specimens (224), gastric aspirate (91), ascitic fluid (108) and 'other' types of specimens (477) were tested.

Smear examination was carried out on all specimens other than urine. Each specimen was inoculated on to 2 slopes each of Lowenstein-Jensen medium (LJ), LJ with added pyruvate (LJP), selective 7H11 oleic acid albumin medium (7H11) and 2 bottles of Kirchner's liquid medium (KL) made selective by the addition of antibiotics.

Before inoculation, the specimens were treated as follows. Early morning midstream urine samples were allowed to stand at 4°C for 4 hours and the lower portion was collected and centrifuged. The deposit was cultured by Petroff's method, as for sputum sample. In the case of CSF, one set of all the four media was inoculated directly before processing. The remaining CSF was centrifuged, the deposit was treated with 5% $H_2 SO_4$ and centrifuged again. This deposit was inoculated onto another set of media. Pus, pus swabs and gastric aspirates were treated as for sputum. If the gastric aspirate was fluid in consistency, it was centrifuged and the deposit treated. Lymph glands and other solid specimens were cut into small pieces, ground and then processed.

Results: The culture positivity rate for *M.tuberculosis* was 14% for all the specimens. Non-tuberculous mycobacteria (NTM) were isolated from 3% of the specimens and 4% of the cultures were contaminated. Pus, specimens obtained at surgery and lymph glands were most often positive by culture. There was a wide variation in the culture positivity rates in the different types of specimens. Thus, the positivity rate was 4-6% in urine, ascitic fluid and 'other' types of specimens, 11-14% in CSF and gastric aspirate and 31-46%

in lymph nodes, pus and operation specimens. The contamination rates for the different categories of specimens ranged from 1 to 8% (see table below).

Type of specimen	Total specimens (A)	Culture positive		Non-tuberculous mycobacteria		Contaminated	
		No.	%	No.	%	No.	%
Urine	818	32	*4*	35	*4*	31	*4*
Cerebrospinal fluid	1428	156	*11*	35	*2*	67	*5*
Lymphnode	567	176	*31*	14	*2*	32	*6*
Pus	94	42	*45*	4	*4*	5	*5*
Operation specimens	224	104	*46*	4	*2*	14	*6*
Gastric aspirate	91	13	*14*	8	*9*	7	*8*
Ascitic fluid	108	6	*6*	4	*4*	4	*4*
Others	477	21	*4*	20	*4*	6	*1*
Total	3807	550	*14*	124	*3*	166	*4*

The proportion of specimens yielding positive cultures of *M.tuberculosis* on different media, when considered singly and in various groups is presented in the table below:

Media	Positive cultures	
	No.	%
LJ	328	*60*
LJP	275	*50*
7H11	163	*30*
KL	339	*62*
LJ, LJP	378	*69*
LJ, KL	514	*93*
LJP, KL	485	*88*
LJ, LJP, KL	546	*99*
All	550	*100*

Considering the media one by one, with respect to all the specimens, the LJ and KL media gave a high proportion of positive cultures (60 and 62% respectively). The LJP medium gave 50%. The 7H11 medium was unsatisfactory, only 30% of the specimens being positive.

For groups of media, a specimen was considered positive if there was growth in at least one of the media. Considering the media two by two, LJ and KL gave 93% positive, and LJP and KL was almost as good (88%). When LJ, LJP and KL were considered together, 546 (99%) of the 550 positives were detected.

Of the cultures which were positive on one medium and not positive on any other medium, only 4 were from 7H11. On the other hand, KL was able to pick up 162 positives which were not positive by any of the other media. Thus, for the isolation of *M.tuberculosis* from specimens other than sputum, inoculation of a set of multiple media consisting of LJ and KL is the best.

(started: 1980; completed: 1984).

Virulence in the guinea-pig of tubercle bacilli isolated from sputum of participants in the BCG trial, Chengalpattu

A total of 32 isolates of *M.tuberculosis* from patients in the BCG trial area were tested at the Centre and also at the Department of Medical Microbiology, University of Wisconsin, Madison, USA (Prof. Donald Smith and colleagues), in order to ascertain whether (a) the findings regarding the low (and broad range of) virulence of Madras isolates were also true of the Chengalpattu isolates; (b) the virulence in guinea pigs correlated with the ability of an isolate to spread hematogenously and to be recovered from the spleen of guinea pigs infected by the intramuscular route; (c) there was a relationship between the hematogenous seeding to the spleen in guinea pigs infected by the intramuscular route (BE-spleen-IM) and in those infected by the respiratory route (BE-spleen-Resp.); and (d) there was a relationship between virulence and susceptibility to the bactericidal action of H_2O_2 or TCH.

The virulence estimation was done as developed in the Centre earlier. To determine the number of bacilli recoverable from the spleen, the spleen was excised, homogenised in distilled water (Madras) or in 2% albumin solution (Madison), diluted and cultured on LJ slopes in Madras and OAA agar in Madison. Susceptibility to TCH and H_2O_2 was determined by the method of Grange et al. (J. Gen. Microbiology, 1978, *108*,1).

The table below gives the reproducibility (three replicates) of the Root Index of Virulence (RIV) in guinea pigs infected via the intramuscular route with one of six Chengalpattu isolates.

Replicate	1	Isolate	34	25	1	11	20	14
		RIV	0.50	0.50	0.74	1.07	1.09	1.40
Replicate	2	Isolate	1	34	25	11	20	14
		RIV	0.71	0.89	0.92	1.02	1.29	1.46
Replicate	3	Isolate	25	34	1	11	20	14
		RIV	0.47	0.84	0.98	1.16	1.36	1.45

Analysis of the RIV data revealed a significant F ratio ($P<0.0005$); however, individual isolates were ranked for virulence in approximately the same order. There was a significant correlation between the RIV of a given isolate and the BE-spleen-IM (32 isolates) both at Madras and at Madison ($r=0.765$ at Madras and $r=0.922$ at Madison, $P<0.001$ in both cases).

Studies carried out in Madison revealed a significant direct correlation ($r=0.807$, $P<0.001$) between BE-spleen-IM and BE-spleen-Resp.

Considering the relationship between RIV and susceptibility to TCH, in Madras there was some correlation ($r=0.565$, $P<0.01$) whereas in Madison the correlation was poor ($r=0.24$, NS). Correlation between RIV and susceptibility to H_2O_2, done only at Madison, revealed a weak relationship ($r=0.480$ for RIV vs 0.2% H_2O_2 and $r=0.549$ for RIV vs .005% H_2O_2).

In conclusion, the study has shown:

1. that the virulence in guinea pigs of the isolates tested from Chengalpattu was similar to that in Madras;

2. that there was high correlation between the RIV and the bacterial content of cultures from the spleen of guinea pigs infected intramuscularly, both at Madras and Madison;

3. there was a high correlation between the number of bacilli recovered from the spleen of guinea pigs infected intramuscularly and the number recovered from the spleen of guinea pigs infected by the respiratory route.

(started: 1985; completed: 1986).

Immunological investigations in tuberculous ascites

Cell mediated immune responses were studied by lymphocyte and macrophage functions in seven patients with bacteriologically and/or histologically confirmed tuberculous ascites. Eight non-tuberculous ascites patients were

included as controls. Anti-PPD antibody levels were estimated by ELISA method using 10μg/ml for PPD and 1 in 80 dilution for ascitic fluid and serum. Macrophages from tuberculous ascitic fluid showed increased production of H_2O_2 when compared with macrophages from controls. Proliferative response of lymphocytes to PPD antigen (50 μg/ml) was greater in ascitic fluid than in peripheral blood in tuberculous patients while the responses were in the opposite direction in control patients. Tuberculous ascitic fluid had higher mean levels of anti-PPD antibodies than ascitic fluid from controls, though their mean levels in peripheral blood were similar in the two groups. It is concluded that the results provide support for the concept of immunological compartmentalization. Mean levels of three selected indices in ascitic fluid are given below:

Index	Mean ± S.D. (No. of Patients)		P-value for the contrast
	TB ascites patients	Non-TB ascites patients	
1. H_2O_2 production (nmol/10^6 macrophages or monocytes)	8.4 ±3.7 (7)	2.1 ±1.5 (6)	<.01
2. Stimulation Index (SI)* to PPD	1.19 ±0.07 (7)	1.05 ±0.07 (7)	<.01
3. ELISA optical density	0.111 ±0.019 (8)	0.057 ±0.049 (8)	<.05

*S.I. = $\frac{\text{Mean of log CPM with antigen}}{\text{Mean of log CPM for controls}}$

(started: 1985; completed: 1985).

Antibody (IgG) levels to different mycobacterial antigens by ELISA

An Enzyme Linked Immuno-sorbent Assay (ELISA) was carried out to assess the antibody levels in tuberculous patients and healthy volunteers to different mycobacterial antigens. A total of 39 previously untreated sputum positive pulmonary tuberculosis patients and 21 adult healthy volunteers from the Centre's staff and blood bank donors were investigated. Sonicate antigens were prepared from 8 tuberculous and non-tuberculous mycobacterial strains. They were used along with PPD-S at a concentration of 5 μg/ml in the assay for coating the ELISA plates. Serum was used at a dilution of 1 in 40.

The mean antibody levels (O.D. values) in tuberculous patients were found to be significantly higher than in the healthy volunteers (controls) to all the antigens from tuberculous mycobacteria (see table below):

	Level of antibody bindings (OD—mean ± SD)		P-Value
	Patients (39)*	Volunteers (21)*	
Tuberculous Mycobacteria:			
1. *7219*	0.345 ±0.099	0.247 ±0.087	0.001
2. South Indian	0.254 ±0.049	0.222 ±0.049	0.02
3. *M.bovis*	0.243 ±0.059	0.211 ±0.055	0.05
4. PPD	0.149 ±0.047	0.113 ±0.060	0.02
Non-tuberculous Mycobacteria:			
M. Kansasii	0.290 ±0.048	0.237 ±0.052	0.001
M. scrofulaceum	0.299 ±0.074	0.237 ±0.071	0.001
M. avium intracellulare-S	0.161 ±0.064	0.184 ±0.069	0.2 (NS)
M. Chelonei	0.151 ±0.083	0.122 ±0.078	0.2(NS)
M. fortuitum	0.124 ±0.059	0.153 ±0.054	0.07 (NS)

* No. in parentheses indicate the numbers tested.
(NS) = Not statistically significant.

It was also found that the mean antibody levels to antigens from *M. kansasii* and *M.scrofulaceum* differed significantly in the two groups of subjects, but the antigens from other non-tuberculous mycobacteria did not. The statistically significant difference in the mean antibody levels to antigens from *M. kansasii* and *M. scrofulaceum* implies that these two non-tuberculous mycobacterial strains share a higher proportion of common antigenic components with tuberculous mycobacteria.

(started: 1984; completed: 1985).

STUDIES IN PROGRESS

Serial estimations of lysozyme in cerebrospinal fluid (CSF) from patients with tuberculous meningitis

Lysozyme, which has antimicrobial activity, was found to be elevated in the CSF of bacteriologically confirmed cases of tuberculous meningitis (1982 annual report). A study is in progress to find out whether serial lysozyme values would be of value for assessing the progress of tuberculous meningitis patients during treatment.

CSF specimens are being collected on admission to treatment and at 1 and 2 months and the lysozyme content estimated by lysoplate assay. The intake is in progress, 40 patients having been admitted so far.

(started: 1984; expected year of completion: 1987).

Homogeneity of colony morphology in strains of *M.tuberculosis*

There have been reports of differences in colony morphology within the strain *M.tuberculosis*, although the most commonly observed form is rough. Mankiewicz and Liivak (ARRD, 1975, *111*,307) reported the isolation of colonies of two distinctly different morphologies in 14% of Eskimo patients; these different types differed in phage lysis as well as drug susceptibility pattern. Other workers have shown differences in morphology among atypical strains also.

A study has been initiated at this Centre to look for differences in colony morphology in South Indian strains and evaluate virulence, phage types and drug-susceptibility patterns. The primary isolate is plated out on OAA agar and incubated. Single colonies are examined for morphological appearance. If different types are observed, these colonies are subcultured on L-J medium and tested for virulence in the guinea-pig, phage typing pattern and drug susceptibility.

So far, positive cultures from 120 patients have been examined. The data are being analysed.

(started: 1985: expected year of completion: 1987).

Bacteriocin typing of mycobacteria

Screening of rapidly growing non-tuberculous mycobacteria (NTM), isolated from South Indian patients, for the production of mycobacteriocin is in progress. As described earlier (1983 annual report) the "streak plate" method is used for demonstrating mycobacteriocin production. Six promising indicator strains were obtained earlier by screening 100 rapid growers against 40 strains of rapid growers. Screening the same 100 rapid growers against 20 more strains has yielded one more effective indicator strain (BI No. 1). This strain could reduce the number of untypable strains from 32 to 23. By using these 7

indicator strains, a total of 8 major bacteriocin types were obtained. These were further subtyped as shown in the table below:

Bacteriocin types		Indicator strain—BI No.							Total No.
		1	25	50	52	54	57	60	
A	1	+	—	—	—	—	—	—	8
	2	+	+	—	—	—	—	—	1
	3	+	+	—	—	—	+	—	1
	4	+	+	+	—	—	—	—	1
	5	+	+	+	—	—	+	+	1
	6	+	+	+	+	—	+	—	1
	7	+	+	+	+	—	—	+	1
	8	+	—	+	+	+	—	—	1
	9	+	—	+	—	—	—	—	3
	10	+	—	+	—	—	+	—	2
	11	+	—	+	—	—	+	+	3
	12	+	—	—	+	—	+	—	5
	13	+	—	—	+	—	+	+	1
	14	+	—	—	—	—	+	+	1
	15	+	—	—	—	—	+	—	2
B	1	—	+	—	—	—	—	—	4
	2	—	+	+	—	—	—	—	4
	3	—	+	+	+	—	+	—	2
	4	—	+	—	+	—	+	—	1
C	1	—	—	+	—	—	—	—	4
	2	—	—	+	—	—	+	—	2
	3	—	—	+	—	—	+	+	2
	4	—	—	+	+	—	+	—	1
	5	—	—	+	+	—	—	+	1
D	1	—	—	—	+	—	—	—	2
	2	—	—	—	+	+	—	—	1
	3	—	—	—	+	—	+	—	3
	4	—	—	—	+	—	+	+	1
E		—	—	—	—	+	—	—	5
F		—	—	—	—	—	+	—	5
G		—	—	—	—	—	—	+	4
H (Untypable)		—	—	—	—	—	—	—	23
									100

Further work is in progress to identify more indicator strains and to reduce the number of untypable strains.

(started: 1984; expected year of completion: 1987).

Cetylpyridinium Chloride (CPC) as a preservative for the storage of sputum samples at ambient temperature

In developing countries, it is quite common to store sputum samples at ambient temperature up to 7 days, before they are processed for culture. Earlier studies conducted in this Centre and elsewhere had shown that there was a significant loss in positivity when the sputum samples were cultured after storing them at ambient temperature beyond 3 days. So a preservative that could prevent the loss of viability of tubercle bacilli would be very useful where sputum specimens are to be transported to a Central laboratory for processing, involving delay. A pilot study is therefore being carried out to study the effect of CPC as a preservative on the storage of sputum samples.

Overnight collection specimens of sputum from pulmonary tuberculous patients are divided into 3 aliquots of 5 ml. each after homogenisation with glass beads. CPC is added to give a final concentration of 5mg/ml. and the aliquots stored at ambient temperature in darkness. The aliquots are randomly allocated for 1, 7 and 14 days of storage. All the aliquots are subjected to direct smear by fluorescence microscopy and culture for AFB by standard Petroff's method.

A total of 47 samples of sputum have been studied so far and the preliminary results are encouraging. It is proposed to undertake a full scale study with more samples.

(started:1985; expected year of completion: 1987).

Production of monoclonal antibodies to *M. tuberculosis* H37Rv and South Indian strain 7219

BALB/C mice were immunised with sonicates of *M. tuberculosis* H37Rv and South Indian strain 7219. Spleen cells of mice thus immunised were fused with Sp2/O myeloma cells. Six such fusions have been done so far for H37Rv and 7 for 7219. From the H37Rv fusions, 183 hybridoma clones were obtained and were screened for antibody production by ELISA and RIA. Of the 183 clones, 8 were positive for antibody production against H37Rv sonicate antigen by ELISA. These clones have been frozen and stored in liquid nitrogen. Limiting dilution and specificity assay will be done for these clones. Of the 139 clones obtained from the South Indian strain fusions, none have been positive for antibody production. Further fusions are under way for the production of more clones against both H37Rv and 7219.

(started: 1985; expected year of completion: 1987)

Production of monoclonal antibodies against heat-killed *M. tuberculosis*

It is well known that the South Indian (S.I.) strain of *M.tuberculosis* is different from the other strains. Hence, raising monoclonal antibodies against the surface as well as somatic antigens of the South Indian strain of *M.tuberculosis* is likely to be useful for immunological investigations.

M. tuberculosis (S.I. strain)—immunized mouse splenocytes were fused with myeloma cells (Sp^2 O Cells). A total of 28 hybrids, producing antibody against the S.I. strain were raised. The culture supernatants of these hybrids were screened against heat killed whole bacilli of S.I. strain, as well as the crude sonicated preparation of the same strain, using Enzyme Linked Immuno-Sorbent Assay (ELISA). Of these 28 hybrids, 6 are producing antibodies against the whole bacilli and the rest are producing antibodies against crude sonicated preparation. One of these hybrids, producing IgM antibody against the whole bacilli, has been cloned by limiting dilution method. Studies on the characterization and specificity of the other hybrids are in progress.

(started: 1985; expected year of completion: 1990).

Kinetics of the delayed type hypersensitivity response in tuberculous guinea pigs

To demonstrate the interrelationship between tuberculous mycobacteria like the low and high virulent South Indian variants of *M. tuberculosis* and various NTM, and between these and BCG, groups of guinea pigs have been inoculated subcutaneously as follows: (i) high virulent South Indian variant of *M. tuberculosis*, (ii) low virulent South Indian variant, (iii) H37Rv, (iv) *M. bovis*, (v) BCG, (vi) *M. avium* and (vii) *M. scrofulaceum*. One uninonculated group is left as control. Skin tests will be done for each guinea pig with sonicates of NTM, PPD-S and saline control. Subgroups of guinea pigs will be superinfected with the organisms mentioned above. At the end of 12 weeks after infection/ superinfection, each animal will be sacrificed and visual scoring and viable count in spleen will be performed.

(started: 1986; expected year of completion: 1987).

Bacteriological investigations of acute respiratory infections in children

Bacteriological investigations were carried out on specimens (nasal swabs, nasal secretions, throat swabs and laryngeal swabs) collected from 151 children below 6 years of age, suffering from acute respiratory infections (ARI) attending the out-patient department of the Institute of Child Health, Madras.

There were 80 (53%) males and 71 (47%) females. The predominant clinical symptoms in the 151 patients were running nose, blocked nose and cough. The duration of the symptoms or combination of the symptoms ranged from 1 day to 7 days in 99 cases and more than 7 days in 29 cases, while in 3 children, the duration was not known. Of the 151 children, 84 (56%) yielded one or a mixture of bacteria which could be considered as potential pathogens of ARI. Of the 94 isolates obtained from these 84 children, 40 (42%) were non-fermenting gram negative bacilli, 20 (21%) *Haemophilus influenzae*, 17 (18%) *B-haemolytic streptococci* (Groups C and G), 10 (10%) *Klebsiella pneumoniae*, 4(4%) *Streptococcus pneumoniae*, 4 (4%) *Neisseria sps* and 1 (1%) *staphylococcus aureus*. The bacteria isolated in relation to the clinical diagnosis are shown in the table on page 51.

Clinical diagnosis	Total No. of children	No. of children with the organisms isolated	Organism isolated													
			Non-fermenters		Beta haemolytic streptococci		Haemophilus influenzae		Strepto. pneumoniae		Staph. aureus		Neisseria sp (pneu)		Klebsiella pneumoniae	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
URI (non-specific)	51	29	15	*29*	6	*12*	10	*20*	0	*0*	1	*2*	0	*0*	2	*4*
LRI (non-specific)	77	42	19	*25*	9	*12*	8	*10*	2	*3*	0	*0*	4	*5*	7	*9*
Asthmatic bronchi Broncho-pneumoniae Bronchitis Acute bronchitis	19	11	4	*21*	2	*11*	2	*11*	2	*11*	0	*0*	0	*0*	1	*5*
Others	4	2	2		0		0		0		0		0		0	
Total	151	84	40 (5)	*27 (3.3)*	17	*11*	20	*13*	4	*3*	1	*1*	4	*3*	10	*7*

Note: Children having more than one organism are added under each type.
Percentages are based on the total no. of children.
Figures in parentheses indicate pure growth.

Duration of symptoms (days)	Total No. of children	No. of children with the organisms isolated	Organism isolated													
			Non-fermenter		B-Haemolytic Streptococci		Klebsiella Pneumoniae		Haemophilus Influenzae		Strepto-Pneumoniae		Staphylococcus Aureus		Neisseria Sp. (Pure)	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1-3	54	31	13 (2)	24 (4)	9	17	2	4	7	13	0	0	0	0	2	4
4-7	65	33	13 (3)	20 (5)	5	8	7	11	10	15	2	3	1	2	2	3
>7	29	17	13	45	3	10	1	3	3	10	0	0	0	0	0	0
Not known	3	3	1		0		0		0		2		0		0	
Total	151	84	40 (5)	27 (3.3)	17	11	10	7	20	13	4	3	1	1	4	3

Note: Children having more than one organism are added under each type.
Percentages are based on the total number of children.
Figures in parenthesis indicate pure growth.

The frequency of isolation of non-fermenters was almost the same in upper respiratory infection (URI) and lower respiratory infection (LRI). Likewise beta-haemolytic streptococci also were isolated with the same frequency from URI and LRI cases. *Klebsiella pneumoniae* was isolated more often from LRI than from URI (9% and 4% respectively), whereas *Haemophilus influenzae* was isolated more frequently from URI than from LRI cases (20% and 10% respectively). All the 4 isolations of *Neisseria sps* and 2 of *Streptococcus pneumoniae* belonged to LRI cases. Mixed infections were noted in 6 patients.

The frequency of isolation of non-fermenters increased when the duration of illness exceeded 7 days (see table on page 52). The isolation of *Klebsiella pneumoniae* was maximum when the duration of illness was 4-7 days. This is an important finding because the initial viral infection must have predisposed for the later bacterial invasion.

Now studies are in progress in the patients admitted to the Institute of Child Health, Madras, to look for both viral and bacterial agents in acute respiratory infections in children, and to assess the significance of prior viral infection on bacterial superinfection.

(started: 1985; expected year of completion: 1987).

Bacteriology of broncho-alveolar lavage obtained from TPE patients

Forty broncho-alveolar lavage samples, collected from 27 Tropical Pulmonary Eosinophilia patients (24 males and 3 females, age ranging from 15-40 years), were examined bacteriologically. Of these, 33 samples from 24 patients were positive by culture. The details of the results are presented in the table below:

Organism isolated	No. of samples
Pseudomonas sp.	16 (40%)
Mixture*	6 (15%)
α-Haemolytic streptococci	3 (7.5%)
Coliforms	2 (5%)
S. Aureus	2 (5%)
Klebsiella	2 (5%)
E. Coli	1 (2.5%)
Neisseria	1 (2.5%)
No growth	7 (17.5%)
Total	40

*Mixture of α-strep, Neisseria, staphylococcus

As can be seen, the organisms most frequently cultured were *Pseudomonas sp.* (40%) followed by mixed growth (15%), α-*Haemolytic streptococci* (7.5%), *S. aureus, Klebsiella* and coliforms (5% each), and *E. Coli* and *Neisseria* (1% each). In 7 of the samples there was no growth.

The mixed growth represents the flora of normal oropharynx. Isolation frequencies of *Pseudomonas aeruginosa* and other non-fermenting gram negative bacilli at various stages of treatment were as follows: 8 isolations before start of treatment, 4 during 1st month after treatment, 3 during 6th month after treatment and 1 during 12th month after treatment. Further studies are in progress to assess the significance of the findings of the study.

(started: 1985; expected year of completion: 1986).

Acute phase proteins in tuberculous patients

Tissue injury or infection results in an increase in the concentration of several plasma proteins of hepatc origin ('acute phase proteins'). These proteins have not been studied in detail in chronic diseases such as tuberculosis and leprosy. An investigation was therefore undertaken to study the changes in the concentrations of several acute phase proteins in adult patients with pulmonary and abdominal tuberculosis and in children with tuberculous meningitis who were admitted to clinical trials at this Centre. The proteins investigated were C-reactive protein, ceruloplasmin, haptoglobin, α_1-acid glycoprotein, α_2-macroglobulin and transferrin, the last mentioned being a negative acute phase reactant. The plasma concentrations of these proteins were determined on admission and at the end of treatment in patients with abdominal tuberculosis and tuberculous meningitis while in patients with pulmonary tuberculosis, the concentrations were determined before, during and at the end of chemotherapy and also at 12 months after stopping treatment. These concentrations were also determined in a group of healthy volunteers. The main aim of the investigations was to examine whether a bacteriological relapse could be predicted on the basis of the concentrations at the end of chemotherapy.

The concentrations of the various proteins were determined using the Radial Immunodiffusion technique employing specific antisera and a minimum of 4 standards. The details of the dilution of the antisera and the serum

samples, the range of the concentration of the standards set up and the incubation periods are listed in the following table:

Acute phase protein	Dilution of antiserum in the gel	Range of standards	Sample dilution	Incubation time (hours)
C-reactive protein	1 in 100	0.6- 7.6	Neat & 1 in 2	72
Ceruloplasmin	1 in 25	2.9-23.0	1 in 5	48
Haptoglobin	1 in 50	2.4-38.8	1 in 20	48
α_1-acid glycoprotein	1 in 25	2.4-19.2	1 in 20	48
Transferrin	1 in 50	4.8-38.0	1 in 20	48
α_2-macroglobulin	1 in 125	2.8-22.4	1 in 40	72

The assays were undertaken in batches after randomisation; each batch had samples from different groups of patients, in addition to standards.

The mean serum concentrations (mg/dl) of the acute phase proteins before start of treatment in patients with pulmonary, abdominal and meningeal tuberculosis and those in healthy volunteers are presented in the following table:

Acute phase protein	Pulmonary T.B.	Abdominal T.B.	Meningeal T.B.	Healthy volunteers
C-reactive protein	7.2	2.1	1.1	0.15
Ceruloplasmin	75.9	62.6	77.5	24.9
Haptoglobin	513	309	509	146
α_1-acid glycoprotein	269	246	245	61
Transferrin	228	229	410	339
α_2-macroglobulin	310	296	491	275
No. of subjects	20	19	11	11

The mean concentrations of C-reactive protein, ceruloplasmin, haptoglobin and α_1-acid glycoprotein were significantly higher ($P<0.01$) in tuberculous

patients than in healthy volunteers. The mean transferrin concentrations in patients with pulmonary and abdominal tuberculosis were significantly lower than in healthy volunteers ($P<0.01$). The mean concentrations of α_2-macroglobulin were similar in pulmonary and abdominal tuberculous patients and healthy volunteers. Comparing the values on admission between patients with pulmonary and abdominal tuberculosis, the mean value of haptoglobin in the latter group was lower than in the former group ($P<0.01$).

The mean concentrations (mg/dl) of the different proteins before (0 month), during (2 months) and at the end of chemotherapy (6 months), and during the follow-up phase (18 months after start of treatment) in the 20 patients with pulmonary tuberculosis are presented in the following table:

Acute phase protein	0m	2m	6m	18m
C-reactive protein	7.2	2.2	0.8	0.7
Ceruloplasmin	75.9	79.1	60.4	28.8
Haptoglobin	513	278	152	160
α_1-acid glycoprotein	269	166	86	90
Transferrin	228	342	333	290
α_2-macroglobulin	310	362	346	350

There was a sharp decrease in the mean concentrations of C-reactive protein, haptoglobin and α_1-acid glycoprotein with effective anti-tuberculosis treatment and the mean values both at 2m and at the end of chemotherapy were appreciably lower than the pre-treatment values ($P<0.01$ for both contrasts). The mean values of these three proteins at 12 months after stopping treatment were similar to those at the end of treatment. The mean values for ceruloplasmin were similar at 0 and 2 months. There was a slight but significant fall by the end of chemotherapy ($P<0.05$), and the mean value at 18m was appreciably lower than that at the end of chemotherapy ($P<0.01$). There was a significant increase in the mean concentrations of transferrin with treatment ($P<0.01$); however, there was a slight fall thereafter and the mean value at 18 m was significantly lower ($P<0.01$) than at the end of chemotherapy (6 m). α_2-macroglobulin did not appear to behave like an acute phase reactant as the differences between the mean levels at the four different time-points were not significant ($P>0.2$).

The mean concentrations (mg/dl) of the different acute phase proteins on admission and at the end of treatment in 19 patients with abdominal tuberculosis are presented in the following table:

Acute phase protein	Pre-treatment	End of treatment
C-reactive protein	2.1	0.8
Ceruloplasmin	62.6	39.8
Haptoglobin	309	119
α_1-acid glycoprotein	246	78
Transferrin	229	291
α_2-macroglobulin	296	346

The changes observed in patients with abdominal tuberculosis were similar to those with pulmonary tuberculosis with appreciable decrease in the concentrations of C-reactive protein, ceruloplasmin, haptoglobin and α_1-acid glycoprotein ($P \leqslant 0.05$) and an increase in that of transferrin ($P < 0.01$).

The mean values of the different proteins (mg/dl) before and at the end of treatment in 11 patients with tuberculous meningitis are presented in the following table:

Acute phase protein	Pre-treatment	End of treatment
C-reactive protein	1.1	0.2
Ceruloplasmin	77.5	40.9
Haptoglobin	509	154
α_1-acid glycoprotein	245	97
Transferrin	410	234
α_2-macroglobulin	491	502

In the 11 patients who completed their chemotherapy, with the exception of α_2-macroglobulin, there was a significant decrease in the levels of all other acute phase proteins including that of transferrin at the end of treatment ($P < 0.01$).

These investigations have to be extended to treatment failures and relapses in order to assess the predictive value of the estimates of these proteins, and to seek a basic understanding of the disease causing mechanisms and their control.

(started: 1983; expected year of completion: 1989).

Self-induction of rifampicin metabolism during daily, thrice-weekly and twice-weekly treatment

Treatment of tuberculosis with intermittent short-course regimens containing rifampicin in addition to streptomycin, isoniazid and pyrazinamide has been shown to be as effective as daily treatment with the same drugs; however the incidence of hepatitis appears to be appreciably less with intermittent regimens. It has been suggested (1983 annual report) that hepatic toxicity during daily treatment with regimens containing rifampicin and isoniazid is possibly due to the induction of isoniazid hydrolase by rifampicin, resulting in an increased formation of hydrazine, a hepatotoxic metabolite of isoniazid. An investigation was undertaken to study the induction of the hepatic microsomal enzyme system in patients treated with intermittent (thrice-or twice-weekly) regimens. Decrease in the plasma half-life of rifampicin and of the increases in the levels of gamma-glutamyl aminotransferase (GGT) in plasma and of the excretion of D-glucaric acid in urine were used as measures of induction.

Patients were allocated at random to one of the following three regimens:

1. 1 $RHEZ_7/11\ EH_7$—Rifampicin (R) plus isoniazid (H) plus ethambutol (E) plus pyrazinamide (Z) daily for 1 month followed by ethambutol plus isoniazid daily for 11 months.
2. 1 $RHEZ_3/11\ EH_7$—Same as above except that the drugs were administered thrice-weekly during the 1st month.
3. 1 $RHEZ_2/11\ EH_7$—Same as above except that the drugs were administered twice-weekly during the first month.

The dosages of the drugs are given in the following table:

Drug	Daily	Thrice-weekly	Twice-weekly
Rifampicin	450 mg	450 mg	450 mg
Isoniazid	300 mg	400 mg	600 mg
Ethambutol	800 mg	1200 mg	1600 mg
Pyrazinamide	1.5 g	1.5 g	2.0 g

All drugs were administered under supervision during the first month.

The investigations were undertaken on the day of admission and at 1, 2 and 4 weeks thereafter. The drugs were administered on an empty stomach and blood at 0, 3, 4½ and 6 hours and urine excreted over the period 0-6 hours were collected. Plasma separated from the blood samples and aliquots of urine were stored at — 20°C. Determination of the activities of GGT in samples collected at 0 hour and the concentrations of rifampicin at 3, 4½ and 6 hours and of D-glucaric acid in urine collected over the 0-6 hour period were undertaken after randomisation of the samples, employing standard methods. In all, 36 patients were admitted to the study. The results are being analysed.

(started: 1985; expected year of completion: 1986).

Xylose absorption test

The xylose absorption test has been used to study the degree of malabsorption, particularly in the jejunal portion of the small intestine. It is proposed to use this test to study the impairment of absorption in patients with abdominal tuberculosis who are being treated at this Centre. A preliminary standardisation experiment has been started with 2 different dose-sizes, namely, 5 g and 10 g of xylose, to determine which of them gave estimates that were associated with less variation.

The investigation was undertaken in healthy volunteers. Xylose 5 g was administered on an empty stomach to a group of 5 volunteers and 10 g to another group of 6 volunteers (body weight range 51-66 kg). Blood at 1½ hours and total urine excreted over the periods 0-2 and 2-5 hours after the dose, were collected. Xylose concentrations in protein-free filtrates from blood and in urine samples were determined by a standard procedure after randomisation. The proportion of the dose of xylose excreted in the urine during the 2 periods are presented in the following table:

	Hour/period of collection	Dose of D-xylose administered	
		5 g	10 g
Blood Concentration (mg%)	1½	16.1 ±2.6*	22.0 ±5.2
Urine Proportion of dose excreted	0-2	16.7 ±1.5	11.1 ±1.3
	2-5	15.7 ±0.7	12.8 ±3.2
	0-5	32.4 ±1.8	23.9 ±4.0
No. of subjects		5	6

* Mean ± standard deviation.

The mean blood xylose concentrations at 1½ hours after 5 and 10 g doses of xylose were 16.1 and 22.0 mg%, respectively ($P < 0.001$). Doubling the dose of xylose did not result in a doubling of the blood concentration, and the mean concentration with the 10 g dose was only about 37% higher than that with the 5 g dose. The difference between the lowest and highest values recorded was 6.1 mg% with the 5 g dose and 14.9 mg% with the 10 g dose, and the coefficients of variation between replicate estimates were 16% and 24%, respectively.

The test employing 5 g xylose was repeated on another occasion in 4 of the 5 volunteers and the mean blood xylose concentrations at 1½ hours on the two occasions were 16.5 mg% and 15.7 mg% respectively ($P > 0.2$).

The mean proportion of dose of xylose excreted in urine over the period 0-5 hours was about 26% lower with the 10 g dose than that with the 5 g dose ($P < 0.001$). The coefficients of variation for replicate estimates were 5.6% and 16%, with the lower and higher doses, respectively. The mean ratio of xylose excreted in urine over the 0-2 to the 2-5 hour period was 1.06 (range: 0.96 to 1.19) after the 5 g dose and 0.91 (range 0.63 to 1.23) after the 10 g dose.

The proportion of dose excreted in urine (over the 0-2 hour period only) after a 5 g dose was determined in 4 of the 5 volunteers on another occasion. The mean values on the two occasions were similar, namely 16.5 and 16.6% respectively.

These findings suggest that estimates obtained with both blood and urine are likely to be more precise with the 5 g dose than with the 10 g dose of xylose. The criterion to determine the degree of malabsorption would depend to a great extent on the range of values obtained in healthy individuals and these ranges (in both blood and urine) are much narrower with the 5 g dose than with the 10 g dose. Moreover, investigations undertaken in the same individuals have shown that the test employing 5 g dose of xylose is likely to yield reproducible results.

The effect of storing the protein-free filtrates at —20°C for different periods of time on xylose concentrations was also investigated. The protein-free filtrates from blood collected at 1½ hours after the 5 g dose were stored for 30 and 45 days and that after the 10g dose was stored for 7 days before estimations were repeated. The mean values on the day of collection and 7 days after storage (following a 10g dose) were 23.3 and 23.4 mg% respectively. The mean values on the day of collection and at 30 and 45 days after storage (after a 5 g dose) were 16.1, 15.7 and 14.6 mg% respectively. The differences were not significant statistically, and these findings suggest that xylose concentrations remain unaffected after storage at —20° C.

Since the number of subjects is small, it is proposed to repeat these tests in a larger number of volunteers to arrive at the normal range in healthy subjects.

(started: 1985; expected year of completion: 1987).

Tuberculous infection and activation of macrophages

An investigation has been initiated to study the bactericidal activity of the macrophage in order to understand the mechanism of action (oxygen-dependent or not) and to postulate a model for pharmaco-regulation of its activity. The investigations have been started with peritoneal and tissue macrophages in mice infected with tubercle bacilli; it is later proposed to undertake *in vitro* investigations for easier manipulation.

Pyrazinamide is a powerful anti-tuberculosis drug and it has been established that its action is restricted to bacilli in an acid environment such as prevalent inside the macrophage. The mechanism of pyrazinamide action has not been delineated so far and it is possible that pyrazinamide might exert its bactericidal action indirectly through the activation of the macrophage. It was decided to undertake a preliminary investigation to study the effect of infecting mice with different dose levels of *M. tuberculosis* (pyrazinamide-sensitive strain) and *M. bovis* (a naturally resistant strain to pyrazinamide) on the release of hydrogen peroxide and the activities of lysozyme, and three other lysosomal hydrolases, namely B-glucuronidase, cathepsin-D and acid phosphatase.

Swiss albino mice were infected intravenously with 0.1 ml containing 10^6, 10^5 and 10^4 organisms of *M. tuberculosis* and *M. bovis* in 7H 9 liquid medium. Six control mice (uninfected) and 8 mice in each of the low (10^4), medium (10^5) and high (10^6) dose infected groups were sacrificed on days 3, 7, 14, 21 and 42 days after infection.

The peritoneal exudate cells were collected immediately after sacrifice and a cell count made after pooling the cells of two animals in each group. A cell-smear was made on a slide for nonspecific 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 the cells), the rest were kept frozen at — 20° C till the assay of the other hydrolytic enzymes. In addition, the liver, lungs and spleen were excised, a 10% homogenate in sucrose-EDTA medium prepared and stored at — 20°C. The viable count of both *M.tuberculosis* and *M. bovis* were set up for each mouse with a portion of the spleen.

The results are being analysed.

(started: 1985; expected year of completion: 1987).

Characterization of antigenic components of different tuberculous and non-tuberculous mycobacteria by immunoblot analysis

A sensitive method was adopted for analysing and characterising the antigenic material with the help of Western blotting and subsequent characterisation by immunological screening. Various mycobacterial sonicate extracts were separated by SDS-polyacrylamide gel, and were transferred electrophoretically on to nitrocellulose paper which retains the exact replica of the original gel pattern. The transferred proteins were made to react with appropriate antisera. This was followed by reaction with either radio - or enzyme-labelled protein-A. After autoradiography or enzyme reaction, only the protein bands which reacted with antisera were expressed.

Though immunized rabbit serum (anti-BCG antibodies) showed reaction against most of the antigenic components, pooled sera from tuberculosis patients showed selectivity in their recognition pattern. It was observed that sera from patients recognised components below 14.3 KD region. Out of 10 patients tested, sera from 5 patients recognised this band but none of the 10 volunteers' sera recognised this lower molecular weight component. It was also found that this component is shared by *M. bovis*, *M. kansasii*, *M. scrofulaceum* and *M. gordonae* but not by *M. chelonei* and *M. fortuitum*. It is proposed to further characterise the individual components (e.g. 14.3 KD) that show stronger reaction with sera of patients with pulmonary tuberculosis than with sera of volunteers, in order to assess their usefulness in immuno-assays.

(started: 1985; expected year of completion: 1990).

Studies in characterization and purification of filarial antigens

Qualitative analysis of antibody response (by immunoblot technique) was carried out in 40 subjects (10 with chronic filariasis, 10 with asymptomatic microfilaraemia, 10 with TPE and 10 normals in endemic area) with *S. digitata* crude extract and *B. malayi* crude extract as filarial antigens. With *S. digitata* antigen, it was found that a band in the molecular weight region of 45,000 was recognised by IgG antibodies of all asymptomatic microfilaraemia patients but by none of the subjects in the other three groups. Further characterization and purification of antigen may help in the screening for asymptomatic microfilariasis.

Using the same technique (immunoblot) and the same antigens, qualitative analysis was carried out prior to and at the end of treatment in 8 TPE patients. It was found that with both antigens, there was a decrease in the IgG antibody binding at the end of treatment, with *S. digitata* against a band in the molecular weight region of 21,000 and with *B. malayi* against a band in the molecular weight region of 60,000. A decrease was also observed in IgA antibody binding in the molecular weight region of 60,000 with *S. digitata*.

Further studies of purification of the antigens are in progress.

(started: 1985; expected year of completion: 1987).

Human Leucocyte Antigen (HLA) typing

In order to understand the influence of human major histo-compatibility complex on, and its association with, diseases such as tuberculosis, leprosy and filariasis, it was decided to establish a HLA laboratory at the Centre.

To set up a HLA laboratory, it is essential to have HLA antisera. It is well known that HLA antigens induce iso-antibodies in humans through trans-plants, blood transfusions, and pregnancies. To procure HLA antisera, 81 post-partum haemorrhage blood samples were collected from the Kilpauk Medical College Hospital, Madras. These sera were screened against HLA-A and B-loci antigens using 30 cell panel members. Of these 81 sera, 3 sera had antibodies present against HLA antigens; one of them showed non-specific activity and the other two reacted weakly with HLA-B5 and BW 35.

This work was carried out in collaboration with the Tamil Nadu Forensic Sciences Laboratory, Madras. HLA sera were also procured from other HLA laboratories such as the Institute for Immunohaemotology, ICMR, Bombay, and the Tissue Typing Laboratory, School of Biological Sciences, Madurai. In addition, some HLA sera were purchased from Biotest Co., West Germany. Now, our laboratory is in a position to start HLA typing.

(started: 1985).

Antigen detection assay for circulating antigens in filariasis

Efforts were made to detect filarial antigen using simple ELISA procedure (see 1984 annual report). During the period, 5 monoclonal antibodies, CA 86, CA 101, CA 90, CA 16 and CA 2, specific for circulating filarial antigen, 8 monoclonal antibodies, 4-5, 4-7, 4-8, 5B C5, 4A E5, 7A E8, b7 and 1BE11, specific for adult filarial parasite and 3 monoclonal antibodies, M1b, M5a, and M3a, specific for microfilariae, were generated.

Monoclonal antibodies CA 101 and CA 16 were employed in antigen detection ELISA after trapping the antigen in circulation, using polyclonal rabbit anti-filarial antibody. The results are given in the table below:

Clinical category	Positivity proportion with	
	CA 101	CA 16
Endemic normals	2/9	3/9
mf +ve	10/11	11/11
Lymphoedema	0/7	4/7
Hydrocele	2/6	2/6
Elephantiasis	0/8	3/8
Acute	0/1	0/1

A monoclonal antibody based assay was also developed with a mixture of CA 16, CA 86 and CA 90. This mixture was also biotinylated and used as a tracer antibody in avidin-biotin ELISA. The assay was repeated in 10 non-endemic serum plasma samples and 6 microfilaraemic individuals. The results are presented in the table below:

Serum No.	Expt. 1	Expt. 2	Expt. 3
B10	—	—	—
B14	—	—	—
B16	—	—	—
B25	—	—	—
B33	—	—	—
B34	—	—	—
B35	—	—	—
B36	—	—	—
NA 70232	—	—	—
NA 72245	—	—	—
84-51	+	+	+
84-50	+	+	+
W23	+	+	+

Monoclonal antibodies CA16, CA90, CA101 and CA2 were found to have specificities for paphoryl–choline determinants. Hence, it is proposed to test all other monoclonal antibodies both as trapping as well as tracer agents in the two-site ELISA. More monoclonal antibodies are also being raised against homologous and heterologous parasites with a view to using them for antigen detection.

(started: 1984; expected year of completion: 1987).

EPIDEMIOLOGICAL STUDIES

STUDIES COMPLETED

Quality control of BCG vaccines

The BCG Vaccine Laboratory, Madras, produces BCG vaccine for national consumption. It is also one of the WHO reference laboratories, and receives samples of BCG vaccine for an independent assessment.

The Epidemiological division is assisting the laboratory by providing routine computer and field services in connection with the WHO-assisted quality control system for BCG vaccine in respect of the vaccines produced and examined there.

During the period under report, data arising from investigation of 30 pools of vaccine were analysed and the results made available to the laboratory. In addition, 6 batches of vaccines produced by the laboratory were assessed in terms of skin lesion and allergy following BCG vaccination.

Resurvey for filariasis in endemic villages

Filariasis is an important public health problem in India. Epidemiological information available on this disease is mostly based on single-point surveys of the endemic population. In order to understand the dynamics of the disease transmission, the determinants of filarial infection and disease and the establishment of endemicity, well-planned longitudinal studies need to be conducted.

In 2 endemic villages with an approximate population of 3000, the first resurvey was conducted employing similar methodology as in the base line survey (annual report, 1984). The objectives of the resurvey were to (1) find out the incidence rates of microfilaraemia and different forms of clinical disease, and (2) study the course of infection and disease in the original cohort. The results are being analyzed.

(started: 1985, completed: 1986).

A pilot chemotherapy study for filariasis

A pilot chemotherapy study was started in early 1983 and a two-year follow-up of the 58 patients included in the study was completed in April 1985. The objectives of the study were to (1) develop the measurement tools, follow-up forms and a surveillance system suitable for a community-based chemotherapy trial, and (2) observe the long-term compliance of the patients for drugs and investigations. The forms and tools were pretested, modified and are now available for undertaking chemotherapy trials in the community. A weekly surveillance system was developed. The drug compliance was high (>80% of patients consumed >80% of the chemotherapy). Compliance for frequent blood test was also high. In the microfilaraemia positive group of 14 patients, the microfilaria either disappeared or were remarkably reduced at the end of chemotherapy, and this effect was maintained for at least 1 year. How-

ever, in 5 patients the microfilaraemia persisted in spite of the chemctherapy (12 days), although with highly reduced counts, suggesting the partial nature of the action of drugs. In the group of 39 patients with clinical filarial disease, the disease did not show any significant change even with prolonged (1 year) antifilarial medication.

(started: 1983; completed: 1985).

Inter-physician agreement in filariasis

A study was conducted (1) to quantify the base-line inter-physician agreement for various clinical manifestations (Phase-I), (2) to standardize the examination procedures by physicians based on Phase-I observations (Phase-II), (3) to study the agreement levels after standardization (Phase-III) and compare them with base line findings and (4) to identify 'soft areas' requiring further study.

About 110 patients in Phase-I and 150 patients in Phase-III were examined by three physicians (2 experienced and one new). The patient pool consisted of village patients (endemic areas survey) and out-patients attending the Government General Hospital, Madras, to have the full spectrum of the disease.

In general, the agreement levels were high (kappa $\geqslant$ 0.7) for many manifestations. Some clinical signs with lower agreement levels were identified, and examinations for these conditions were standardized. The agreement levels improved remarkably after standardization, and high levels of kappa ($\geqslant$0.6) were achieved. However, lymph gland manifestations and some of the genital manifestations still remained as 'soft areas' with lower kappa values (0.3 to 0.5).

(started: 1986; completed: 1986).

Inter-observer variation in eliciting signs and symptoms of Acute Respiratory Infections (ARI)

An earlier study had shown that there was a lot of variation in eliciting signs and symptoms due to ARI even between two paediatricians (1984 annual report). The purpose of the present study was (a) to see if the use of standard methods to elicit signs and symptoms would improve inter-observer agreement; (b) to identify signs and symptoms with good observer agreement, and (c) to reconstruct the algorithm for field use, using only these signs and symptoms.

This study was conducted in the Institute of Child Health, Madras. Three paediatricians independently examined 231 consecutive children attending the out-patient department and recorded information on signs and symptoms on precoded forms. They also recorded independently their clinical diagnosis and their management decisions. The data were analysed for agreement on all these factors (reliability) and also for comparison of clinical management decisions with algorithm decisions and clinical diagnosis with algorithm decisions (validity). The kappa value was found to range from 0.12 to 0.96 for agreement between paediatricians with regard to signs and symptoms. The symptoms with a kappa value of $\geqslant$ 0.5

and the signs with a kappa value of $\geqslant$ 0.4 (except for whoop and sore throat) were picked out and the algorithm reconstructed using them. The overall agreement between paediatricians using this revised algorithm was found to be 0.62-0.65, which was a definite improvement over the value obtained in the previous study (0.56). Thus an algorithm was developed which could be tested under field conditions.

(started: 1985; completed; 1985).

Field testing of algorithm for Acute Respiratory Infections

The algorithm developed in the above study was used in the field by health workers on a population of about 350 children. Each child was seen independently by a paediatrician, on the same day that it was seen by a health worker. Symptoms and signs were recorded independently by the health workers and medical officer on precoded forms. The health worker recorded the algorithm decision, but did not initiate treatment. The medical officer did not use the algorithm but recorded the clinical diagnosis and initiated treatment. The information on signs and symptoms were compared for agreement, and the clinical diagnosis and decision were compared with the algorithm decision to assess the validity and reliability of the algorithm. It was found that the reliability of the algorithm was between 0.59 and 0.62, except for one health worker who did badly (K =.49). When compared to clinical management decisions by a paediatrician, the algorithm was found to be 78% sensitive and 71% specific. Using clinical diagnosis as the standard, the algorithm was found to be 77% sensitive and 73% specific. The overall accuracy was 75% using either factor as the standard.

(started: 1985; completed: 1986).

Factors associated with risk of death in Acute Respiratory Infections

A retrospective study was carried out utilising the case sheets of 1303 children admitted to the Institute of Child Health, Madras, with acute respiratory infections over a 1-year period. Information was collected on clinical condition, nutritional status, immunisation status, X-ray status, antibiotics used, and other associated diseases, and compared with the outcome. A 10% sample of case sheets was also checked for accuracy of data collection.

The main conditions were found to be pneumonia and broncho-pneumonia. The case fatality rate was 15% for pneumonia and 16% for broncho-pneumonia. The most important risk factors identified were vitamin A and B deficiency, presence of concomitant diarrhoea and malnutrition. Risk of death did not seem to be determined by age, sex, birth order or number of siblings. There was a slightly lower risk in those immunised.

(started: 1984; completed: 1985).

BCG prophylaxis in tuberculosis and leprosy

The final round of 15-year follow-up of the BCG prophylaxis study in tuberculosis was continued. During the resurvey, 2,64,008 registrations were made and 1,04,283 individuals needed x-ray examinations. Of these, 93,709 (90%) were X-rayed and 210 sputum positive cases were detected from among 8235 persons subjected to sputum examinations.

In addition, 125 sputum-positive pulmonary tuberculosis cases were detected from among 3331 in the selective follow-up group, which comprised of symptomatics and persons showing abnormal shadows in their chest X-rays among those who were sputum negative during previous examinations.

In the Project's central clinic at Tiruvallur and at the health institutions in the study area, 5068 persons were X-rayed and 405 sputum positive cases of pulmonary tuberculosis were diagnosed.

In the BCG prophylaxis study in leprosy, the concluding round of 15-year follow-up was continued. During the period under report, 2,37,996 registrations were made in 87 panchayats. Out of 1,81,126 due for examination, 1,63,715 (90%) persons were examined for evidence of leprosy and 3,378 (2.1%) new cases of leprosy (2,647 definite and 731 suspected) were diagnosed.

(started: 1968; expected year of completion: 1986).

Short-course chemotherapy in pulmonary tuberculosis under programme conditions (Tiruvallur)

The study of short-course chemotherapy in pulmonary tuberculosis under programme conditions (see 1984 annual report) was continued.

Patients who are willing to attend the health centre twice a week for drug administration are offered a fully supervised twice-weekly regimen of 6 months' duration, namely, rifampicin, isoniazid and pyrazinamide for 2 months followed by rifampicin and isoniazid for 4 months, with a supplement of streptomycin where feasible. Patients unwilling to attend the health centre twice a week are offered a daily self-administered regimen of 6 months' duration with weekly attendance at the health centre for collecting drugs, the regimen being rifampicin, isoniazid and pyrazinamide for 2 months followed by rifampicin and isoniazid for 4 months. Patients who refuse even this regimen are offered one of the conventional NTP regimens.

In all, 510 patients have been admitted to the study so far, 156 to the twice-weekly regimen and 354 to the daily regimen. The drug compliance rates (80% or more of the required doses) were 51% and 63% for patients on the twice-weekly and the daily regimen respectively. The follow-up of the patients is under way and information on bacteriological status, drug sensitivity and symptomatology is being systematically collected and recorded.

(started: 1983; expected year of completion of follow-up: 1987).

Expansion of base line surveys

In order to study the natural history of filariasis, it is estimated that longitudinal follow-up of an endemic population of at least 15,000 is required. To fulfil this objective, the filariasis base-line surveys are being extended to other endemic villages. As a part of this effort, one more endemic village with a population of about 600 was surveyed during the year. The preliminary results show that this village is highly endemic, with microfilaraemia and disease rates of more than 15% each.

(started: 1986; expected year of completion: 1987).

Surveillance of a fixed population for epidemiological features of ARI

A pilot study of surveillance for ARI in the community was undertaken in one village (Koppur) in children between 0-5 years of age. The total number of eligible children was 316.

After a complete house-to-house census, each child is seen once in 15 days to collect information (on precoded forms) on the presence or absence of ARI. Those children with ARI are seen again after a week and information on their clinical condition, compliance status and preference for other types of treatment collected. Information on socio-economic status and the knowledge, attitudes and practices of the population with respect to ARI is also being collected. The algorithm developed in the previous study is being used in this surveillance.

Interim analysis of the data shows that the morbidity at any time point is 45%, of which half are common colds. Compliance with treatment given by health workers is practically universal and 70% of children had recovered or improved. However, in 14% of these children, the episode had recurred by one week.

Nine surveillance rounds have been completed so far. The study is in progress.

(started: 1985; expected year of completion: 1988).

A study of causative organisms in ARI

A descriptive study was undertaken in the Institute of Child Health to identify the organisms responsible for causing acute respiratory infections in children between 0-5 years. Children attending the outpatient department of the Institute with ARI were referred by a medical officer for investigations. Specimens such as laryngeal swabs, throat swabs, nasal swabs and nasal secretions were collected by a health worker, and transported to the laboratory in the Centre, for identification of the organism. Information on clinical status and diagnosis, and other socio-economic factors was also collected from these children.

The first phase of this study was limited to children attending as out-patients, upper respiratory specimens, and bacteriological isolations. One hundred and fifty-one children had been admitted by March 1986 and 324 specimens collected. The findings are reported on page 50.

The second phase of the study is on children admitted to the hospital, and includes blood for serology in addition to the other specimens. This phase is in progress.

A laboratory for bacteriological and virological studies has been developed at the Centre for this purpose.

(started: 1985; expected year of completion: 1987).

APPENDICES

TRAINING PROGRAMMES

WHO fellows

Dr. Zakir Ahmed and Dr. Zapa Lenghaia, India, from 29-4-85 to 10-5-85.

Mrs. Pleampis Theskorn and Mr. Pramoth Purach-Kanjana Thailand from 20-5-85 to 31-5-85.

Dr. S.L. Kapoor India from 8-7-85 to 19-7-85.

Mr. Dudley Yariyari, Papua New Guinea from 30-9-85 to 4-10-85.

Miss Shameema Hussain, Maldives from 18-11-85 to 29-11-85.

Trainees

The following underwent training in different departments as follows:

Bacteriology

Graduate Medical Laboratory Technologists from the Voluntary Health Services Medical Centre, Madras.

B.Sc. students from the Loyola College, Madras.

Laboratory Technicians from the Chest Clinic, Pondicherry—3 batches.

Senior Technicians from Arogyavaram Medical Centre, Arogyavaram, Chittoor District, Andhra Pradesh.

Medical Officer from TB Sanatorium, Pondicherry.

Immunology

Dr. Cariappa Annaiah from Radda Barnen Research Laboratory at Schieffelin Leprosy Research & Training Centre, Karigiri, North Arcot District, Tamil Nadu.

Others

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

Medical students

Chengalpattu Medical College, Chengalpattu—1 batch

Medical College, Trichur, Kerala—1 batch

Post-graduate students

MD (TB) and DTCD students from the Medical College, Calicut, Kerala.

MD student from S. V. Medical College, Tirupati, Andhra Pradesh.

MD student from Andhra Medical College, Visakhapatnam, Andhra Pradesh.

MD student from the Rangaraya Medical College, Kakinada, Andhra Pradesh.

Nursing and para-medical students

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

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

Students doing Diploma in Community Health Nursing— 2 batches.

ICMR-WHO SEMINAR

A 3-day Seminar (25th to 27th July, 1985) was organised at the Centre under the joint auspices of the WHO and the ICMR to disseminate knowledge on current trends in short-course chemotherapy and its implementation under District Tuberculosis Programme conditions. The seminar was inaugurated by Mr. R. Shanmugham, I.A.S., Commissioner & Secretary, Health & Family Welfare, Govt. of Tamil Nadu, Madras. The topics covered and the speakers are mentioned below:-

Subject	Speaker
National TB Programme	Dr. N.K. Menon, Madras.
Tuberculosis problem—situation analysis	Dr. P. Chandrasekhar, TB Specialist, National Tuberculosis Institute, Bangalore.
Concept of monitoring and surveillance in tuberculosis	-do-
Relative importance of improvements in the efficiency levels of case-finding, case-holding and chemotherapy under the NTP	Dr. S. Radhakrishna, Director, Institute for Research in Medical Statistics, Madras.
Problems relating to the NTP in Tamil Nadu	Dr. Naina Muhammed, Dy. Director of Medical Services (TB), Govt. of Tamil Nadu.
Symptoms and signs of pulmonary tuberculosis or History of pulmonary tuberculosis through the ages	Dr. K.V. Thiruvengadam, Professor of Medicine, Madras Medical College, Madras.
Sputum microscopy in tuberculosis	Dr. C.N. Paramasivan, Assistant Director, Tuberculosis Research Centre, Madras.
Conventional regimens of chemotherapy under NTP	Dr. R. Parthasarathy, Dy. Director, Tuberculosis Research Centre, Madras.
Principles of short-course chemotherapy	Dr. S.P. Tripathy, Senior Deputy Director-General, ICMR, New Delhi.
Scope, need and value of operational studies under field conditions	Mr. P.R. Somasundaram, Assistant Director, Tuberculosis Research Centre, Madras.
Problems of defaulters and 'lost' cases in DTP	Dr. T. Santha Devi, Assistant Director, Tuberculosis Research Centre, Madras.

Subject	Speeker
Bactericlogy in short-course chemo-therapy programme—Pondicherry and North Arcot District	Dr. R. Prabhakar, Director, Tuberculosis Research Centre, Madras.
Toxicity and hypersensitivity reaction to anti-TB drugs and its management with special relation to STC	Dr. K.C. Mohanty, Department of TB and Respiratory Diseases, J.J. Group of Hospitals, Bombay.
STC in Bombay City TB control pro-gramme	-do-
Short-course chemctherapy-patients' acceptance	Dr. K. Jagannath, Superintendent, Govt. TB Sanatorium, Tambaram.
Sputum-negative pulmonary tuberculosis	Dr. K.V. Krishnaswami, Director (Retd.) Institute of TB & Chest Diseases, Madras.
Short-course chemotherapy in relation to pulmonary tuberculosis in children	Dr. Vimlesh Seth, Associate Professor of Paediatrics, All India Institute of Medical Sciences, New Delhi.
Failure regimens	Dr. Rani Balasubramanian, Senior Research Officer, Tuberculosis Research Centre, Madras.
Abdominal tuberculosis	Prof. N. Madanagopalan, Gastro-entero-logist, Govt. General Hospital, Madras.

STAFF DEVELOPMENT PROGRAMME

1. Dr. Manjula Datta and Dr. R.V.S.N. Sarma participated in the III INCLEN meeting at Cavate, Philippines during January 27th—Feb. 2nd, 1985.

2. Dr. C.N. Paramasivan participated in the workshop in connection with the short-term training course on Hybridoma Technology under the National Biotechnology Board at the School of Biological Sciences, Madurai-Kamaraj University, Madurai, during April 15th-May 4th, 1985.

3. Dr. V. Kumaraswami was awarded a WHO fellowship for 6 months from April, 1985 to undergo training in Clinical Immunology at the Royal Postgraduate Medical School and Hammersmith Hospital, London, UK.

4. Dr. P.R. Narayanan was awarded a fellowship for 1 year under the National Biotechnology Board Overseas Junior Associateship, Department of Science and Technology, Govt. of India, to work on genetic engineering at the Laboratory of Viral Diseases, National Institutes of Health, Bethesda, Maryland, USA., from June 28, 1985.

5. Mr. Abdul Ravoof was awarded a 1-year fellowship under the Colombo Plan to undergo training in the immunology of tuberculosis at the Royal Postgraduate Medical School and Hammersmith Hospital, London, UK., from July 1, 1985.

6. Mr. P.V. Krishnamurthy was awarded a WHO fellowship for 18 months from July 1985 to acquire training in scientific data management at McMaster University, Canada.

7. Dr. T. Santha Devi, Dr. C.N. Paramasivan, Mr. P.R. Somasundaram, Mr. M.S. Krishnamurthy and Mr. A.M. Diwakara participated in the First ICMR Workshop on "Scientific Communication in Biomedicine" held at Hyderabad during November 20-21, 1985.

8. Dr. C.N. Paramasivan participated in the training course on "Modern aspects of monoclonal antibody production by hybridomas", held at the National Institute of Immunology, New Delhi, during December 2—14, 1985.

9. Mrs. Ambujam Ganesh was awarded a WHO fellowship for 3 months from January, 1986 to visit different centres in the USA for acquiring field experience in tuberculosis and leprosy.

10. Dr. Manjula Datta and Dr. R.V.S.N. Sarma participated in the Centre for Diarrhoeal Diseases/Tropical Diseases Research workshop on "Methods for Research in Tropical and Diarrhoeal Diseases" at the National Institute for Cholera and Enteric Diseases, Calcutta, during February 17—25, 1986.

11. Mr. R.S. Vallishayee participated in the International Epidemiological Association Regional Scientific Meeting, held at M.L.B. Medical College, Jhansi, during February 25–28, 1986.

PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

Name of conference, venue and date	Title of paper	Name of staff member
Indian Immunological Society Conference, Hyderabad, 2—4 January, 1985	Characterisation studies of monoclonal antibodies against *M. avium intracellulare*	Dr. C.N. Paramasivan
Symposium on 'Laboratory aspects of tuberculosis' at the 39th National Conference on Tuberculosis & Chest Diseases, Cuttack, 28—31 January, 1985	Role of sputum microscopy in tuberculosis	-do-
-do-	Non-tuberculous mycobacteria: An over view	-do-
-do-	Immunodiagnosis of tuberculosis	Dr. Rajiswamy
39th National Conference on Tuberculosis & Chest Diseases, Cuttack, 28—31 January, 1985	Short-course Chemotherapy under District Tuberculosis Programme	Dr. N. M. Sudarsanam
-do-	Short-course Chemotherapy in tuberculous lymphadenitis	Dr. Rajeswari Ramachandran
National Biotechnology Board Short-term training course on Hybridoma technology, Madurai-Kamaraj University, Madurai, 15 April—4 May, 1985	Application of monoclonal antibodies in parasitic diseases	Dr. Ramesh S. Paranjape
-do-	Screening assay in hybridoma technology	-do-
-do-	Purification and characterisation of monoclonal antibodies	-do-
-do-	Monoclonal antibodies —a general review	Dr. C.N. Paramasivan
-do-	A review on monoclonal antibodies available against mycobacteria	-do-

Annual meeting of the Society for Clinical Trials, New Orleans, 12—15 May, 1985	Clinical trials in 3rd world	Dr. Manjula Datta
Seminar on Occupational Health and Hygiene, Madras, 6 July, 1985	Occupational lung diseases	Dr. V. K. Vijayan
Conference of Trained Nurses' Association of India, Hyderabad, 9-10 October, 1985	The last chapter of my service book	Mrs. J. L. Monga
XXVIth Annual Conference of the Association of Microbiologists of India, Madras, 10—12 October, 1985	Cerebrospinal fluid lysozyme in the diagnosis of tuberculous meningitis	Dr. N. Selvakumar
-dc-	Observation on the cultivation of *M. leprae* and *M. tuberculosis* in medium 'V' and 'V_1'	Mr. Daniel Herbert
XXVIth Conference of Indian Society of Gastroenterology, Madras, 1—5 November, 1985	Clinical trial in abdominal tuberculosis	Dr. Rani Balasubramanian
40th National Conference on Tuberculosis & Chest Diseases, Shillong, 16—18 November, 1985	Changes in pulmonary function in Bhopal gas tragedy victims	Dr. V. K. Vijayan
-do-	The role of para-medical personnel in NTP	Mrs. J. L. Monga
-do-	Chemotherapy studies in TB meningitis in children	Dr. Padma Ramachandran
WHO/SWG/Filariasis XIIth Meeting on Immunopathology of Filariasis, Thanjavur, 19—22 November, 1985	Clinical bancroftian filariasis; an appraisal of some issues	Dr. V. Kumaraswami
V National Congress on Respiratory Diseases, Jaipur, 13—15 December, 1985	Broncho-alveolar lavage in Tropical Pulmonary Eosinophilia	Dr. V. K. Vijayan
IX Annual Conference of the Indian Society of Psychiatric Social Work, Madras, 14—16 December, 1985	Social work a reality	Mrs. Beena E. Thomas

IX Annual Conference of the Indian Society of Psychiatric Social Work. Madras, 14—16 December, 1985.	Social work intervention with reference to tuberculosis	Mrs. Sudha Ganapathy
-do-	Meaning of illness to patients and their families with reference to tuberculosis of spine	Mrs. K. Thilakavathi
12th Annual Conference of Indian Immunology Society, Trichur, 14—16 December, 1985	Isolation of antigens from *M. tuberculosis* H37 Rv using monoclonal antibody affinity column	Dr. Rajiswamy
-do-	Reduced lymphocyte response to mitogens in patients with Bancroftian filariasis	Miss. C.R. Vanamala
-do-	Oral BCG induced protective immunity and the influence of environmental mycobacteria on the immune response to BCG Vaccination	Mrs. Sujatha Narayanan
Annual Conference of Association of Physiologists and Pharmacologists of India, Calcutta, 26—28 December, 1985	Small airways disease in pulmonary tuberculosis patients treated with short course chemotherapy	Mr. K. V. Kuppu Rao
II National symposium on Hepatitis B virus infection, Madras, 22 January, 1986	ELISA and its application in the detection of hepatitis	Dr. C.N. Paramasivan
The XIV All India Biennial Conference of Indian Association of Leprologists, Jabalpur, 23–25 January, 1986.	A double–blind controlled clinical trial to assess the role of anti-histamines in the treatment of multibacillary leprosy.	Dr, A. Thomas
International Epidemiological Association Regional Scientific Meeting, M.L.B. Medical College, Jhansi, India, 25—28 February, 1986	Measurement of observer agreement	Mr. R.S. Vallishayee

LIST OF PUBLICATIONS

Papers published

1. Rajajee, S., Pushpa, V. and Narayanan, P.R. Surface markers of lymphoblasts in acute lymphoblastic leukaemia. *Indian Journal of Haematology*, 1984, *2*, 234-235.

2. Kumaraswami, V. and Narayanan, P.R. Eosinophils and the lung in tropical pulmonary eosinophilia. *Lung India*, 1985, *3*, 25-26.

3. Ramesh S. Paranjape, Kumaraswami, V., Prabhakar, R. and Narayanan, P. R. Increased serum levels of anti-filarial (IgA) antibodies in patients with tropical pulmonary eosinophilia. *Lung India*, 1985, *3*, 27-29.

4. Prema Gurumurthy and Mannering, G.J. Membrane bound cytochrome P-450 determines the optimal temperatures of NADPH—cytochrome P-450 reductase and cytochrome P-450—linked monooxygenase reactions in rat and trout hepatic microsomes. *Biochemical and Biophysical Research Communications*, 1985, *127*, 571-577.

5. Paramasivan, C.N., Govindan, D., Prabhakar, R., Somasundaram, P.R., Subbammal, S. and Tripathy, S.P. Species level identification of non-tuberculous mycobacteria from South Indian BCG trial area during 1981. *Tubercle*, 1985, *66*, 9-15.

6. Kailasam, S., Jayasankar, K., Kannapiran, M., Krishnamurthy, M.S., Krishnamurthy, P.V. and Raghupati Sarma, G. Serum protein profile in patients with pulmonary tuberculosis. *Indian Journal of Medical Research*, 1985, *81*, 551-557.

7. Parthasarathy, R., Prabhakar, R. and Somasundaram, P.R. Efficacy of 3-month regimen in pulmonary tuberculosis. *American Review of Respiratory Disease*, 1985, *131*, 801-802.

8. Rajajee, S. and Narayanan, P.R. Immune response to BCG vaccination in children. *Journal of Tropical Paediatrics*, 1985, *31*, 85-89.

9. Raji Swamy and Narayanan, P.R. Immunology of tuberculosis—An overview. *Lung India*, 1985, *3*, 65-68.

10. Kannapiran, M., Krishnamurthy, P.V. and Raghupati Sarma, G. Uric acid disposition during intermittent chemotherapy of pulmonary tuberculosis with regimens containing pyrazinamide and rifampicin. *Indian Journal of Medical Research*, 1985, *82*, 116-121.

11. Raghupati Sarma, G. Genetic control of drug metabolism and drug action in man. *Biomedicine*, 1985, *8*, 3-11.

12. Chandra Immanuel, Jayasankar, K., Narayana, A.S.L. and Raghupati Sarma, G. Self-induction of rifampicin metabolism in man. *Indian Journal of Medical Research*, 1985, *82*, 381-387.

13. Selvakumar, N., Acharyulu, G. S. and Prabhakar, R. Cerebrospinal fluid lysozyme in the diagnosis of tuberculous meningitis. *Indian Journal of Medical Research*, 1985, *82*, 479-481.

14. Selvaraj, P. and Pitchappan, RM. Effect of oestradiol dipropionate on the immune system of the pigeon, *Columba livia*. *Developmental and Comparative Immunology*, 1985, *9*, 669-677.

15. Ramesh S. Paranjape, Kumaraswami, V., Prabhakar, R., Subramaniam, S. and Narayanan, P.R. Anti-filarial IgG antibodies in patients with Bancroftian filariasis and tropical pulmonary eosinophilia. *Indian Journal of Experimental Biology*, 1985, *23*, 676-678.

16. Usha Raghavan, Basheer Ahamed, Sujatha Narayanan, Kumaraswami, V. and Thiruvengadam, K.V. Urticaria—some observations. *Aspects of Allergy and Applied Immunology*, 1985, *18*, 81-90.

17. Narayanan, P.R. and Paranjape, R.S. Detection of circulating filarial antigens. *Annals of National Academy of Medical Sciences (India)*, 1985, *21*, 168-172.

18. Kuppu Rao, K.V., Vijayan, V.K. and Prabhakar, R. Small airways disease in pulmonary tuberculosis .patients treated with short course chemotherapy. *Indian Journal of Physiology and Pharmacology*, 1985, *29*, (suppl. 1), 36.

19. Paramasivan, C.N., Daniel Herbert and Prabhakar, R. Non-tuberculous mycobacteria—An overview. *Lung India*, 1986, *4*, 7-12.

20. Paranjape, R.S., Acharyulu, G.S., Krishnamurthy, P.V., Ramachandran, P., Ravoof, A., Narayanan, P.R., Tripathy, S.P. and Prabhakar, R. Cell mediated immunity in tuberculous meningitis. *Indian Pediatrics*, 1986, *23*, 127-134.

21. Narayanan, P.R., Vanamala, C.R., Raja Alamelu, Kumaraswami, V., Tripathy. S.P. and Prabhakar, R. Reduced lymphocyte response to mitogens in patients with Bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1986, *80*, 78-84.

22. Padma Ramachandran, Duraipandian, M., Nagarajan, M., Prabhakar, R., Ramakrishnan, C.V. and Tripathy, S.P. Three chemotherapy studies of tuberculous meningitis in children. *Indian Journal of Tuberculosis*, *33*, 56-65; *Tubercle*, 1986, *67*, 17-29.

23. Paranjape, R.S., Rabia Hussain, Nutman, T.B., Hamilton, R. and Ottesen, E.A. Identification of circulating parasite antigen in patients with Bancroftian filariasis. *Clinical and Experimental Immunology*, 1986, *63*, 508-516.

Papers accepted for publication

1. Raghupati Sarma, G., Chandra Immanuel, Kailasam, S., Narayana, A.S.L. and Venkatesan, P. Rifampin-induced release of hydrazine from isoniazid: A possible cause of hepatitis during treatment of tuberculosis with regimens containing isoniazid and rifampin. *American Review of Respiratory Disease*.

2. Tuberculosis Research Centre, Madras and National Tuberculosis Institute, Bangalore. A controlled clinical trial of 3- and 5- month regimens in the treatment of sputum-positive pulmonary tuberculosis in South India. *American Review of Respiratory Disease.*

3. Rajajee, S. and Narayanan, P.R. Contact sensitisation to DNCB in paediatric population. *Indian Journal of Paediatrics.*

4. Rajajee, S., Pushpa, V., Narayanan, P.R. and Sundaravalli, N. Delayed cutaneous hypersensitivity to DNCB in iron deficiency anaemia. *Indian Pediatrics.*

5. Parthasarathy, R., Raghupati Sarma, G., Janardhanam, B., Padma Ramachandran, Santha, T., Sivasubramanian, S., Somasundaram, P.R. and Tripathy, S.P. Hepatic toxicity in South Indian patients during treatment of tuberculosis with short-course regimens containing isoniazid, rifampicin and pyrazinamide. *Tubercle.*

6. Selvakumar, A., Selvaraj, P., Damodaran, C. and Chandrasekaran, P. Screening of sera from South Indian pregnant women for the presence of HLA antibodies. *Journal of the Forensic Science Society of India.*

7. Rathnasabapathy, S.V., Paramasivan, C.N., Shanmugasundaram, N. and Jagannathan, K. Secondary bacterial flora in patients with pulmonary tuberculosis. *Lung India.*

8. Paramasivan, C.N., Daniel Herbert, Narayana, A.S.L., Prabhakar, R. and Somasundaram, P.R. Evaluation of homogenising substances in estimating the viability of tubercle bacilli. *Indian Journal of Medical Microbiology.*

9. Acharyulu, G.S., Narayanan, P.R., Krishnamurthy, P.V. and Prabhakar, R. Elisa: Its reproducibility and its use in tuberculosis case finding. *Indian Journal of Tuberculosis.*

10. Vijayan, V.K., Jain, S.K. and Misra, N.P. Changes in pulmonary functions in victims of Bhopal tragedy. *Indian Journal of Tuberculosis.*

JOURNAL CLUB

Meetings of the Journal Club were held each week. Scientific articles of interest, comprising a wide range of subjects, were reviewed by different staff members. The relevance of each article to the Centre's activities was discussed at the end of each review. In all, 41 articles were reviewed by 36 staff members.

Under the auspices of the Journal Club, guest lectures by eminent scientists were also arranged.

GUEST LECTURE

Dr. S. Sriramachari, Additional Director-General, Indian Council of Medical Research, New Delhi, gave a talk on "Pathological changes in the victims of the Bhopal tragedy".

LECTURES BY VISITING SCIENTISTS

Subject	Speaker
Tuberculosis survey in Britain	Prof. Wallace Fox, Director, MRC TB & Chest Diseases Unit, London, U.K.
Functional aspects of T-cell cloning for parasitic diseases	Dr. H.D. Engers, WHO Immunology Research & Training Centre, Geneva.
Mechanism of action of anti-tuberculous drugs	Prof. D.A. Mitchison, Royal Postgraduate Medical School, Hammersmith Hospital, London.
Can filaria teach us how to control allergy?	Dr. Eric A. Ottesen, Chief, Section of Clinical Parasitology, National Institute of Health, Bethesda, Maryland, U.S.A.
Tropical pulmonary eosinophilia	Prof. F.E. Udwadia, Professor of Chest Diseases, Grant Medical College, Bombay.
State of clinical infectious diseases in the U.S.A.	Dr. Garry Weil, Asst. Professor of Medicine, Washington University School of Medicine, St. Louis, Missouri, U.S.A.
Yellow oleander poisoning	Dr. S. Subash, Asst. Professor of Medicine, Department of Gastro-enterology, Madras Medical College, Madras.
Immunology of BCG vaccination	Dr. Linda Walker, British Medical Research Council, London, U.K.
Mechanism of action of gonadotropin releasing hormone in the interruption of pregnancy in the rat	Dr. R. Sridaran, Asst. Professor of Physiology, Morehouse School of Medicine, Atlanta, Georgia, U.S.A.
Leuko-agglutination test: Its prognostic value in chronic diseases—TB and Cancer	Dr. R.M. Nicholls, Head, Department of Immunogenetics, University of New South Wales, Australia.
Assessment of pulmonary function	Prof. Denison, Medical Research Council, Brompton Hospital, London, U.K.
Forensic techniques relevance to diagnosis	Prof. J.W. Thorpe, Lecturer, Forensic Sciences Unit, Strathclyde University, U.K.

Use of recombinant DNA technology to analyse polymorphism	Dr. Rajan, Albert Einstein College of Medicine, New York, U.S.A.
Epidemiology of tuberculosis	Dr. Stefan Grzybowsky, Emeritus Professor of Medicine, University of British Columbia, Canada.
Case holding and case finding in tuberculosis	Dr. T. Shimao, Director Emeritus, Research Institute of Tuberculosis, Anti-Tuberculosis Association, Japan
The necessity to maintain research and training facilities for tuberculosis in the world	-do-
Macrophage activation mechanism	Dr. T. Hashimoto, Director, Institute of Basic Medical Sciences, University of Tsukuba, Japan.
Effect of interferon in tuberculosis	Dr. D.B. Lowrie, British Medical Research Council, London, U.K.
Leprosy trial in Venezuela	Dr. P.G. Smith, Head, Tropical Epidemiology Unit, London School of Hygiene & Tropical Medicine, London, U.K.
Tuberculosis control in Beijing	Dr. Zhang Li-xing, Deputy Director, Beijing TB Centre, Beijing, China.

DISTINGUISHED VISITORS

1. Dr. Halfdan Mahler, Director-General, WHO, Geneva.
2. Dr. U Ko Ko, Regional Director, WHO, SEARO, New Delhi.
3. Prof. David Denison, Brompton Hospital, London.
4. Dr. S. Pattanayak, WHO, SEARO, New Delhi.
5. Dr. Daw Yin Mya, WHO, SEARO, New Delhi.
6. Dr. Howard Eugene, Department of Pathology, University of Geneva, Switzerland.
7. Dr. Kenneth J. Bart, Centers for Disease Control, Atlanta, Georgia, USA.
8. Drs. Anna-Kari Bill, Anders Hernborg and Ingela Sjogren from the Swedish Embassy and the Department of Lung Medicine, Uppsala University, Sweden.
9. Dr. P. Diesh, Public Health Adviser and Mr. W.B. Rogers Beasly, USAID, New Delhi.
10. Dr. Margaret Braithwaite, Brompton Hospital, London.
11. Dr. Mary C. Wilson, Mt. Auburn Hospital, Cambridge, Massachusetts, USA.
12. Mr. M.J. Sexton and Mr. D.W. Baker, Overseas Development Authority, London.
13. Miss Anne Cockitt, British Council, London.

CONSULTANTS

During the period under review, the following scientists visited the Centre as WHO consultants.

1. Prof. Wallace Fox, Director, MRC Tuberculosis and Chest Diseases Unit, London, U.K. (2 visits)

2. Prof. Stefan Grzybowski, Emeritus Professor of Medicine, University of British Columbia, Vancouver, Canada.

3. Prof. D.A. Mitchison, Professor of Bacteriology, Royal Postgraduate Medical School, Hammersmith Hospital, London, U.K.

4. Dr. T. Shimao, Director Emeritus, Research Institute of Tuberculosis, Anti-TB Association, Japan.

5. Dr. T. Hashimoto, Director, Institute of Basic Medical Sciences, University of Tsukuba, Japan.

PRIZES AND AWARDS RECEIVED BY STAFF MEMBERS

Dr. R. Prabhakar was the recipient of the Dr. P.V Benjamin Memorial Gold Medal Oration Award at the 13th Andhra Pradesh State Tuberculosis and Chest Diseases Workers' Conference, Kakinada, 5th and 6th October, 1985 and delivered an oration on "Laboratory aspects in tuberculosis—an overview".