TUBERCULOSIS RESEARCH CENTRE

CHETPUT MADRAS-600 031

REPORT ON RESEARCH ACTIVITIES DURING 1990

INDIAN COUNCIL OF MEDICAL RESEARCH

NEW DELHI

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The contents of this report should not be reviewed, abstracted or quoted

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PREFACE

The Centre in the recent past has diversified its research activities with an accent on operational research for effective implementation of short course chemotherapy for pulmonary tuberculosis under the district tuberculosis programme in 18 districts of 10 States. The research activities in this context are based on the findings of monitoring of the programme, which identify the lacunae in it. Major constraints encountered in the programme are in case finding and case holding and detailed studies on these components are being carried out employing various strategies at the district level, utilising the available local resources. In addition, efforts towards development of human resources are made by providing in-service training to the personnel involved in this programme through Group Educational Activities and periodic evaluation. Furthermore, the community is activated by health education programmes through audio and visual media, and by promoting leadership in the community. An additional strategy of utilisation of NSS volunteer force from educational institutions is being tried to establish a link between the community and the governmental agencies, which could help in developing optimum utilisation of the health facilities for diagnosis and treatment. Similarly, an ambitious research programme is ongoing in the tribal area situated in a hilly terrain in North Arcot district, utilising the educated youths among the tribal population for case finding and drug delivery. In another study, a novel method for improving case holding is being tried by using a "satisfied customer" approach, namely, motivating new patients with the help of patients who had been treated successfully. Encouraged by the successful outcome of maternal and child health programmes at the rural level, the village Dais (traditional midwives), who are close to the rural community, are being trained for case finding and case holding in the tuberculosis programme. Similarly, in another study, utilisation of multi-purpose workers (MPWs) for retrieval of treatment defaulters is being tried. A pilot study to elucidate the reasons for mal-distribution of anti-tuberculosis drugs in the districts has been initiated, and if need be, large-scale studies in various districts will be carried out concurrently, so that recommendations could be made for adequate drug supply to peripheral health institutions. Sociological studies to estimate the level of health education about tuberculosis prevailing among the employees of some public organisations and NSS student volunteers before they are trained are being carried out, which will enable us to plan suitable intervention measures, if necessary, to improve awareness about the disease.

In addition to large-scale operational research studies, the Centre continues to make invaluable contributions to the National TB Programme in the form of applied and basic research in tuberculosis. Some of the signal contributions in recent years have been in the form of laboratory methodologies that could be reliably and easily applied under field

conditions. Clinical trials carried out at this Centre have shown that short course regimens (6-8 months) for treatment could be applied successfully not only to pulmonary tuberculosis, but also to extra-pulmonary forms of the disease. Short course regimens have been effective in the ambulatory treatment of tuberculosis of the spine, tuberculous lymphadenitis in children and abdominal tuberculosis. A clinical study with short course regimens for tuberculoma of the brain is in progress; this will be of great interest in the management of the disease without recourse to surgery, which is a highly specialised procedure not practicable or suitable for developing nations with limited resources and expertise in neurosurgical techniques.

The number of tablets/capsules to be consumed in a single dose of Short Course Chemotherapy regimens is usually large, which could affect its acceptability and give rise to adverse reactions. A controlled clinical trial has been initiated to find out whether splitting up the 4 oral drugs into two 2-drug combinations and giving them on alternate days would improve acceptability and reduce adverse reactions without affecting efficacy. This is being supported by **in vitro** studies and **in vivo** studies in mice. If successful, convenient means of dispensing the drug combinations in blister-packs will be considered for application under field conditions. Completely oral regimens of chemotherapy containing ethambutol are being evolved to overcome operational constraints in administration of streptomycin injections, to successfully treat patients with drug-resistant organisms and prevent the emergence of drug resistance while on treatment.

Laboratory studies to determine the bioavailability of individual anti-tuberculosis drugs from triple- and double-drug formulations are being carried out to ensure high standards in pharmaceutical preparations. Studies are under way for developing immuno-diagnostic tests and efforts in this direction are made by investigating the various antigenic bands derived from mycobacteria, using monoclonal antibodies. Development of DNA probes for quick, accurate and easy diagnosis of tuberculosis has been a high-priority area of research. Successful outcome of research in these areas could make far-reaching contributions to the National TB Programme. Furthermore, laboratory investigations undertaken in cases of tuberculous meningitis have revealed that adenosine deaminase levels could be used as an additional aid for diagnosis.

Large-scale epidemiological studies have been initiated to develop surveillance methodology and diagnosis of childhood tuberculosis under field conditions, utilising the basic health workers. Considering the global importance of dual infections with HIV and tuberculosis, long-term follow-up studies have been initiated in high-risk groups with HIV infection for observing the natural history of the disease and for evidence of

development of tuberculosis. In addition, the prevalence of HIV infection among patients with tuberculosis is also being estimated in specialised institutions and chest clinics. Voluminous data generated from the multi-disciplinary research activities of the Centre are computerised for speedy and accurate collation and analysis.

The Scientific Advisory Committee, which met on the 29th and 30th of June 1990 under the chairmanship of Dr S.P. Pamra, and the newly-constituted Epidemiology Sub-Committee, which met on the 28th June 1990, gave valuable guidance and helpful suggestions regarding the research activities of the Centre.

The Centre will continue to render able support to the National Tuberculosis Programme by playing a leading role in the identification of priority areas for research, in addition to formulation and successful implementation of research and development programmes.

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OPERATIONAL RESEARCH STUDIES

STUDIES COMPLETED

Knowledge about tuberculosis among educated subjects

Lack of health awareness is a well-recognised lacuna in the utilisation of health facilities. Lack of knowledge about existing health facilities leads to poor utilisation. To educate the community, the Government and the TB Association have taken up publicity measures, using films, slides, posters, flip charts etc. Information is given in mass media like press, video and television for greater publicity. Educated individuals in a community have access to most of these propaganda material. An evaluation of the existing level of awareness among the educated is undertaken in this study.

Methodology: With this in mind we considered educated individuals i.e., among general public with literacy levels of high school and above, and gave them a structured questionnaire on tuberculosis. Of these, one group consisted of college students and the other group, of employed individuals from various sources like banks, colleges, Accountant General's Office, TV Station etc. (convenience sample).

The questions included whether they had watched programmes on TV relating to tuberculosis, cause of TB, symptoms of TB, methods of diagnosing TB, spread of infection in the community, duration of treatment for TB, precautions to prevent the spread of infection, role of isolation, diet, etc., what they would do if they suspected that somebody in their area had got TB, role of bed rest, management of pregnant mother with TB and lactating mother with TB.

Results: A total of 446 questionnaires from 103 students and 343 employed personnel were analysed. Of these, 248 (55.2%) were males and 198 (44.4%) were females; 30.3% were less than 25 years of age, 31% were from 25-35 years, 24.4% from 35-45 years and 13.5% were above 45 years.

The findings are presented in the table on page 13.

Health institutions, books and magazines and radio, TV media seems to be the main sources of information. More than 75% were aware that TB is caused by a germ. Cough was considered to be a predominant symptom (75%); blood spitting was mentioned by 13.3%.

		% of subjects
Source	Health Inst.	19.3
	Books, Magazines	39.5
	Radio, TV, Movie	23.0
	Other TB patients	7.0
	Relatives, friends	11.1
Cause of TB	TB germs	78.0
	Smoking	9.2
	Poor hygiene	2.2
	Others	10.5
Symptoms of TB	Cough	74.9
	Blood spitting	13.3
	Chest pain	1.7
	Fever	2.7
	Loss of weight	4.3
Diagnostic tests	Sputum examination	45.4
	X-ray chest	33.1
	Blood test	12.6
	Mantoux	5.6
	Physical examination	2.4
Method of spread	Cough and spit	72.7
	By water	18.6
	No idea	1.7

		% of subjects
Duration of treatment	2 months	4.6
	6-8 months	17.6
	12-18 months	21.3
	3 years	6.5
	Till symptoms disappear	28.0
	No idea	18.4
Precautions to be taken by TB pts.	By covering the mouth	73.4
	By burning sputum	15.9
	Stop smoking	4.3
	Stop drinking	0.2
	No idea	4.3
Is nutritious diet necessary for TB pts?	No	3.6
	Yes	87.0
	No idea	9.4
Is bed rest essential?	No	28.0
	Yes	58.2
	No idea	13.8
Can mother with TB breast feed her child?	No	61.1
	Yes	14.3
	No idea	24.6

Only 45.4% mentioned sputum examination as a method of diagnosis; x-ray chest was mentioned by 33.1% of patients. In all, 72.7% were aware that coughing and spitting resulted in spread of the disease; 28.0% mentioned that treatment could be stopped when symptoms disappear, while 18.4% had no idea about the duration of treatment.

Conclusion: This study among the educated shows how cough as a symptom of pulmonary tuberculosis is known to 75%, but sputum examination as a method of diagnosis to only 45%. About duration of treatment, there seems to be no clear-cut idea for most of them. In all, 73% were aware of the need for covering the mouth while coughing to prevent the spread of the disease; 87% are of the opinion that nutritious diet was necessary in addition to drugs, and 58% thought bed rest was

essential; 61% felt that a mother with TB should not breast feed her child. This study shows that knowledge about TB is poor even among educated people, and that the campaign against TB, especially to create awareness, needs further strengthening.

(started:1990; completed:1990).

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

"Dais" are traditional birth attendants, conducting deliveries at home in the villages. Primary Health Centres in Tamil Nadu are conducting annual training courses for the village Dais, to teach them to conduct deliveries in a proper way under aseptic conditions. A Dai often lives in the same village and is expected to have good rapport with the villagers. Hence, a pilot study was undertaken in Sriperumbudur to explore the feasibility of utilising the services of Dais for the improvement of the District Tuberculosis Programme.

The aims of the study are :

1. to elicit the opinion of the community regarding the quality of services rendered by Dais;

2. to find out whether the services of Dais could be utilised for the improvement of case-finding in the District Tuberculosis Programme.

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

In order to study the operational aspects of primary health care delivered to the community through Dais, a health visitor and a clinic nurse from the Centre visited 13 villages in Sriperumbudur area. The Dais in these villages were given practical training in identifying chest symptomatics and in collecting sputum specimens from them, for transportation to the Centre.

In order to assess the opinion of the community about Dais and the services rendered by them, a Medical Social Worker interviewed the head of the household or a responsible person in each family in 24 randomly selected villages representing the 12 clusters. A total of 466 houses were visited.

		No.	%
Availability of Dais	Yes	367	79
	No	32	7
	No idea	67	14
Services of Dais	Pre-natal	51	11
	Conducting deliveries	346	74
	Post-natal	3	1
	Treatment for minor complaints	132	28
	Immunisation	25	5
	Others	35	8
	No idea	51	11
Usefulness of service	Very useful	61	13
	Useful	252	54
	Not useful	13	3
	No opinion	71	15
	Not applicable	69	15
	Total	466	100

Analysis of the 466 respondents are given on page 15; 85% of the respondents interviewed were between the age of 15 years and 54 years, 53% of them were illiterates, and 44% were engaged in agricultural work. For any health complaints, 83% of the respondents had utilised Govt. health facilities, 84% had mentioned that they were visited by health personnel at home, and 45% had mentioned that Dais visited them. The other health personnel who visited were Village Health Nurses (VHNs), Community Health Assistants (CHAs, 'Prepare'), Health Visitors and Doctors; 79% had stated that Dais' services were available for them including 51% who had mentioned that they were available any time. When enquired about the type of services rendered by Dais, 74% had mentioned about Dais conducting deliveries, and 28% said that minor complaints were treated by Dais. In all, 84% had mentioned that the Dais' residences were in their respective villages, and 67% of the respondents had mentioned that Dais' services were useful for them. When the heads of the house-holds were visited and interviewed for collecting information on Dais, the social worker also made a preliminary assessment about the awareness of tuberculosis in general, using a questionnaire. After this, a separate one page hand-out in local language (Tamil) containing basic and important information on tuberculosis was given to them to read or get read. The same family member was interviewed a second time (post assessment) within a period of two weeks, using the same questionnaire, to assess the utility of the hand-out in spreading the message among the public.

The questionnaire was based on information relating to the spread of the disease, treatment and prevention aspects. The preliminary findings of the responses before and after distribution of the pamphlet is given on page 17. It is encouraging to note that there was an increase in the awareness of the disease in general in all aspects after distribution of the hand-out.

Health education to the community : An exhibition on TB was conducted at Sriperumbudur on 28.05.90. About 300 villagers, mainly women, came for the exhibition. A film show on tuberculosis, role-play and an exhibition were held in three villages at 7.00 PM, with a view to meet the male members of the community. About 200 persons, mainly males, attended.

Health education of the health personnel in 'Prepare' : Village Dais were given training in identification of chest symptomatics in the community and proper collection of sputum and transportation of the specimens with proper labelling. Training of Dais was repeated on 5 occasions. In addition, periodic training of village animators, community health assistants and village youths on tuberculosis was done at the villages.

Expected right answer	% among those who had heard of TB when first interviewed		% among those who had not heard about TB when first interviewed	
	First interview	Second interview	First interview	Second interview
	Before giving pamphlet %	After giving pamphlet %	Before giving pamphlet	After giving pamphlet %
Category of people affected by TB				
i. Rich and poor	64	92	No idea	77
ii. Rural and urban	56	87	-do-	73
iii. Children and adults	54	90	-do-	81
Need not pay for investigations and treatment	89	100	-do-	96
Preventive measure while coughing is to cover the mouth	25	62	-do-	42
Family members need to be investigated	90	99	-do-	90
Measures to prevent TB is to take BCG vaccination	22	57	-do-	29
No. of persons interviewed	**189**		**48**	

Case finding by village Dais : A total of 238 sputum specimens were collected till December 1990 and examined by smear and culture for **M.tuberculosis**. Of these, 78 specimens had been collected by the Centre's staff along with Dais, of which 4 were positive by smear for AFB. The other 160 sputum specimens were collected by Village Dais independently, of which 24 were found to be smear positive for AFB and 26 of 28 were positive by culture for **M.tuberculosis**. Sensitivity tests to isoniazid and rifampicin were done on all positive cultures. Results are available on 35 cultures (23 pretreatment and 12 during treatment); 8 had resistance to isoniazid and one had resistance to rifampicin.

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

The Centre's Medical Officer/Health Visitor visited these patients at random and did pill count to check about drug regularity and collected urine samples for tests. Minor irregularities were observed in drug consumption and suitable measures were taken for that. Out of 28 patients, only one refused treatment, in spite of repeated motivation by the Centre's Medical Officer and Health Visitor and 'Prepare' Medical Officer and their team. One patient complained of giddiness after taking treatment in the first month of treatment but with reassurance, he felt better. No further complaints were made.

Efforts are also being made to identify and collect sputum samples from chest symptomatics missed by Dais, by visiting a sample of households from each hamlet where treatment is initiated for sputum smear positive patients, and also interview a sample of chest symptomatics already identified by the Dais whose sputum smear was negative.

The study is in progress.

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

Involvement of voluntary agencies in improving the District Tuberculosis Programme

Attempts were made to study the effect of involvement of voluntary agencies in the programme (see 1989 annual report). For this purpose, two agencies working in Madras City were identified. Training programmes by way of lectures and demonstrations were organised for the community workers working in these agencies. In spite of repeated visits and frequent meetings, the referrals of chest symptomatics from these two agencies were not satisfactory. Hence the study has been suspended.

(started:1989; suspended:1990)

Condition of microscopes in the districts

During the year 1990, information was collected on the condition of microscopes from 7 districts, and is presented in the table below.

	No.of micro-scopes	No. with defe-cts	Coa-rse adj-ust-ment	Fine adj-ust-ment	Sta-ge	Sub-sta-ge	Eye pie-ce	Low power obje-ctive	High power obje-ctive	Oil imm-ersion	Con-den-sor	Mir-ror	Any other
N.Arcot	15	10	5	1	3	-	4	-	-	2	2	1	1
Pondy	5	3	1	-	-	1	-	-	1	1	-	1	-
Vidisha	4	4	2	-	1	-	-	2	1	1	1	-	-
Ujjain	4	2	-	1	-	2	-	1	1	1	1	-	-
Sagar	5	4	1	1	1	-	1	-	1	2	2	2	-
Baroda	5	3	1	1	2	1	-	-	-	1	-	-	-
Nagpur	6	1	-	-	-	-	-	-	-	1	-	-	-
Total	**44**	**27**	**10**	**4**	**7**	**4**	**5**	**3**	**4**	**9**	**6**	**4**	**1**

It is observed that 27 (61%) of 44 microscopes needed corrective measures. In the previous (1989) annual report it was reported that 124 (60%) of 206 microscopes needed repairs. These findings suggest that there is need for frequent supervision and a service facility to correct the defects of the microscopes, to effect better case finding.

(started: 1989; completed 1990)

STUDIES IN PROGRESS

Short course chemotherapy under District Tuberculosis Programme

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

The regimens being prescribed are:

1. **$2RHZ_2/4HR_2$** : Rifampicin 600 mg plus isoniazid 600 mg plus pyrazinamide 2.0 g given twice a week for 2 months, followed by rifampicin 600 mg plus isoniazid 600 mg twice a week for the next 4 months, all doses being given under supervision.

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

3. **$2RHZ/4RH_2$** : Rifampicin 450 mg plus isoniazid 300 mg plus pyrazinamide 1.5 g daily for 2 months, followed by rifampicin 600 mg plus isoniazid 600 mg twice a week for 4 months; in the first two months, the drugs are collected once in 15 days for self-administration and in the next 4 months, all doses are administered under supervision in the clinic.

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

Policy A: Regimen 1 with regimen 2 as an alternative.

Policy B: Regimen 2.

Policy C: Regimen 3 with regimen 2 as an alternative.

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

The programme of short course chemotherapy is integrated with the District Tuberculosis Programme; hence, implementation and running of the programme is the responsibility of the staff of the District Tuberculosis Centres and the PHIs (Peripheral Health Institutions). The Centre's staff make periodic monitoring visits to the districts; during 1990, 11 districts were visited at least once by a team from the Centre.

Implementation of the programme : By 1990, 10 districts had implemented short course chemotherapy in 100% of District Tuberculosis Programme implemented PHIs, 3 in 90-99%, 4 in 75-89% and the other district had implemented 55%.

Sputum examination and intake to SCC : The average sputum examination per month from the inception of SCC ranged from 782 to 3538 in the six Policy A districts, 643 to 2238 in Policy B districts and 576 to 1625 in Policy C districts, and the percentage of positivity ranged from 4% to 14%, 7% to 9% and 5% to 10%, respectively. The percentage of eligible patients started on SCC ranged from 47-74 (median 63) in Policy A districts, 29-72 (median 50) in Policy B districts and 42-79 (median 62) in Policy C districts. Considering all the 18 districts, the admissions ranged from 40-59% in 9 districts and 60-79% in 8 districts. In the other district, only 29% were started on SCC.

Detailed analyses on smear positivity in the districts over the years have shown that there was some variation in a few districts but there was no clear-cut trend. Considering all the 18 districts in 1990, the smear positivity was less than 5% in 2 districts, 5-9% in 13 districts and 10-14% in the remaining 3 districts.

Considering the intake to SCC, there seems to be an improvement in the proportion put on SCC over the years in most of the districts. In 1990, 80% or more were put on SCC in 6 districts, 70-79% in 3, 60-69% in 4, 50-59% in 3 and less than 50% in 2 districts, namely 47% and 33%.

The reasons for not starting patients on SCC are given in the table on page 22.

As seen from the table, there is a need to improve the organisational aspects, particularly ensuring uninterrupted supply of SCC drugs and implementation of SCC in all PHIs.

Treatment completion rate : From the inception of SCC, treatment completion rate for 5 different cohort periods (ending June, 1989) are available. In the current cohort, the SCC treatment completion rate is

	No.	%
Total new patients with positive sputum	23,823	
Eligible for SCC	19,165	
Of these, patients not started on SCC	5,196	27%
Reason for not putting on SCC not given	739	15%
Reason for not putting on SCC		
(a) **Attributable to patients**	1,783	35%
Living too far away	645	
Likely to migrate	486	
Too old or sick	270	
Clinic hours not convenient	104	
Initial defaulter	103	
Others	175	
(b) **Organisational/Administrative**	1,926	37%
SCC drugs not available	833	
SCC not implemented	684	
Awaited to start SCC	264	
Others	145	
(c) **Others and non-specific**	748	14%

53% and ranged from 174 to 507 patients (median 322) in Policy A districts, 49% and 89 to 452 patients (median 225) in Policy B districts, and 61% and 109 to 563 patients (median 426) in Policy C districts. The median completion rates for Policy A districts were 46%, 57%, 51%, 54% and 54% for the 5 cohort periods, 48%, 42%, 52%, 48% and 50% for Policy B districts, and 52%, 46%, 52%, 51% and 60% for Policy C districts.

For 2 concurrent cohort periods, comparison of treatment completion rates for SCC and standard regimens are also available and are given in the table on page 23.

Considering standard chemotherapy, there was an increase in the treatment completion rates by 20-30% in 4 districts, 10-19% in 4 districts, and by 7% and 3% in 2 districts; in 4 districts, the treatment completion rate was less by 1%, 2%, 8% and 20%. In the other 4 districts, information was not available for comparison.

District	SCC				Standard Rx			
	7/86-6/87 @		7/87-6/88@@		7/86-6/87		7/87-6/88	
	No. of pts.	≥80% Rx.(%)	No. of pts.	≥80% Rx.(%)	No. of pts.	≥80% Rx.(%)	No.of pts.	≥80% Rx. (%)
Policy A								
N.Arcot	1215	46	915	44	463	27	433	45
Puri	322	52	425	54	218	33	299	32
Baroda	804	50	770	46	281	14	498	35
Thane	1101	75	356*	94	113	22	NA	
Ujjain	270	44	256	54	445	24	364	36
Dehradun	770	75	576	59	503	35	NA	
Median	**787**	**51**	**520**	**54**	**363**	**26**	**348**	**36**
Policy B								
Karnal	311	49	171	46	486	28	365	44
Kanpur	302	75	197	76	708	20	651	43
Nagpur	902	73	792	65	NA		534	68
Rajkot	398	55	339	50	577	17	300	43
Raichur	282	41	309	30	333	37	162	17
Sagar	196	24	352	25	335	24	238	16
Median	**306**	**52**	**324**	**50**	**486**	**24**	**332**	**43**
Policy C								
Pondicherry	417	67	392	69	213	29	NA	
Vidisha	358	26	349	22	159	31	273	34
Aurangabad	200	48	304	53	416	27	380	57
Varanasi	327	68	416	86	469	49	550	56
Sabarkantha	837	33	677	43	607	15	497	13
W.Godavari	256	55	405	49	650	22	696	35
Median	**342**	**52**	**400**	**50**	**442**	**28**	**438**	**35**
Total	**9268**	**56**	**8003**	**53**	**6976**	**26**	**6240**	**40**

* : DTC admission only; NA - Not available.
@ : 7/86 - 6/87 : July 1986 to June 1987.
@@ : 7/87 - 6/88 : July 1987 to June 1988.

Considering the different policies, the median proportions completing 80% or more of treatment with SCC regimens are very similar in the 2 cohorts, ranging from 50% to 54%. Considering the standard regimens, there is an increase between the 2 cohorts from 26% to 36% in Policy A, 24% to 43% in Policy B, and 28% to 35% in Policy C; thus there is an appreciable increase in Policy B, the proportion in the 1987-88 cohort approaching the proportion with SCC regimens.

The median treatment completion rate from the inception of SCC with regimen 1 in the 6 districts with Policy A is 58% (range 45-71%), 52% (range 25-86%) with regimen 2 in 16 districts and 70% (range 38-84%) with regimen 3 in the 6 Policy C districts. Among those who had completed 80% or more of chemotherapy and had their sputum examined at 6/8 months, the median positivity was 1.5% for regimen 1, 1.2% for regimen 2 and 1.8% for regimen 3.

Two districts where SCC implementation was poor were visited by the Centre's staff to find out the lacunae. Two other districts have been visited to find out details regarding the drug position in the various centres in order to suggest a system of distribution. The details are reported elsewhere.

Further, operational studies to improve case-finding and case-holding components are also in progress.

(started:1983)

Training programmes and monitoring

A training programme for the DTC and PHI staff in Baroda district was undertaken, attended by 28 participants. In addition to demonstrations to Lab. Technicians and Drug Distributors, visits to PHIs for on-the-spot training were undertaken. Due to various constraints (financial, weather, socio-political climate), some of the other planned tours had to be postponed and later cancelled.

In the previous years, workshops under the auspices of WHO and ICMR were held at this Centre and could cater to only a few personnel involved in the programme. In 1990, a different approach was adopted by organising workshops at regional levels. The main aim was to enable a larger proportion of PHI personnel to actively participate in these workshops.

The first workshop was held at Ujjain (Madhya Pradesh), involving the districts of Ujjain, Vidisha, Sagar, Baroda and Nagpur. In this

workshop programme, apart from the usual lectures and practical training for paramedicals, panel discussions were introduced. The panels included district and state level officers, and covered the important components of the District TB Programme.

In the second workshop, held at Pondicherry, the participants were from two districts - Pondicherry and North Arcot. For this workshop, there were further modifications, as follows:

(a) Management experts participated, focussing on the importance of management concepts in DTP.

(b) Participants were divided into two groups, along with the Centre's resource persons, to enable them to freely interact. The points focussed upon during these discussions were then presented to a panel, many of whose members were state and district level authorities, for expert opinion. Further details of these two workshops are given in an appendix (see page 141).

Monitoring activities continued throughout the year. With a view to expedite the perusal of 'Monthly Reports on Tuberculosis' received from the districts, six desk monitoring teams had been formed, each comprising of a doctor, a bacteriologist, a statistician and a medical social worker. Each team was assigned three districts - one under each treatment policy. Reports prepared by these teams were discussed in subsequent meetings, and necessary follow-up action initiated. A total of 10 meetings were held during the year.

As part of the monitoring activities, some more PHIs and the DTC were visited in four districts, as detailed below:

	Type of institution			
	DTC	XC	MC	RC
No. visited	4	9	16	2

Computerised analytical data based on the Monthly Reports on Tuberculosis received from the 18 districts were prepared. Copies of these reports have been sent to the concerned district authorities and supervisors of the programme at the directorate level.

As there were frequent requests for drug supplies, visits to two districts were planned and carried out with a view to obtain first-hand information regarding drug issues at the various PHIs and the DTCs of

these districts. Based on the observations, follow-up action by way of correspondence with the concerned authorities has been initiated.

Four booklets were printed and distributed to the PHIs in the 18 districts; the titles are as follows:

1. Some facts about Tuberculosis.
2. Tuberculosis - Some important points (in Tamil).
3. Role of voluntary organisations in TB Control.
4. District TB Programme - Relevant aspects of chemotherapy.

Hand-bills were printed in Kannada and Hindi, giving important points regarding tuberculosis, and distributed primarily to the public of Raichur, Nagpur, Vidisha, Sagar and Ujjain districts.

(started : 1988).

Augmentation of case-holding component of DTP by utilisation of the services of the MPW

The District Tuberculosis Programme has been in operation for 3 decades. Treatment default and discontinuation of treatment are major obstacles for achieving efficiency of DTP. Case-holding has to be augmented to raise the level of achievement from the present status. Various interventional strategies are being planned to improve case-holding. Involvement of Multi Purpose Workers is one such strategy planned.

Aims and objectives : To assess the value of utilisation of the Multi Purpose Worker (MPW) in improving the case-holding component, for all patients initiated on treatment under the DTP.

Materials and methods : Intensive training to Multi Purpose Workers attached to 6 peripheral institutions (PHIs) of West Godavari District was given. A total of 201 MPWs, ranging from 17 to 62 per PHI, were involved in the study.

The local staff at the selected health facilities were given practical training as the first phase of the study. The MPW supervisor was asked to make a note of defaulters, fill up the basic information in a form for each defaulter and to hand over the form to the MPW at the time of the weekly meeting. MPWs were asked to contact defaulters during their routine visit and to find out the reason for default and motivate the patient to attend the PHI. They were provided with forms and were instructed to record the date of visit and the reason for default.

Results : During the period May'89 to February'90, a total of 206 forms were issued by MPW supervisors for defaults; all the 206 patients were visited by MPWs. The following table gives the effect of one visit by the MPW.

	No. of pts.	%
Home visits made by MPW	206	100
Retrieved : within 4 days	68	
in 5-9 days	12	
Total retrieved	**112**	**54**

The following table gives important reasons for default :

Reason	No. of defaults
No drugs in PHI	2
Feels O.K.	4
Distance	2
Private treatment	15
Migrated	32
Expired	14
Total	**73**

This study had to be suspended within a year due to shortage of drugs in the district, and also due to repeated natural calamities.

(started:1989; completed:1990).

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

The National Service Scheme is a major youth programme in the country with the objective of personality development through community service. NSS activities provide students and teachers with opportunities to participate in the development programmes in various sectors including health, both in rural and urban areas. Hence, it was decided to have a task force consisting of NSS volunteers to motivate the community for the Madurai city TB Programme. At the same time it was also decided to activate the TB work in health posts available in the city, with a view to provide health facility near patients' homes.

Aims and objectives :

1. Activation of corporation dispensaries in Madurai for anti-TB work to provide diagnostic and treatment benefits nearer their houses.

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

3. To activate case-finding and case-holding components of Madurai city TB Programme, utilising NSS task force for identifying chest symptomatics through home visits and motivating them to attend for treatment.

4. Utilising NSS volunteers for health education of the community by street plays, dramas etc.

Study area selected : Sellur area in Madurai was chosen for the pilot study. The study was started in ORS Zone I utilising the Aruldaspuram dispensary which was the health post catering to this population. The population covered was 25,000, residing in 19 streets.

Conduct of the study : This consisted of 5 major aspects.

1. **Sensitising the community through health education**: Health education for the students and the community was given through film shows, exhibition and talks. Hand bills about the facts of tuberculosis were distributed in the community. The students were divided into small groups and informal discussions were held regarding identification of chest symptomatics and on facts about tuberculosis.

2. **Activities of corporation dispensaries**: The Chief Medical Officer, Madurai Corporation and the Commissioner of the Corporation were approached regarding activation of corporation dispensaries for

case-finding and case-holding. Several corporation technicians were trained for sputum microscopy. A laboratory with microscope was established in Aruldaspuram dispensary for sputum microscopy. The doctor and paramedical workers of the dispensary were briefed about case-finding, microscopy, treatment regimen, toxicity, treatment card entries and defaulter retrieval.

3. **Enumeration** : A total of 569 NSS students from Meenakshi College and Lady Doak College, Madurai did a door-to-door enumeration survey of ORS Zone I of Sellur and in two days' time, covered the population of 25,000. During their enumeration, 1,251 chest symptomatics were identified and were asked to attend the Aruldaspuram dispensary for sputum examination.

4. **Camp details** : A sputum camp was held in Aruldaspuram Dispensary for 3 days. A total of 372 chest symptomatics attended the camp and sputum smear examination was done for all these symptomatics. They were asked to give a second sputum specimen for examination if the first smear was found to be negative. A total of 287 first smears and 124 second smears were examined. Of these, 411 smears, 23 (from 22 patients) were found to be positive and the patients started on treatment.

5. **Case-holding** : Students visit the patients receiving treatment to check regularity in drug collection, toxicity, drug consumption etc. So far, 75 visits were made by students over a period of 2 months to these 22 patients. The study is ongoing.

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

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

The aim of the study was to estimate the case potential from the out- patient department of a Taluk Headquarters Hospital (XC) and a PHC (MC).

Centres with a large outpatient attendance from two consecutive quarterly reports were selected. Symptomatics as defined in the D.T.P. (those with cough for more than 15 days, chest pain for more than one month, haemoptysis at any time) among those aged more than 15 years were the subjects. These patients were identified by the Centre's Medical Officer in the routine O.P.D. on four consecutive days in Gudiyatham Taluk Headquarters Hospital (XC) and three consecutive days in Odugathur PHC (MC). Medical Officers of the institution were requested to identify symptomatics and refer for sputum examination as usual. The Centre's

Medical Officer looked after male outpatients on one day and female outpatients on another day, the other outpatients being looked after by the PHI Medical Officer. The visits were without prior intimation, and the sputa were examined by the Centre's Lab. Technician. The findings are presented in the table below :

Exam.by MO of	PHI	Period	New OP	New sputum examined		Smear-positive	
			No.	No.	%	No.	%
TRC	Gudiyatham	17.8.90 to 20.8.90	1318	38	2.9	4	10.5
PHI	-do-	-do-	3538	17	0.5	1	(6.0)
Both			4856	55	1.1	5	9.1
TRC	Odugathur	21.8.90 to 23.8.90	265	12	4.50	0	(0.0)

These figures may be compared with routine data obtained from the Quarterly Report of April-June 1989 (see table below).

PHI	New out patients	New sputum examination		Smear-positives	
	No.	No.	%	No.	%
Gudiyatham	76772	1783	2.0	16	0.9
Odugathur	8510	5	0.1	1	(20.0)

At Gudiyatham, the percentage of sputum examination is the same as compared in the quarterly routine report (2.9% and 2.0%), while sputum positivity increased from 0.9% to 10.5%. At Odugathur the percentage of sputum examination was 4.5% as compared with 0.1% in the quarterly routine report. This shows that the symptomatic selection by

the Centre's doctor was better at Gudiyatham, since the positivity rate was much higher.

(started: 1990; expected year of completion: 1991).

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

The Jawadhu hills area is thickly wooded and situated about 900 m above mean sea level, with a population of about 90,000. Some of the villages and hamlets (consisting of 15-20 huts each) are remote from the roads (2-10 km) and have to be reached on foot. During the rainy season, landslips and swollen streams are likely to make them unapproachable. Health care delivery for this population needs to be strengthened, more so because of the physical inability of health workers to reach the interior villages. Regarding National Health Programme, the coverage is about 50% for family planning and 60% for triple vaccine.

A tour was undertaken to get familiarised with the terrain, and to find out the feasibility of developing strategies for improvement of case-finding and case-holding components of DTP, which are especially poor in this tribal area. A total of 8 staff members undertook the tour from the 10th to 12th of January 1990.

The observations were as follows:

(a) There were no facilities available for sputum microscopy at Jamnamarudur PHC.

(b) Quite a number of patients (diagnosed during survey by the Centre's Epidemiology Unit) who were started on treatment had stopped taking treatment prematurely.

(c) Awareness about tuberculosis is poor in these villages.

It was decided to undertake a pilot study in Jawadhu Hills to augment case-finding and case-holding components of District Tuberculosis Programme.

The aim was to find out the feasibility of involving one or more literate youth and the ooran (leader) from each hamlet for identification of chest symptomatics in the community, proper collection of sputum from them, drug distribution to sputum positive patients and documentation of drug supply.

As a preliminary step, 19 roadside hamlets situated around Jamnamarudur have been selected and one literate youth from each hamlet was identified with the help of oorans. Awareness of oorans and these literate youths on tuberculosis has been assessed using a questionnaire. Analysis revealed that the awareness is very poor.

During the next visit to Jawadhu Hills, an exhibition on tuberculosis was conducted and training in case finding and drug delivery were given to literate youths already identified. The pharmacist of Jamnamarudur PHC was also instructed to help the literate youths. A handbook on tuberculosis was also issued to all the literate youths. In future, it is planned to visit Jawadhu Hills at least once a month. Literate youths will identify chest symptomatics, collect a sputum specimen from them and bring the sputum specimens to Jamnamarudur PHC on a scheduled day. The Centre's Lab. Technician will examine the sputum specimens by smear and report the results the same day or the next day. All smear positive patients will be started on a Short Course Regimen - 2EHRZ/6TH in the village the next day by the literate youth, in the presence of the Centre's Physician. Literate youths will supply 15 days' drugs to patients.

The Centre's Physician and Social Worker will do the following during the visit:

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

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

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

The study is in progress.

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

Bacteriological investigations for Short Course Chemotherapy under District Tuberculosis Programme conditions in Raichur district

To assess the efficacy of Short Course Chemotherapy (SCC) regimens under programme conditions, it is important to have reliable information on the sputum culture and sensitivity test results of patients on admission, at the end of treatment and during follow up. Our Centre has taken the responsibility of obtaining this information from 1985 onwards in two neighbouring districts of Madras, namely, North Arcot and

Pondicherry which correspond to treatment policies A and C, respectively (1989 annual report). To assess the same in a district with Policy B, a feasibility study has been undertaken at Raichur district from July 1990.

Initially, DTC Raichur and PHC Matmari were taken up for this study as these two places are geographically close to each other and also among the PHCs, Matmari has a relatively large number of SCC patients. The sputum specimens are sent by post to the Centre, for examination by smear, culture and sensitivity testing.

In all, sputum specimens were collected from 52 sputum positive pretreatment patients and were subjected to bacteriological investigations. All but one were smear positive at the Centre, while culture was positive in 46 (88%) and negative or contaminated in 6 patients. The other specimen was negative by smear but positive by culture.

Drug sensitivity test results were available on 44 cultures; 29 (63%) were fully sensitive to streptomycin (S), isoniazid (H) and rifampicin (R), while the remaining were resistant to one or more drugs, as set out in the table below.

Sensitive to SHR	Resistant to					
	S	H	R	SH	HR	SHR
29	2	8	1	2	1	1

Sputum specimens are being collected from these patients at 3, 8, 12, 18 and 24 months and the intake to this study is continuing.

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

SCC under District Tuberculosis Programme - survey of unimplemented PHIs in the districts

During the monitoring of SCC under DTP, the progress of implementation of PHIs is being scrutinised. There was slow progress of implementation in 4 districts (less than 75% implemented). In May 1990, one district (Policy A, initiated in 1985) had 30% of unimplemented PHIs, another (Policy C, initiated in 1984) had 49%, and a third (Policy B) and a fourth (Policy C) (both initiated in 1984) had 44% and 27%, respectively. With a view to obtain first-hand information about the reasons for this, a visit was undertaken to the former 2 districts, the districts being chosen for operational convenience. A team consisting of a Medical Officer and

a Statistician, with two motor vehicle drivers covered 5,850 km by road for the study.

Observations made during the visit to the PHIs have been recorded and are being processed. Subsequent to the PHI visits, discussions were held with the concerned DTOs and their staff. The detailed analysis of the observations will be presented later.

On subsequent review of the progress of implementation in December 1990, it has been observed that the proportion of unimplemented PHIs in the 2 districts visited had been reduced to 18% and 20%, respectively. The figures in the other two districts, however, remain at about the same level.

(started: 1990).

A study of the supply and stock position of anti-tuberculosis drugs in the districts

The District Tuberculosis Centres are required to keep a stock of anti-tuberculosis drugs for a six-month period. These drugs are supplied to the peripheral health institutions on a three-month indent basis, such that at any given time, a buffer stock of three months' drug requirement will be available. Despite this, inadequate supplies of anti-TB drugs is one of the common reasons quoted for patients not completing treatment, both with short course and standard regimens. This study was therefore undertaken to assess the actual position of anti-TB drugs in the districts.

Methodology: Two districts, West Godavari and North Arcot, were chosen initially based on operational feasibility. A team from the Centre after correspondence with the State administrators, visited all the peripheral health institutions in the districts, verified the stock registers and obtained details of drug receipts and issues. Over a three-month period, the receipt by the DTC and subsequent issue to the PHIs for that period was also collected. For the same period, the number of treatment cards in each centre, and the number of doses consumed or collections made as on each card were also recorded.

The data from North Arcot is shown on page 35. The table gives the position of the drugs according to the stock registers as on 1.1.90. The supply refers to the period between 1.1.90 to 31.3.90.

Name of the drug	DTC supply	PHI's opening balance	PHI's receipt	PHI's issue	Closing balance
Rifampicin (150 mg)	43,395	42,302	44,061	52,477	33,886
Pyrazinamide (0.5 g)	24,090	27,961	24,644	31,553	21,052
Ethambutol (400 mg)	3,02,490	68,626	3,00,986	1,22,218	2,48,816
Isoniazid (100 mg)	20,10,100	21,53,115	19,83,379	20,36,394	20,91,646
Thioacetazone (50 mg)	4,45,500	3,40,120	5,91,709	5,18,897	4,12,962
Streptomycin (0.75 g)	23,859	9,214	31,564	29,912	8,157
Others (H150+T75)	6,31,000	1,88,679	6,02,000	4,00,050	3,90,629
Others (H75+T37.5)	8,300	72,890	22,000	67,903	26,987

It is seen that there is a discrepancy between DTC supply and PHI receipts, as also between the receipts, issues and closing balance.

The actual stock in each PHI at the end of the three-month period, i.e., 31st March 1990, was compared with the estimated requirement of drugs for the next three months. This was done assuming that the number of patients in each PHI over the next three months was unlikely to be very different from the previous three months as the overall completion rate remained the same. The requirement was estimated as follows.

Rifampicin: If in a centre in a given month there are n twice-weekly regimen patients who are regular,then they would have collected a total of n X 9 packets each month. If in addition, a total of y collections have been made by all the defaulters for the three-month period, then the total no. of packets required by the patients for three-months would be (3 X 9n)+y. As there are 3 capsules of rifampicin of 150 mg each in a packet, the total requirement of rifampicin capsules (x) would be three times this, i.e.,

$$x = 3(27n+y)$$

Isoniazid requirement was calculated similarly. If n_1 patients on the twice-weekly SCC regimen had collected their drugs regularly, and if each packet had 6 tablets of INH of 100 mg each, the total number of INH tablets collected by these patients in 3 months would be n_1 X 9 X 6 X 3. If y is the total doses collected by the defaulters in 3 months, then the total tablets collected by the defaulters is 6y. Therefore patients receiving SCC would have collected x_1 doses which is equal to

$$x_1 = 6(27n_1 + y_1)$$

In addition, patients on standard treatment would also require INH. If there are n_2 regular patients on the twice-weekly standard regimen and y_2 is the total number of doses collected by the defaulters, then the patients receiving the twice-weekly standard regimen would have collected x_2 doses, similar to the calculations above, namely

$$x_2 = 6(27n_2 + y_2)$$

If there are n_3 regular patients on daily standard regimens, they would have collected 3 packets each. For a daily dose of 300 mg, each packet would contain 3 x 30 tablets. If there are a total of y_3 collections made by defaulters, then the patients on daily standard regimens would have collected x_3 doses which can be expressed as,

$$x_3 = 3(90n_3 + y_3)$$

The total number of INH tablets of 100 mg required by patients on standard regimens for 3 months is equal to

$$x_2 + x_3 = 3(54n_2 + 90n_3 + 2y_2 + y_3)$$

The total requirement of INH for the next 3 months is

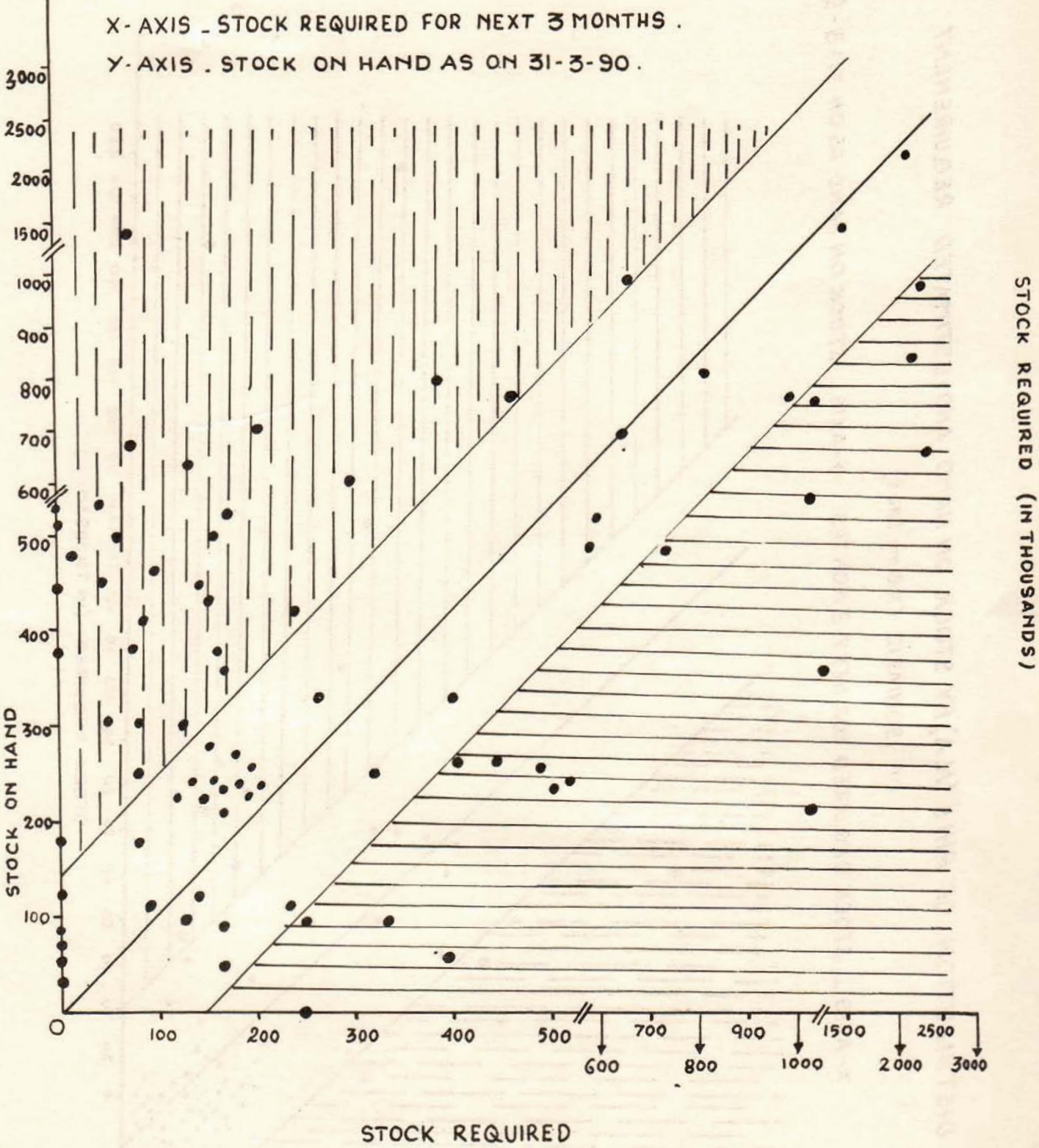
$$x_1 + x_2 + x_3 = 3(54n_1 + 54n_2 + 90n_3 + 2y_1 + 2y_2 + y_3)$$

This estimated requirement of drugs was plotted against the actual stock on hand in the different centres (see diagrams on pages 37 and 38).

It is seen that for both rifampicin and INH, the drug position does not always match the requirement. There are several centres that have excess stock (upper part of diagrams) as well as several with less stock as compared to the estimated requirement (lower part of diagrams).

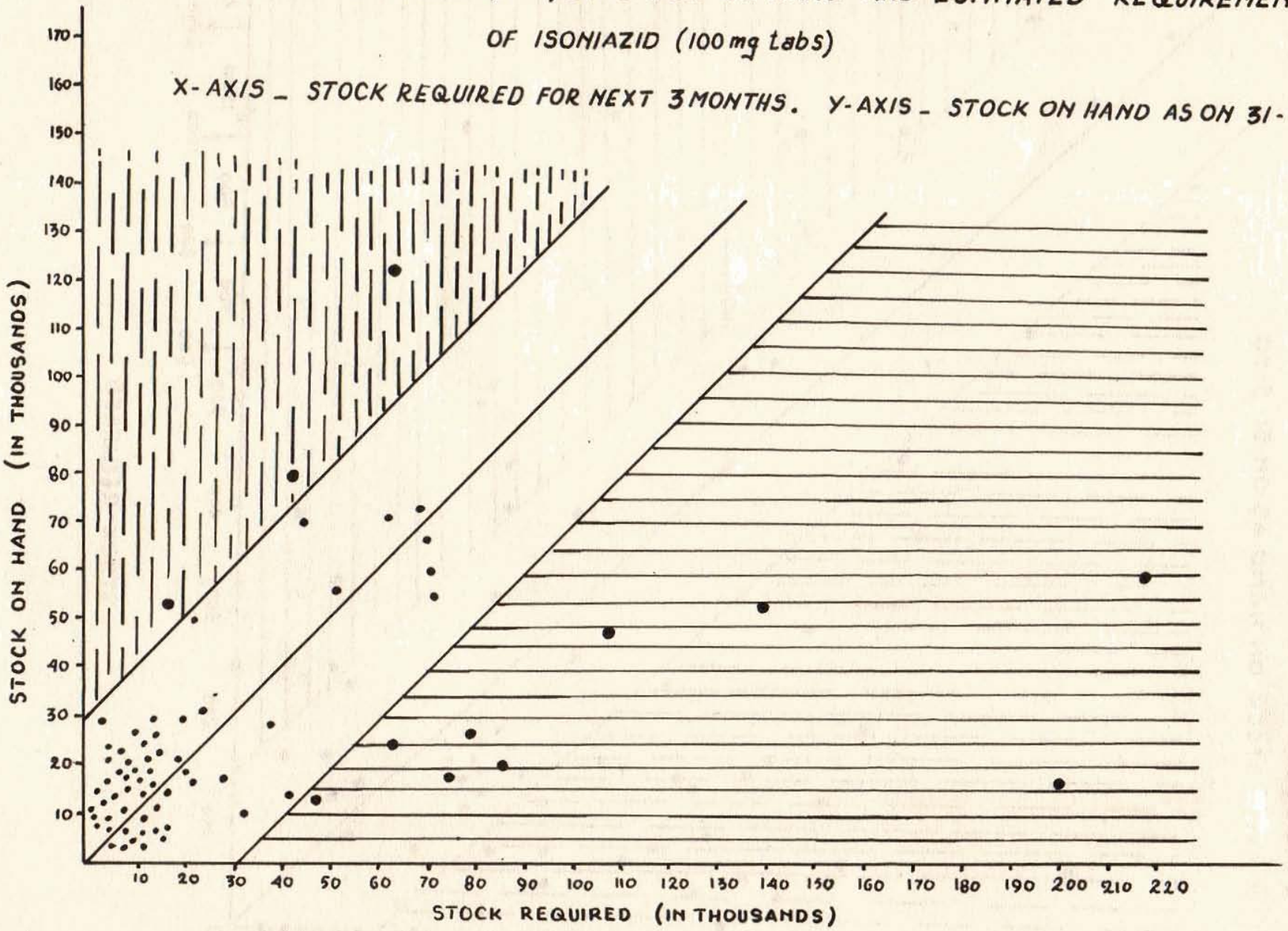
The table on page 39 gives the exact position in the centres.

DISTRIBUTION OF PHIs (N.A.) BY STOCK ON HAND AND ESTIMATED REQUIREMENT OF RIFAMPICIN (150 mg caps)



DISTRIBUTION OF PHIs (N.A.) BY STOCK ON HAND AND ESTIMATED REQUIREMENT OF ISONIAZID (100 mg tabs)

X-AXIS – STOCK REQUIRED FOR NEXT 3 MONTHS. Y-AXIS – STOCK ON HAND AS ON 31-3-90.



Name of PHI	Rifampicin (150 mg)		INH (100 mg)	
	Stock on hand	Stock required	Stock on hand	Stock required
Deficit				
Ambur	516	816	-	-
C.M.C. Vellore	348	1395	24010	60750
Gudiyattam	1092	2577	47821	104646
Thiruppathur	651	2589	57202	214794
Walajapet	762	1260	-	-
RUHSA-Kavanur	240	555	17030	71220
Arani	846	2511	14467	197268
Cheyyar	552	1305	-	-
Polur	195	1278	-	-
Kalambur	237	543	-	-
Thiruvannamalai	771	1053	54638	133956
Adiyur	-	-	26230	77370
St.Thomas Hospital, Chettupattu	-	-	20433	82620
Total	6210	15882	261831	942624
Excess				
Arakonam	1047	693	-	-
Arcot	720	207	78620	41274
Natrampalli	504	162	-	-
Pudupadi	507	-	-	-
Chengam	693	81	52506	16614
St.Thomas Hospital, Chettupattu	822	405	-	-
Kattampoondi	501	-	-	-
Kilpennathur	733	486	-	-
Mallavadi	1640	81	-	-
Melpallipattu	522	-	-	-
Northampoondi	648	138	-	-
Pudhupalayam	549	45	-	-
S.V. Nagaram	603	312	-	-
Vanapuram	534	177	-	-
Vazhur	504	60	-	-
Sholinghur	-	-	66668	43494
Thimiri	-	-	51050	14310
Wandavasi	-	-	124198	61020
Total	9977	2847	373042	176712

It can be seen that for both rifampicin and INH, there appears to be considerable stagnation of drugs in centres with less number of treatment cards. Redistributing these drugs can reduce to a great extent the shortage of drugs in the centres.

However, it must be noted that even if all the excess stock in centres in the lower half of the graph are transferred to the upper half, there would still be a deficit of about 2500 capsules for rifampicin and 5 lakh tablets for INH. This reflects a deficiency in the supply to the district and this needs attention.

The data from the other district are being analysed.

(started 1990; expected year of completion : 1991).

CLINICAL STUDIES

STUDIES COMPLETED

Five-year follow-up of patients with smear-positive pulmonary tuberculosis treated with intermittent short-course chemotherapy

A randomized controlled clinical trial of fully supervised intermittent chemotherapeutic regimens of 6 months' duration in the treatment of sputum positive pulmonary tuberculosis has been conducted at the Centre. The regimens investigated were as follows:

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

3. 2RSHZthrw/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 the RSHZ is administered twice a week during the first 2 months and the dosage of pyrazinamide is 70 mg/kg

Further, half the patients in regimens 1, 2, 4 and 5 were allocated randomly to receive streptomycin 0.75 g with each dose of rifampicin plus isoniazid in the continuation phase.

In all, 1371 patients were admitted to the study. After 125 exclusions, there remained 1246 patients (1023 with initially drug-sensitive bacilli and 223 with initially drug-resistant bacilli) for the analyses of efficacy.

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*12 mg/kg in the latter half of the study.

Of the 1023 patients with drug-sensitive bacilli, 2(0.2%) had an unfavourable response during chemotherapy. One developed miliary tuberculosis and the other had positive sputum cultures at 5 and 6 months. The remaining 1021 patients had a favourable response at the end of chemotherapy; 1004 were assessed for relapse. The bacteriological relapses requiring treatment during a five-year follow-up are presented in the following table :

Initial Rx. (2 months)	Continuation Rx. (4 months)	No. assessed	Total No.	%	Relapse requiring treatment: Year of relapse after stopping Rx. 1st	2nd	3rd	4th	5th (upto 54m)
RSHZ thrice weekly	SRH ow	102	6	6	2	1	3	1	0
	RH ow	103	5	5	3	1	0	1	0
	SRH tw	99	5	5	1	1	2	1	0
	RH tw	99	8	8	2	2	1	1	2
	SH tw	95	6	6	2	1	1	2	0
	Any	498	30	6.0	10	6	7	5	2
RSHZ twice weekly	SRH ow	109	7	6	4	2	1	0	0
	RH ow	103	7	7	7	0	0	0	0
	SRH tw	98	6	6	1	1	2	2	0
	RH tw	94	7	7	5	0	1	0	1
	SH tw	102	11	11	7	1	1	1	1
	Any	506	38	7.5	24	4	5	3	2

Thirty (6%) of 498 thrice-weekly patients had a relapse; the rates were similar for the various continuation regimens and ranged from 5 to 8%. Thirty-eight (7.5%) of 506 twice-weekly patients had relapsed during the same period, the rates ranging from 6 to 11% for the various continuation regimens. None of the differences in the relapse rates, i.e., between thrice-weekly and twice-weekly series in the initial phase or any two of the continuation regimens is statistically significant.

Ten of the 30 relapses in the thrice-weekly and 24 of the 38 in the twice-weekly series occurred during the first year of follow-up. The remaining relapses in both groups were spread over a period of 2-5 years. In a vast majority of cases, the bacilli were drug sensitive at the time of relapse.

There were 223 patients with bacilli initially resistant to one or more drugs; 62 had bacilli resistant to streptomycin alone, 64 to isoniazid alone and 97 to both drugs. Analyses have shown that of 62 patients with resistance to streptomycin alone, 1 had an unfavourable response and 8 relapsed. Since streptomycin resistance did not pose a problem when short course chemotherapeutic regimens were prescribed, these patients were not included for analyses; the findings in patients with initial resistance to isoniazid with or without streptomycin resistance are presented in the table below.

	Total	Unfavourable response		Total	Relapse		Total	Over-all failure	
		No.	%		No.	%		No.	%
SRH tw	39	6	15	33	0	0	39	6	15
RH tw	29	5	17	21	1	5	26	6	23
SRH ow	31	9	29	21	5	24	30	14	47
RH ow	28	11	39	16	1	6	27	12	44
SH tw	34	20	59	14	5	36	34	25	74

The results with thrice-weekly and twice-weekly series during initial phase are similar and are hence amalgamated and presented. The unfavourable response rates and the overall failure rates, that is, unfavourable response plus relapses, are tabulated according to the regimen in the continuation phase. Considering the regimen where streptomycin, rifampicin and isoniazid were given twice a week in the continuation phase, the proportion of patients who had unfavourable response was 15% of 39 patients and the overall failure rate was also 15%. When the same drugs were given without streptomycin, unfavourable response occurred in 17% of 29 patients and the overall failure rate was 23%, similar to the regimen containing streptomycin. In contrast, when the same three drugs were given once a week, unfavourable response occurred in 29% of 31 patients and the failure rate also increased to 47% - significantly higher than the regimen where drugs were given twice a week ($P < 0.02$). When the same drugs were given once a week without streptomycin, the failure rate was similar to the regimen containing streptomycin. These findings clearly show that streptomycin as a third drug in the continuation phase has not conferred any additional benefit. Considering the regimen where no rifampicin was given in the continuation phase, the proportion of patients who had unfavourable response was 59% and the overall failure rate was 74%. The differences between the regimen without rifampicin in the continuation phase and the rifampicin-containing once-weekly regimens ($P < 0.02$) and twice-weekly regimens ($P < 0.00001$) are highly significant.

To conclude, results of 5-year follow-up have shown that fully intermittent regimens containing streptomycin, rifampicin, isoniazid and pyrazinamide in the initial phase of 2 months are effective in patients with drug sensitive organisms. However, in patients with organisms resistant to isoniazid initially, the response is better with regimens containing rifampicin twice-weekly or thrice-weekly for 2 months and twice-weekly in the continuation phase. Streptomycin administered in addition to rifampicin plus isoniazid in the continuation phase, irrespective of the rhythm, did not confer any benefit.

(started: 1980; completed:1990).

Collaborative clinical trial of tuberculous lymphadenitis in children followed up to 54 months after stopping treatment

The 60-month follow-up of patients admitted to the short course chemotherapy study of tuberculous lymphadenitis in children was completed this year. This trial was conducted by the Centre in collaboration with the paediatric surgery departments of the Institute of Child Health and Hospital for Children and the Government Stanley Hospital, Madras (see annual report 1985-86). The subjects were children aged 1-12 years with biopsy-proved tuberculous lymphadenitis (by histopathology or culture). Patients admitted to the study were treated with a fully supervised intermittent 6-month regimen consisting of streptomycin, isoniazid, rifampicin and pyrazinamide thrice a week for 2 months followed by streptomycin and isoniazid twice a week for 4 months. For patients with significant residual lymphadenopathy (exceeding 10 mm) at the end of treatment, a repeat lymph node biopsy was done and the specimen subjected to histopathological and bacteriological examinations. Patients were assessed at regular intervals at the Centre and by the paediatric surgeon.

In all, 197 patients were admitted to the study. After excluding 29 patients, data are available for 168 patients who have been followed up for 60 months from the start of treatment.

In all, 50 patients had repeat lymph node biopsies at the end of treatment. Of these, 2 had positive lymph node cultures and were retreated. These were considered as failures of chemotherapy. Six patients had lymph node histology suggestive of tuberculosis with negative cultures. One of these patients developed a cervical cold abscess 2 months after stopping chemotherapy and was retreated on clinical grounds, while the other 5 have completed post-treatment follow-up uneventfully.

In the follow-up phase, 5 more patients required retreatment for tuberculosis - one each for abdominal tuberculosis in the 4th month of follow-up, tuberculous meningitis in the 36th month, pulmonary and lymph node tuberculosis in the 38th month, spinal tuberculosis in the 42nd month and hilar adenitis in the 43rd month.

In all therefore, 8 (5%) of 168 patients required retreatment, for failure of chemotherapy, relapse of lymph node tuberculosis or for development of tuberculosis at other sites; the remaining 160 (95%) patients had a favourable response at the end of treatment and throughout the period of follow-up.

(started:1980; completed:1990).

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

The detailed findings of the short-course chemotherapy study on tuberculous meningitis patients have already been presented (1988 annual report). To summarise, 215 patients aged between 1 and 12 years were admitted to the study. They were randomly allocated, after stratification according to clinical severity, in equal proportions to the following 2 regimens.

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

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

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

In all, 215 patients were admitted to the 2 regimens (107 to regimen I, 108 to regimen II). Of these, 1 died of a non-tuberculous cause and 29 patients were discharged against medical advice before completing therapy. The response to the allocated regimen could not be assessed in 35 patients, as their treatment had been modified because of the development of hepatitis or ocular changes. The analysis of response to treatment was therefore based on 150 patients, after excluding the above 65. The results showed that 40 patients (27%) had died of tuberculous meningitis, 52 (35%) had neurological sequelae and 58 (39%) had a complete recovery. The response was similar in the two regimens.

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

The follow-up investigations include (a) a complete examination with special reference to the central nervous system, (b) a chest radiograph at 3-monthly intervals for patients who had persistent abnormality at the end of treatment, till they become normal, and (c) cerebro-spinal fluid (CSF) examination for cell count, biochemical characteristics and bacteriological examination for **M.tuberculosis** every three months for patients with abnormal CSF findings at the end of treatment.

In addition, between 48 and 60 months, the following investigations are done: (d) electro-encephalogram; (e) psychometric evaluation; (f) hearing assessment ; and (g) radiographs of skull for evidence of calcification.

Of the 110 survivors at the end of 9 months, 10 died subsequently and the remaining 100 patients (1 with severe, 28 with moderate, 14 with mild sequelae and 57 with complete recovery) have completed the 36th monthly examination, i.e., 27 months of follow-up after stopping treatment. The table given below shows the status at 36 months compared to the status at 9 months.

Status at 9 mths	No.of pts.	Deaths after 9 months due to		Status at 36 months				
		TBM sequelae	Non TB causes	No.eli-gible	Status			Complete recovery
					Severe	Mod.	Mild	
Severe sequelae	5	4	0	1	1	0	0	0
Moderate sequelae	30	1	1	28	0	25	2	1
Mild sequelae	17	2	1	14	0	5	6	3
Complete recovery	58	0	1	57	0	3	4	50
	110	7*	3	100	1	33	12	54

* 5 died between 10 and 24 months.

The one patient with severe sequelae eligible for 36th monthly examination showed no improvement. Of the 28 patients with moderate sequelae, in 25 the status remained same, 2 patients improved to mild sequelae while 1 patient recovered completely. On follow-up of the 14 patients with mild sequelae, in 5 patients the status changed to moderate sequelae, in 6 there was no improvement, while the remaining 3 patients recovered completely.

Of the 57 patients with complete recovery, in 3 the status changed to moderate sequelae and in 4 to mild sequelae, while in the remaining 50 the recovery was maintained. Only 1 patient, who had mild sequelae at the end of treatment, had a relapse in the 12th month (with sensitive organisms) and died in the 31st month.

The 5-year follow-up is continuing.

(started 1982; expected year of completion 1992).

Screening for renal involvement in sputum positive pulmonary tuberculosis patients

This study was started to estimate the frequency of mycobacteriuria among sputum-positive pulmonary tuberculosis patients and to assess the renal functions among those with a urine culture positive for **M.tuberculosis.**

When the sputum smear was reported as positive for AFB, patients were asked to bring the entire quantity of early morning urine on 3 consecutive days in sterile bottles, and the specimens were examined by culture for **M.tuberculosis**. One specimen was examined by culture for non-tuberculous organisms also. Patients who had a positive urine culture for **M.tuberculosis** were investigated by haematological, biochemical and radiological tests and followed up for evidence of renal damage.

A total of 158 patients have been screened. Of these, urine culture was positive for **M.tuberculosis** in 5 patients. One patient could not be followed-up; a second patient refused hospitalisation and no information is available on renal function for this patient. The remaining 3 patients were referred to the Nephrology Department, Govt. General Hospital for further investigations. Intravenous pyelogram was reported to be normal in all the 3 patients. Ultrasonogram was normal in 2 patients; the other patient showed minimal increase in cortical echogenecity and minimal pelvicalyectasis in the ultrasonogram, suggestive of bilateral renal disease. Repeat urine culture for **M.tuberculosis** at the end of chemotherapy has been negative in the 4 patients who were followed up.

(started:1987; completed:1990).

STUDIES IN PROGRESS

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

Earlier studies at this Centre have shown that short-course regimens of 5 to 7 months' duration are highly effective in the treatment of sputum-positive pulmonary tuberculosis. All the regimens tried so far have been fully supervised, included intramuscular streptomycin and were studied in patients who had not received significant previous chemotherapy. These conditions are difficult to apply in the field and hence a prospective study is in progress to investigate three fully oral regimens of 6 or 8 months' duration, with varying frequencies of attendance and different rhythms of drug intake. Patients are randomly allocated, irrespective of previous chemotherapy, to one of the following regimens:

1. **$2EHRZ_7$(ow)/$6EH_7$(tm):** This is a fully self-administered daily regimen of 8 months' duration. Ethambutol 600 mg, isoniazid 300 mg, rifampicin 450 mg and pyrazinamide 1.5 g daily are prescribed for the first 2 months, followed by ethambutol 600 mg and isoniazid 300 mg daily for the next 6 months. The patients are required to attend the clinic once a week during the first 2 months and twice a month during the next 6 months for drug collection.

2. **$2EHRZ_2$/$4EHR_2$(tw) or $2EHRZ_2$/$4EHR_2$(ow):** This is a twice-weekly regimen of 6 months' duration. The patients receive ethambutol 1200 mg, isoniazid 600 mg, rifampicin 450 mg and pyrazinamide 2.0 g during the first two months, and ethambutol, isoniazid and rifampicin in the same dosages during the next 4 months. Half the patients, by random allocation, receive fully supervised chemotherapy at the clinic, necessitating twice weekly attendance throughout. The other half attend only once a week, when one dose is given under supervision and the other dose is supplied for self-administration.

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

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

The intake to the study was completed in October 1990. A total of 1204 patients (601 in Madras and 603 in Madurai) have been admitted to the study. Of these, 842 patients have completed 10/12 months of follow-up and the interim findings are presented here. After excluding 75 patients, there remain 767 patients in the analysis. Of these, 592 patients had organisms sensitive to isoniazid and rifampicin, 153 had organisms resistant to isoniazid and 22 had organisms resistant to isoniazid and rifampicin.

Three sputum specimens are collected every month till the end of chemotherapy and 2 specimens thereafter up to 24 months; they are examined by smear and culture for **M.tuberculosis**. Month by month culture negativity based on multiple specimens in patients with initially sensitive organisms is given in the table below.

Month	Percentage culture negative		
	$2EHRZ_7/6EH_7$ (1)	$2EHRZ_2/4EHR_2$ (2)	$2HRZ_2/4HR_2$ (3)
1	33	23	27
2	89	74	74
3	91	91	87
4	96	97	89
5	97	97	89
6	95	97	88
Total patients (range)	203-202	196-195	193-191

At 2 months, 89% of patients in regimen 1, and 74% in regimens 2 and 3 had all the 3 cultures negative. The difference between regimen 1 and regimens 2 and 3 is statistically significant (P=0.0001). From the 4th month onwards, culture negativity is similar in regimens 1 and 2 but significantly lower in regimen 3 (P=0.01).

An unfavourable response (see top table on page 50) is defined as a total of 2 positive cultures during the last 2 months of treatment; this occurred in 3% of 203 patients initially drug sensitive in regimen 1, 2% of 196 in regimen 2 and 11% of 193 in regimen 3; the difference between regimen 3 and the other 2 regimens is significant (P=0.01).

	Regimen	Total assessed	Unfavourable No.	response %
(1)	$2EHRZ_7/6EH_7$	203	7	3
(2)	$2EHRZ_2/4EHR_2$	196	3	2
(3)	$2HRZ_2/4HR_2$	193	22*	11

Includes 1 tuberculous death.

Relapse requiring treatment: Bacteriological relapse requiring treatment is defined as 2 or more positive cultures in a 2-month period, one of which is at least 20 colonies or more, associated with a positive smear. The relapses up to 18 months (10/12 months of follow-up) in patients with initially sensitive organisms are presented in the table below.

Regimen	Total assessed	Relapse requiring Rx.		Month of relapse after stopping Rx.			
		No.	%	1-3	4-6	7-9	10-12
(1) $2EHRZ_7/6EH_7$	190	6	3	6	0	0	0
(2) $2EHRZ_2/4EHR_2$	192	17	9	8	6	3	0
(3) $2HRZ_2/4HR_2$	169	14	8	9	2	2	1

Of the 190 patients assessed in regimen 1, 3% had a relapse, 9% of 192 in regimen 2 and 8% of 169 in regimen 3.

The response and relapse during the 10/12 month follow-up of patients with initially isoniazid-resistant organisms is given on page 51.

Thirteen per cent of 62 patients in regimen 1, 22% of 41 in regimen 2 and 60% of 50 patients in regimen 3 had an uníavorable response. The difference between the initially 4-drug regimens and the 3-drug regimen attains statistical significance (P=0.001). Relapse occurred in 6% of 54 in regimen 1, 27% of 30 patients in regimen 2 and 11% of 18 in regimen 3.

There were 22 patients (5 in regimen 1, 8 in regimen 2 and 9 in regimen 3) who had resistance to both isoniazid and rifampicin. All except one

Regimen	Total assessed	Unfavourable response		Total assessed for relapse	Relapse requiring Rx.		Month of relapse after stopping Rx			
		No.	%		No.	%	1-3	4-6	7-9	10-12
(1)$2EHRZ_7/6EH_7$	62	8	13	54	3	6	3	0	0	0
(2)$2EHRZ_2/4EHR_2$	41	9	22	30	8	27	8	0	0	0
(3)$2HRZ_2/4HR_2$	50	30	60	18	2	11	1	1	0	0

had an unfavourable response; the patient with a favourable response relapsed in the first month of follow-up. Thus none of the 22 patients with isoniazid and rifampicin resistance could be successfully treated with any of the 3 regimens.

Information on more patients and for a longer period of follow-up will be available in due course.

(started:1986; expected year of completion:1995).

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

Several highly effective rifampicin-containing short course chemotherapy regimens of 6-8 months' duration have been evolved for the treatment of pulmonary tuberculosis. In almost all these regimens, four drugs, namely, rifampicin, isoniazid, pyrazinamide and streptomycin or ethambutol are given together in a single dose, either daily or intermittently. The number of tablets/capsules to be consumed in a single dose is therefore large and the incidence of adverse reactions such as arthralgia and jaundice is high with daily regimens. One of the measures that might help to overcome these difficulties is to split the four oral drugs into two 2-drug combinations, each combination given on alternate days, thus making each two-drug combination intermittent.

The Centre is investigating, both at Madras and its unit at Madurai, a regimen of rifampicin and ethambutol on one day and isoniazid and pyrazinamide on the next day, each combination given thrice a week for the first 2 or 3 months, followed by rifampicin and isoniazid twice weekly for the next 4 and 3 months, respectively. Since both the drug combinations will be given intermittently, the toxicity is expected to be low while the efficacy is unlikely to be affected. If the findings are

promising, this will be a major step towards the possible use of blister packs for drug delivery in TB Programmes. These two regimens are to be compared with a control regimen of rifampicin, isoniazid, pyrazinamide and ethambutol given in a single dose thrice a week for the first 2 months, followed by rifampicin and isoniazid twice a week for the next 4 months. This will provide information as to whether the regimen will be equally effective when all 4 drugs are given together or when they are given as two 2-drug combinations on alternative days.

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

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

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

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

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

A total of 28 patients in Madras and 23 patients in Madurai have been admitted up to December 1990.

The study is in progress.

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

Collaborative clinical trial of tuberculous lymphadenitis at Madurai

A controlled clinical trial of tuberculous lymphadenitis in children aged 1-12 years is being carried out at the Centre's Unit in Madurai in collaboration with the Paediatric Surgery Department (Dr. A.J.Thiruthuvathas) of the Government Rajaji Hospital, Madurai. The study was extended to adults, in collaboration with the Surgery Department (Dr. D. Anantharaj and Dr. M.N. Kamaluddin) in 1989.

Patients, with or without a history of previous chemotherapy, are considered for the study, provided the clinical diagnosis of TB lymphadenitis is confirmed by either histopathology or culture of lymph node biopsy. The histopathology slides are read by Dr V. Ananthalakshmi, Professor of Pathology, Madurai Medical College and bacteriological investigations are done at the Centre at Madras.

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

The patients are assessed clinically at regular intervals at the Centre's Unit and by the surgeons. At the completion of chemotherapy, the patients are assessed by an independent observer who recommends a repeat lymph node biopsy if the patient has significant residual lymphadenopathy. So far, 56 child and 100 adult patients have been admitted and the intake is continuing.

(started:1988; expected year of completion of intake:1991).

Collaborative study of abdominal tuberculosis

As mentioned in previous annual reports (1985-86; 1989) the Centre is carrying out a collaborative study of abdominal tuberculosis. The objectives of this study are:

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

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

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

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

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

Patients with bacteriological, histopathological or radiological confirmation, as well as those with a clinical condition highly suggestive of abdominal tuberculosis, are admitted to the study. Patients are allocated at random to either a 6-month daily regimen or a standard 12-month daily regimen, the details of which are given below:

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

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

Between August 1983 and December 1990, 898 patients were registered and of these, 192 patients were admitted to the study; information up to 1 year is available for 182 patients (91 2RHZ/4RH, 91 SEH/EH).

Characteristics on admission: The mean age was 30 years (range 13-72 years); there were 83 males and 99 females. An initial induration of 10 mm or more to 1 TU of PPD (RT23 with Tween 80) was seen in 75% of the patients. Of the 182 patients, 129 (71%) had intestinal lesions, 79 (43%) had peritoneal tuberculosis, 25 (14%) had hepatic tuberculosis, 17 (9%) had mesenteric tuberculosis and 2 had retroperitoneal tuberculosis; 60 patients had combined lesions.

In all, 139 patients (76%) had presented with pain in the abdomen, in association with tenderness of abdomen or alteration in bowel habits or increased borborygmi or distension of abdomen or anorexia.

Confirmation of diagnosis: Of the 129 patients with intestinal tuberculosis, 111 (86%) had radiographic evidence by barium contrast studies, of whom 53% had either direct histopathological or bacteriological confirmation, or tuberculosis elsewhere in the body (indirect evidence).

Status at the end of Rx	2RHZ/4RH		SEH/EH	
	No.	%	No.	%
Symptom free	75	95	64	88
Clinically improved but still symptomatic	3	4	4	5
Change of Rx for clinical deterioration	1*	1	3	4
TB death	0	0	2	3
Total patients with assessable response	79	100	73	100
Response not assessable				
Non-tuberculous death	4		6	
Received less than 75% of Rx	3		8	
Interruption of 25% or more of Rx	3		0	
Rx modification	2		4	
Total patients	91		91	

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

Of the remaining, 16 patients had histopathological confirmation and two patients were admitted on the basis of laparotomy findings of multiple strictures of the ileum and peritoneal tubercles.

Of the 79 cases of peritoneal tuberculosis, peritoneal biopsy was positive for **M.tuberculosis** in 8, ascitic fluid was positive for **M.tuberculosis,** by smear or culture in 11, and histopathology was positive in 49. Three patients had exudative ascites with sputum positive for pulmonary tuberculosis and 6 had exudative ascites with peritoneal tubercles; 2 patients were admitted on clinical grounds.

Considering the 25 patients with hepatic tuberculosis, all except 6 had histopathological confirmation, while all but 2 of the 17 patients with mesenteric tuberculosis had the diagnosis confirmed by histopathology.

The table on page 55 presents the status at the end of treatment.

Out of 79 patients in the rifampicin series and 73 in the non-rifampicin series, 75 (95%) and 64 (88%) respectively were symptom-free at the end of treatment. Three patients (4%) in the rifampicin series and four patients (5%) in the non-rifampicin series had improved clinically but were symptomatic at the end of treatment. One in the rifampicin series and 3 in the non-rifampicin series had a change of chemotherapy for clinical deterioration, and 2 in the non-rifampicin series had died of tuberculosis.

The patients are being followed up routinely after the end of treatment.

(started: 1983; expected year of completion: 1991).

Collaborative study of brain tuberculoma

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

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

All cases admitted to the study are randomly allocated to one of the following 9-month regimens:

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

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

Chemotherapy consists of 3 drugs, rifampicin, isoniazid and pyrazinamide for 3 months, daily in the first regimen and thrice-weekly in the second regimen, followed by 2 drugs, rifampicin and isoniazid, twice-weekly for 6 months in both the regimens.

The following investigations are done on admission: CT scan, x-ray (chest and skull), CSF culture, culture of sputum and urine, Mantoux, liver function tests and haematological examinations. CT scan is repeated at 1 month, 2 months and every 2 months thereafter till 2 consecutive scans are normal. If the size of the mass at the second monthly scan is more than 80% of the mass on admission, a biopsy of the mass is done for histopathology and culture examinations.

In all, 144 patients (121 from General Hospital and 23 from Railway Hospital) have been admitted to the study. The following analysis is based on 115 patients who have completed treatment after excluding 29 cases for various reasons. The table on page 58 gives the characteristics on admission.

Of the total of 115 patients, 58 were allocated to the daily regimen and 57 to the intermittent regimen; 46 (40%) were males and 69 (60%) females; 34% of the patients were less than 13 years of age, and only 4% were above the age of 44; 91 patients (79%) had a single lesion in the CT scan and 40 patients (35%) had papilloedema. The characteristics in the 2 regimens were similar.

Clinical presentation: Of the 115 patients, 20 presented with papilloedema only, 20 patients had papilloedema with neurological deficit, 18 patients had only neurological deficit and 57 patients had only symptoms.

Factor on admission	Daily		Intermittent		Both	
	No.	%	No.	%	No.	%
Sex:						
Male	24	41	22	39	46	40
Female	34	59	35	61	69	60
Age(years):						
12 or less	17	29	22	39	39	34
13-24	21	36	24	42	45	39
25-34	12	21	4	7	16	14
35-44	7	12	3	5	10	9
45 or more	1	2	4	7	5	4
Lesions:						
Single	45	78	46	81	91	79
Multiple ≤5 lesions	11	19	11	19	22	19
>5 lesions	2	3	0	0	2	2
Papilloedema	20	34	20	35	40	35
Total	58	100	57	100	115	100

Histopathology: Of these 115 cases, 11 patients had surgery done and the specimens histopathologically verified to be tuberculomas.

The table on page 59 shows the progress as assessed by CT scan in 88 patients for whom readings were available.

Scan reading results were available in 88 patients (42 daily, 46 intermittent). Tuberculomas totally disappeared in 69 (78%) of the cases. In 13 (15%), varying degrees of decrease in the size of the lesions were observed. In 2 patients lesion remained static. In 2 other patients, lesions had increased in size and in another 2 patients, new lesions appeared; one patient, who had increased lesion at the end of treatment, refused surgery, and continues to have hemiparesis and papilloedema. All the other 5 patients, including the two patients who had new lesions, the other patient who had increased lesion and the patient whose lesion remained static were clinically doing well.

Scan progress (0-9 months)	Daily No.	Daily %	Intermittent No.	Intermittent %	Both No.	Both %
Lesion disappeared	33	79	36	78	69	78
Lesion decreased						
by ≤ 50%	2	14	3	15	5	15
> 50%	4		4		8	
Static	2	5	0	0	2	2
Lesion increased						
by ≤ 50%	1	2	1	2	2	2
New lesion appeared	0	0	2	4	2	2
Total	42	100	46	99	88*	99

* Excluding 24 patients (15 daily, 9 intermittent) for whom scan was not available for reading and 3 patients (1 daily, 2 intermittent) who died of unknown reasons at 3rd, 2nd and 5th month respectively.

The patients are being followed up till 60 months.

(started: 1983; intake completed: 1990).

Failure and retreatment regimens for patients who fail or relapse on short course chemotherapeutic regimens

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

The chemotherapeutic regimens are as follows :

a) **Patients with bacilli sehsitive to isoniazid and rifampicin**: Such patients are admitted by random allocation to either $3EmbHRZ_2/6HR_2$, or $3EmbHRZ_2/9HR_2$, namely ethambutol 1200 mg plus isoniazid 600 mg (with pyridoxine 10 mg) plus rifampicin 450 mg plus pyrazinamide 2.0g twice a week for the first 3 months, followed by isoniazid plus rifampicin

in the same dosages for either the next 6 months (9m regimen), or the next 9 months (12m regimen). Every dose is given under supervision.

So far, 88 patients have been admitted to these regimens. Of 66 eligible cases who have completed chemotherapy, 21 are excluded -- 2 for change of regimen due to adverse drug reaction, 1 for non-tuberculous death, 1 for early death, 10 for receiving less than 75% of chemotherapy and 7 for admission to study after 60 months of follow-up. Of the remaining 45 (21 in 9m regimen and 24 in 12m regimen), 41 (20 in 9m regimen and 21 in 12m regimen) have shown a favourable bacteriological response. Of the 4 patients showing an unfavourable bacteriological response, 3 (all 12m regimen) had change of chemotherapy for deterioration and 1 (9m regimen) had active disease at the end of chemotherapy.

b) **Patients with bacilli resistant to isoniazid**: Such patients are admitted to $6SEmbRZ_2/6EmbRZ_2$ or $6KEmbRZ_2/6EmbRZ_2$ and prescribed streptomycin 0.75 g, or if the bacilli are resistant to streptomycin, kanamycin 1.0 g, plus ethambutol 1200 mg plus rifampicin 450 mg plus pyrazinamide 2.0 g twice a week for the first 6 months, followed by ethambutol plus rifampicin plus pyrazinamide in the same dosages for the next 6 months (total 12 months). Every dose is given under supervision.

$6SEmbRZ_2/6EmbRZ_2$: So far, 43 patients have been admitted to this regimen. Of the 29 eligible cases who have completed chemotherapy, 8 are excluded, 2 for change of regimen due to adverse drug reaction, 1 for non-tuberculous death, and 5 for receiving less than 75% of chemotherapy. Of the remaining 21, 17 have shown a favourable bacteriological response; of the 4 showing unfavourable bacteriological response, 2 had change of chemotherapy for deterioration, and 2 had active disease at the end of chemotherapy.

$6KEmbRZ_2/6EmbRZ_2$: So far, 45 patients have been admitted to this regimen. Of the 32 eligible cases who have completed chemotherapy, 5 are excluded, 1 for change of regimen due to adverse drug reaction, 1 for non-tuberculous death and 3 for receiving less than 75% of chemotherapy. Of the remaining 27, 21 have shown a favourable bacteriological response; of the 6 showing unfavourable bacteriological response; 5 had change of chemotherapy for deterioration and 1 had active disease at the end of chemotherapy.

c) **Patients with bacilli resistant to isoniazid and rifampicin**: Such patients are admitted to $3S_3EmbEthZ_7/9EmbEthZ_7$ or $3K_3EmbEthZ_7/9EmbEthZ_7$ and prescribed streptomycin 0.75 g thrice a week or, if the bacilli are resistant to streptomycin, kanamycin 1.0 g thrice a week, plus daily ethambutol 600 mg plus ethionamide 500 mg plus pyrazinamide 1.5 g for the first 3 months, followed by daily ethambutol

plus ethionamide plus pyrazinamide in the same dosages for the next 9 months (total 12 months). Throughout the 12 months, the patients attend thrice a week, when they receive that day's dose under supervision and are supplied with drugs for the following day(s) for self-administration.

$3S_3EmbEthZ_7/9EmbEthZ_7$: So far, 17 patients have been admitted to this regimen. Of the 10 eligible cases who have completed chemotherapy, 7 are excluded, 4 for change of regimen due to adverse drug reaction and 3 for receiving less than 75% of chemotherapy. Of the remaining 3, none showed a favourable bacteriological response, and all the 3 had change of chemotherapy for unfavourable bacteriological response.

$3K_3EmbEthZ_7/9EmbEthZ_7$: So far, 28 patients have been admitted to this regimen. Of the 25 eligible cases who have completed chemotherapy, 10 are excluded, 3 for change of regimen due to adverse drug reaction, 1 for non-tuberculous death and 6 for receiving less than 75% of chemotherapy. Of the remaining 15, 6 have shown a favourable bacteriological response; of the 9 showing unfavourable bacteriological response, 8 had change of chemotherapy for deterioration and 1 had active disease at the end of chemotherapy.

The intake to all the regimens is continuing.

(started:1987).

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

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

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

A pilot study to investigate the feasibility of patient-to-patient motivation by utilising a tuberculosis patient who has been regular for treatment to talk to a patient who is being started on treatment has been initiated.

The patients are treated with a 6-month fully supervised regimen with twice weekly attendance. Eight patients have so far been admitted to the pilot study.

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

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

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

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

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

In addition, electrocardiograms are recorded in each patient every year. A total of 175 patients were tested 3-6 years after treatment, and are being followed up yearly.

(started: 1982; expected year of completion:1995).

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

Since wide changes in pulmonary function in normal subjects are known to occur due to ethnic variation, physical activity, environmental conditions, altitude of dwelling, tobacco smoking, age, height, sex and

socio-economic status, a comprehensive study of pulmonary function is being carried out in South Indian subjects residing at Madras. Ethnic South Indians are of Dravidian stock and live in tropical climate at sea level and rice is their staple diet. In all, 85 of a planned total of 160 subjects aged between 7 and 14 years have been studied. Although the study population is not random, attempts were made to obtain a representative cross-section of normal subjects of Madras city. To achieve this, the subjects included relatives of patients attending the Centre and the subjects were eligible for the study if they were ethnic South Indians, with no structural deformity of the thoracic cage, who had been free from respiratory infection for at least three months. None of the subjects had any cardio-respiratory disease, as assessed by detailed history, physical examination, full-plate chest radiographs and 12-lead electrocardiogram.

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

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

Correlation coefficients between pulmonary function and various physical parameters will be obtained, separately for males and females. Normative prediction equations will be developed using the data.

The study is being continued, and the elderly (more than 40 years of age) are also being investigated.

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

Follow-up studies in Tropical Eosinophilia (TE)

Earlier broncho-alveolar lavage and pulmonary function studies had shown that there was intense eosinophilic alveolitis with diffusion defect in Tropical Eosinophilia (J.Clin.Invest, 1987, 80: 216-255). As it had been shown that untreated TE patients presenting with symptoms of long duration could develop intestinal fibrosis, this study was planned to observe the natural history of TE in patients presenting with symptoms of shorter duration (less than 6 months) who had been treated for 3 weeks with Diethyl Carbamazine Citrate (6mg/kg body-weight). In all, 161 patients have been admitted to the study. The follow-up of these patients at 1, 3, 6, 12, 24, 36, 48 and 60 months, utilising the technique of broncho-alveolar lavage and pulmonary function is highly satisfactory.

(started: 1984; expected year of completion of intake: 1992).

Controlled clinical study of multi-drug therapy in multibacillary leprosy

As mentioned in the previous (1988, 1989) annual reports, the Centre is undertaking a controlled clinical trial to assess the relative efficacies of pyrazinamide and rifampicin in combination with clofazimine and DDS in the treatment of multibacillary leprosy, at the Govt. Royapettah Hospital, Madras.

The following 4 regimens are being investigated:

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

II. **NLEP + Addn. of PZA**: Rifampicin, clofazimine and dapsone as in regimen I, plus pyrazinamide 35 mg/kg body-weight daily for the first 3 months followed by 50 mg/kg body-weight twice-weekly for the next 9 months.

III. **NLEP + Extn. of Rif.**: Rifampicin 12 mg/kg body-weight daily for the first 3 months and 12 mg/kg body-weight twice-weekly for the next 9 months followed by 12 mg/kg body-weight once a month, with clofazimine and dapsone as in regimen I.

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

So far, 64 patients have been admitted to the study - 16 patients to each of the four regimens. It is proposed to admit 60 patients to each regimen.

(started:1988; expected year of completion of intake:1996).

A controlled clinical trial of Dapsone with or without clofazimine as continuation chemotherapy beyond 5 years

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

Patients who have completed 60 months of their treatment were stratified according to the average BI value at 57,58,59 and 60 months as 0.5 or more and less than 0.5, and they were randomly allocated to one of two regimens, namely, clofazimine 50mg with dapsone 100mg daily (CD group) or dapsone 100mg daily (D group) for a further period of 2 years.

Patients in interim analysis: In all, 210 patients (104 rif., 106 non-rif.) were admitted to the trial. Of these, 33 patients have been excluded at 60 months (7 died; 10 migrated; 2 had anti-Tb Rx; 14 absconded or got discharged); 8 other patients were not allocated as they had not reported to the clinic and were continuously absent. The remaining 169 patients were allocated to the two regimens - 86 to CD and 83 to D. Of these, 12 patients have been excluded (1 died; 3 migrated; 1 patient had his treatment terminated due to reaction to DDS and 7 failed to attend for treatment for over 6 months) and the findings in the remaining 157 (80 CD and 77 D) patients are presented here.

Clinical Progress: Clinical progress was assessed by an independent assessor who was unaware of the regimen or bacteriological results of the patients, using scores based on semi-quantitative assessments (as described in the previous annual reports). The table on page 66 presents the independent assessor's classification of clinical progress.

Regimen (61-84 months)	Progress	Period (months) 0-60 No.	0-60 %	0-72 No.	0-72 %	0-84 No.	0-84 %
CD (n=80*)	Improvement marked	72	90	70	88	71	93
	moderate	6	8	7	9	3	4
	slight	2	2	2	2	2	3
	No change	0	0	0	0	0	0
	Deterioration	0	0	1	1	0	0
D (n=77*)	Improvement marked	69	91	67	88	69	92
	moderate	6	8	7	9	4	5
	slight	1	1	2	3	0	0
	No change	0	0	0	0	1	1
	Deterioration	0	0	0	0	1	1

* 1 patient not assessed for 0-60m and 0-72m in D group; 4 patients and 2 patients not assessed for 0-84m in CD group and D group, respectively.

Over 0-60 months, moderate or marked improvement was reported in 78 (98%) in the CD group and 75 (99%) in the D group. The corresponding figures were 77 (96%) and 74 (97%) over 0-72 months, and 74 (97%) and 73 (97%) over 0-84 months, respectively. Thus, there was excellent clinical improvement in both series.

Bacterial indices: The main bacterial indices (BI) for the 2 groups at 60, 72 and 84 months are shown in the following table:

Regimen (61-84 m)	BI	Months 60	72	84
CD	Mean	1.16	0.71	0.50
	Range	(0.00-2.83)	(0.00-2.83)	(0.00-1.50)
D	Mean	1.02	0.65	0.50
	Range	(0.00-2.67)	(0.00-1.83)	(0.00-1.67)

The mean BI was 1.16 for the CD group and 1.02 for the D group at 60 months, 0.71 and 0.65, respectively, at 72 months and 0.50 in both the groups at 84 months.

In summary, the interim findings at 84 months show that patients in both regimens have shown similar improvement clinically and bacteriologically. No benefit was seen with the addition of clofazimine for 2 years after 60 months.

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

A controlled study of the efficacy of BCG vaccination in the prevention of tuberculosis in child contacts of patients with pulmonary tuberculosis

This study was undertaken in order to assess the efficacy of BCG vaccination in preventing tuberculosis in the high-risk group of child contacts of patients with sputum-positive pulmonary tuberculosis. It is a double-blind study, in non-BCG-vaccinated close family contacts aged less than five years, who had no evidence of tuberculosis as assessed by a full-plate chest radiograph and a clinical examination by the Centre's physicians. The contacts were allocated at random in equal proportions to BCG administration or to a placebo, after stratification based on the size of the induration to the tuberculin test, into the following categories: (a) 10 mm or more to 1 TU, (b) less than 10 mm to 1 TU and 10 mm or more to 20 TU, and (c) less than 10 mm to both 1 TU and 20 TU.

All these children were kept under close surveillance for a period of five years. They were assessed at the clinic with a chest radiograph and general clinical examination every month for the first 3 months, and subsequently once in 3 months till 12 months, and every 6 months till the end of 5 years. Throughout the 5-year period, at least one home visit was made by a health visitor every month to check on the welfare of the child.

Intake to the study was started in September, 1974 and stopped in April, 1985 and a total of 611 child contacts have been admitted to the study.

The radiographs were read by an independent assessor who was provided with relevant bacteriological and clinical information. Of the 611 children, 57 were found to have evidence of tuberculosis on admission - a prevalence of 9.3%. The following table gives the attack rate of tuberculosis in the 2 series.

As can be seen from the table on page 68, the attack-rate of tuberculosis appears to be similar in the BCG vaccination and placebo series,

	0-9mm			10 mm or more		
	Nil	BCG given	Total	Nil	BCG given	Total
Tuberculosis	39	46	85	43	42	85
Doubtfully tuberculous	5	3	8	2	1	3
Non-tuberculous	22	26	48	17	14	31
Normal	85	77	162	63	63	126
Total	151*	152*	303	125*	120*	245

* A total of 6 x-rays have to be read. (One patient each in 0-9 mm of Nil and BCG groups and two patients each in 10 mm or more of Nil and BCG groups).

irrespective of the initial tuberculin reaction size (P>0.2). Detailed analyses are being undertaken and will be reported in due course.

(started: 1974; expected year of completion: 1991).

LABORATORY STUDIES

STUDIES COMPLETED

CSF Adenosine Deaminase (ADA) and lysozyme (LYSA) levels in CSF of patients with tuberculous meningitis (TBM)

ADA is an enzyme that catalyses the deamination of adenosine into ionosine and ammonia. This was reported to be useful as a diagnostic aid in TBM (Journal of Infectious Diseases, 155, 603, 1987). Earlier we reported the usefulness of LYSA levels in CSF as an additional aid in the diagnosis of TBM (Selvakumar et al., IJMR, 82, 479, 1985). So an investigation was carried out to assess the usefulness of ADA levels in CSF for the diagnosis of TBM and to compare it with lysozyme assay. The findings are presented here.

ADA assay: The ADA assay was performed as described by Giusti et al. (1974). The CSF samples were kept in the deep-freeze (-20°C) for 7 to 10 days before the assay was done. The assay was set up on a routine basis and the details of the samples were not known. On each day of the assay, a pooled sample of CSF was included as a control. The ADA content was expressed in U/l of CSF. The coefficient of variation was found to be 20% for the pooled CSF sample with a mean ADA level of 10.12 U/l in 9 consecutive assays.

Lysozyme assay: The lysozyme content was estimated by the turbidity measurement as described by Jain et al. (1979). A pooled CSF sample was included to monitor the variation in the assay. The lysozyme content was expressed in mcg/ml of CSF. The coefficient of variation for the pooled CSF sample was 15% with a mean LYSA level of 1.43 mcg/ml in 7 consecutive assays.

The frequency distribution, mean, standard deviation and range of ADA values in different groups are given in the top table on page 70. The mean level of ADA in bacteriologically confirmed TBM was 11.6 which was significantly higher than those in the other three groups ($p<0.0001$). In the confirmed TBM group, 25 (96%) of 26 had ADA values of 4 or more. All the 17 samples in the control group and 7 of 10 in the Non-TBM group had values less than 4. If an ADA level of 4 or above was considered as a diagnostic criterion for TBM, the sensitivity and specificity of the test would be 96% and 89% respectively, considering only confirmed TBM.

ADA (U/l)	Groups			
	Confirmed TBM	Clinical TBM	Non-TBM	Control
0-	0	25	3	17
2-	1	10	4	0
4-	6	15	0	0
8-	10	8	3	0
12-	9	3	0	0
Total	26	61	10	17
Mean	11.6	4.5	4.4	0.8
SD	6.2	4.3	4.0	0.5
Range	2.5-25.4	0.1-20	1.0-10.9	0.0-1.6

The frequency distribution, mean, standard deviation and range of LYSA levels in the different groups are given in the table below.

LYSA (mcg/ml)	Confirmed TBM	Clinical TBM	Non-TBM	Control
0-	3	38	5	15
1-	1	4	4	2
2-	2	6	0	0
4-	11	9	0	0
8-	9	4	1	0
Total	26	61	10	17
Mean	6.3	2.1	2.2	0.5
SD	3.4	2.8	3.9	0.6
Range	0.0-10.8	0.0-10.5	0.3-13.1	0.0-1.6

The mean level of LYSA in confirmed TBM was 6.3 which was significantly higher than those in the other three groups ($P<0.0001$). In bacteriologically confirmed TBM, 22 (85%) out of 26 had LYSA values of 2 or more. All the 17 samples in the control group and 9 out of 10 in the Non-TBM group had values less than 2. If a LYSA value of 2 or above was considered as a diagnostic criterion for TBM, the sensitivity and specificity

of the test would be 85% and 96% respectively, considering only confirmed TBM.

The results of ADA and LYSA tests in bacteriologically positive and negative TBM groups are presented in the table below.

Results	Bact.pos.TBM		Bact.neg.TBM	
	Number	%	Number	%
ADA & LYSA positive	22	85	15	5
ADA positive, LYSA negative	3	11	11	18
LYSA positive, ADA negative	0	0	4	7
Both negative	1	4	31	51
Total	26	100	61	100

In bacteriologically positive TBM, 22 (85%) out of 26 were positive by both ADA and LYSA whereas only 15 (25%) out of 61 were positive by both tests among bacteriologically negative TBM. By adopting either of the test criteria, 3 (11%) more cases (all ADA positive, Lysa negative) among bacteriologically positive TBM and 15 (25%) additional cases (11 ADA positive, Lysa negative and 4 Lysa positive, ADA negative) among bacteriologically negative TBM could be diagnosed. Considering both groups, ADA appears to be better since it is able to pick up 14 cases missed by LYSA, compared with only 4 cases picked by LYSA though missed by ADA.

(started:1988; completed:1990).

Bacteriocin typing of M.tuberculosis cultures

Screening of **M.tuberculosis** cultures for the production of bacteriocin yielded 16 types. When pretreatment cultures from 10 patients were tested to check the reproducibility and consistency of this typing, only 5 of them showed consistent results (1988 annual report). It was proposed to screen pretreatment cultures from more patients, after subculturing them

on to 7H9 medium so as to bring them to the log phase. These log phase cultures were inoculated on to LJ medium and screened with a set of 9 indicator strains.

Forty cultures of 17 patients were tested and only 9 patients' cultures gave consistent results. Moreover, all of them belonged to type I. Since cultures from 8 of the 17 patients did not give consistent bacteriocin types and cultures from 9 patients, although showing consistent results, belonged to bacteriocin type I, it was considered that this typing procedure may not give additional information. Hence the study has been discontinued.

(started: 1988; terminated: 1990).

Pyrazinamide susceptibility in patients with initial resistance to isoniazid and rifampicin

In the study of oral SCC regimens (see page 48), patients were treated with EHRZ or HRZ during the first two months. However, in patients who are resistant to isoniazid and rifampicin on admission, these drugs will have very little effect. Pyrazinamide susceptibility tests were performed on the pretreatment cultures of such patients as well as those obtained at the end of the first and second months. As controls, the pretreatment and second month cultures of some patients whose organisms were susceptible to isoniazid and rifampicin on admission were also tested.

In all, cultures from 13 patients with HR resistance on admission and 12 patients with bacilli susceptible to HR were tested. All cultures were coded to conceal their identity. Proportion susceptibility tests on acidified L-J medium (pH 4.85) employing pyrazinamide concentrations of 50 and 100 mg/l were set up. The standard strains, **M.tuberculosis**, H37Rv and **M.bovis** BCG were tested as sensitive and resistant controls, respectively, with each batch of medium. A growth of 5% or more on 50mg/l or 1% or more on 100 mg/l was indicative of resistance to pyrazinamide (Tripathy and others, 1970).

The standard strains H37Rv and BCG were tested on three different occasions. H37Rv was found to be sensitive and BCG resistant to pyrazinamide on all occasions by either definition.

HR sensitivity on admission	No. tested	Pyrazinamide resistant*		
		On admission	1m	2m
Sensitive	12	0	0	0
Resistant	6	0	0	0
	3	0	0	3
	1	0	1	1
	3	3	3	3

* ≥ 5% on 50 mg/l or ≥ 1% on 100 mg/l

All 12 patients who had cultures with organisms susceptible to H and R on admission, were found to be excreting pyrazinamide susceptible organisms on admission and at 2 months. Of the 13 patients with organisms resistant to H and R on admission, cultures from 6 were susceptible to pyrazinamide on admission and at 1 and 2 months while in the case of 3 patients, pyrazinamide resistance was observed on admission itself. One patient became pyrazinamide-resistant at the first month, while cultures from 3 patients were found to be resistant at the 2nd month. Thus, 7 of 13 HR-resistant patients were resistant to pyrazinamide also at the end of 2 months of treatment with pyrazinamide.

(started : 1990; completed : 1990).

Effect of haptoglobin on hemoglobin-supported growth and siderophore production of mycobacteria

Haptoglobin is an acute phase protein. In patients with tuberculosis, the serum concentration of this protein is 3-4 times higher than in healthy subjects and the concentrations return to normal limits with anti-tuberculosis treatment (1985-86 annual report). Haptoglobin has a bacteriostatic effect and it has been suggested that it exerts its effect by forming a complex with hemoglobin and thereby preventing the bacteria from utilizing hemoglobin-bound iron which is essential for the survival of microorganisms. Earlier investigations have shown that hemoglobin iron supports the growth of mycobacteria as well as, if not slightly better than, free iron (1989 annual report). An investigation was therefore undertaken to study the effect of the addition of haptoglobin to a medium containing hemoglobin on the growth of 4 strains of mycobacteria and the production of exochelins and mycobactins, siderophores essential for the sequestration of iron.

The 4 strains investigated were a saprophyte, M. smegmatis, and avirulent (H37Ra), virulent (H37Rv) and South Indian low virulent (SILV) strains of **M. tuberculosis**. These strains were inoculated into a synthetic medium (pH 6.8) containing hemoglobin 29 mcg/ml (equivalent to a free iron concentration of 0.1 mcg/ml) or hemoglobin (29 mcg/ml) plus haptoglobin 58 mcg/ml. (The concentration of haptoglobin to be added to the medium was calculated on the basis of a binding capacity of 50%). At the end of an incubation period at 37°C (8 days for M. smegmatis and 35 days for the 3 M. tuberculosis strains), the cell dry-weights and the concentrations of exochelins and mycobactins were determined after coding the flasks as described earlier (1989 annual report). The experiment was set up in quadruplicate and the mean values are presented in the table below.

Strain	Mean value of the following			
	Addition to the medium	Cell dry weight (g/100 ml)	Exochelin concn. (mg/g dry wt)	Mycobactin concn. (mg/g dry wt)
M. smegmatis	Hb alone	0.960	9.34	25.78
	Hb + Hg	0.070	29.08	58.16
H37Ra	Hb alone	0.186	36.30	42.21
	Hb + Hg	0.030	66.38	132.76
H37Rv	Hb alone	0.274	36.52	61.99
	Hb + Hg	0.036	96.86	116.09
SILV	Hb alone	0.230	26.18	45.72
	Hb + Hg	0.032	56.18	112.36

Hb : Hemoglobin Hg : Haptoglobin

The co-efficient of variation for replicate estimates ranged from 3 to 17% for cell dry-weight, 3 to 21% for exochelin concentrations and 4 to 31% for mycobactin concentrations. An appreciable decrease in the growth of all 4 strains of mycobacteria, ranging from about 84% with H37Ra to 93% with M. smegmatis and a 2-3 fold increase ($P < 0.001$) in the production of both exochelins and mycobactins were observed in the presence of haptoglobin. Thus, despite a significant increase in the production of both siderophores, mycobacteria are unable to utilise iron from the haptoglobin-hemoglobin complex and this has resulted in the severe inhibition observed in the growth. These findings substantiate the

claim that haptoglobin exerts its bacteriostatic effect by withholding hemoglobin-iron from the microorganisms.

(started: 1989; completed: 1990).

Effect of anti-tuberculosis drugs on the growth and siderophore production by high virulent and low virulent strains of M.tuberculosis.

Among a number of mechanisms proposed for the action of isoniazid and ethambutol against tubercle bacilli is the capacity of these two drugs to chelate metal ions essential for the growth of these micro-organisms. An investigation was therefore undertaken to study the effect of sub-MIC levels of these drugs on the growth in vitro and the production of both exochelins and mycobactins by the high virulent H37Rv and the South Indian low virulent (SILV) strains of **M. tuberculosis** under iron-deficient (0.02 mcg/ml) and iron-rich (4.0 mcg/ml) conditions. A similar investigation was undertaken with rifampicin and pyrazinamide, which are not known to have any metal ion binding properties.

The two strains were inoculated into a synthetic medium (pH 6.8) containing iron 0.02 or 4.0 mcg/ml. The MICs of the 4 drugs against **M. tuberculosis** in a liquid medium were 0.05 mcg/ml for isoniazid and rifampicin, 2.0 mcg/ml for ethambutol and 10.0 mcg/ml for pyrazinamide. The concentrations of the drugs employed in the present experiment were 0, 0.0125, 0.025 and 0.05 mcg/ml for isoniazid and rifampicin, 0, 0.5, 1.0 and 2.0 mcg/ml for ethambutol and 0, 2.5, 5.0 and 10.0 mcg/ml for pyrazinamide. The experiment was set up in quadruplicate and after incubation for 35 days at 37°C, the cell dry-weight and the production of exochelins and mycobactins was determined as described earlier (1989 annual report) after coding the flasks. The investigation with isoniazid and ethambutol was undertaken on one occasion and that with rifampicin and pyrazinamide on another.

The mean cell dry-weights for the 2 strains with all 4 drugs is presented in the table on page 76. There was no growth in the presence of the MIC for any of the drugs.

As expected, there is a significant decrease in the growth of both H37Rv and SILV with increasing concentrations of anti-tuberculosis drugs in the medium. However, the growth under iron-rich conditions (4.0 mcg/ml) is substantially higher ($P < 0.001$) than under iron-deficient conditions (0.02 mcg/ml), both in the presence and in the absence of anti-tuberculosis drugs. Further, the effect of all 4 drugs, at the concentrations tested, appears to be similar. The growth of SILV under iron-deficient or iron-rich conditions is significantly lower than that of H37Rv ($P < 0.01$).

The mean values for exochelins and mycobactins in the presence of the different concentrations of the 4 drugs under iron-deficient and iron-rich conditions are presented in the table on page 77.

There was a 2-3 fold increase in the release of exochelins with an increase of the concentration of the anti-tuberculosis drugs in the medium under both iron-deficient and iron-rich conditions. The production of mycobactins was, however, less pronounced, with an increase

Drug	Concentration in medium (mcg/ml)	Mean cell dry-weight (g/100 ml) according to strain and iron concentration (mcg/ml)			
		H37Rv		SILV	
		0.02	4.0	0.02	4.0
Isoniazid	0	0.109	1.077	0.087	0.910
	0.0125	0.046	0.314	0.045	0.205
	0.0250	0.012	0.077	0.011	0.057
Ethambutol	0	0.109	1.077	0.087	0.910
	0.5	0.069	0.397	0.060	0.258
	1.0	0.017	0 093	0.019	0.087
Rifampicin	0	0.125	1.074	0.089	0.897
	0.0125	0.040	0.283	0.043	0.308
	0.0250	0.013	0.086	0.018	0.073
Pyrazinamide	0	0.125	1.074	0.089	0.897
	2.5	0.070	0.309	0.073	0.315
	5.0	0.017	0.088	0.020	0.088

averaging 50-100% with increasing concentration of the drugs. As observed with free iron and hemoglobin, the production of both exochelins and mycobactins was substantially less under iron-rich conditions than under iron-deficient conditions.

Drug and concentration in medium (mcg/ml)		Mean exochelin and mycobactin concentrations (mg/g dry-weight) according to strain and iron concentration (mcg/ml)							
		Exochelins				Mycobactins			
		H37Rv		SILV		H37Rv		SILV	
		0.02	4.0	0.02	4.0	0.02	4.0	0.02	4.0
Isoniazid	0	34.4	9.5	31.5	8.1	82.6	22.6	54.7	20.1
	0.0125	54.3	19.1	38.6	13.4	108.9	36.1	66.3	30.5
	0.0250	85.2	26.2	90.1	17.7	145.5	42.3	90.1	39.0
Ethambutol	0	34.4	9.5	31.5	8.1	82.6	22.6	54.7	20.1
	0.5	58.5	16.5	38.5	13.7	120.4	30.4	67.1	30.2
	1.0	74.7	21.7	52.7	23.2	136.3	40.4	105.5	40.1
Rifampicin	0	35.2	9.8	30.9	8.1	83.4	22.9	53.6	21.4
	0.0125	49.5	17.7	46.6	13.0	104.8	35.4	69.8	27.7
	0.0250	80.6	23.4	55.0	27.4	161.3	42.7	110.0	45.0
Pyrazinamide	0	35.2	9.8	30.9	8.1	83.4	22.9	53.6	21.4
	2.5	46.7	14.5	41.0	12.8	104.3	27.5	61.3	27.8
	5.0	59.3	22.8	51.4	22.8	118.7	34.2	102.9	42.6

These findings demonstrate that, corresponding to the decrease in the growth, there is an appreciable increase in the production of both siderophores with increasing concentrations of all 4 drugs in the medium. This effect is not restricted to isoniazid and ethambutol but is also observed with rifampicin and pyrazinamide which are not known to have any capacity to bind metal ions. In general, under conditions inimical to growth such as the presence of anti-tuberculosis drugs or the presence of haptoglobin in the presence of hemoglobin or that of transferrin and lacatoferrin, proteins that bind free iron (see 1989 annual report), the reaction of the bacteria appears to be to increase the production of both siderophores, probably in an attempt to ensure their survival.

(started: 1989; completed: 1990).

Studies on the mechanism of pyrazinamide action

It is well known that an acid environment (around pH 5.6) such as that prevalent within the macrophage is necessary for activity of pyrazinamide (PZA) against **M. tuberculosis** in vitro. Susceptible mycobacterial strains such as **M. tuberculosis** and **M. microti** are known to produce the enzyme pyrazinamide deamidase in the acid environment of the host's phagolysosome, which converts pyrazinamide to the antimicrobially active metabolite, pyrazinoic acid (PZC). However, ammonia liberated during this reaction increases the intracellular pH, and thereby reduces antimicrobial activity of PZA. The equilibrium of this balanced system is thought to be dependent to some extent on the pH of the host's phagolysosome. Strains resistant to PZA such as BCG do not produce pyrazinamide deamidase, do not convert PZA to PZC and therefore, are not vulnerable to PZA.

The capacity of human tubercle bacilli to become drug susceptible with environmental change suggests that, in the acidic medium, some system in or on the bacilli is activated, or that there is a change in the state of the drug. Accordingly, an investigation was undertaken to study the possible role of pyrazinamide deamidase in PZA-susceptibility and antimicrobial activity of the drug and its principal metabolic product PZC, at neutral and acid environments. M. microti, a PZA-susceptible strain and M. bovis BCG, a PZA-resistant strain were used in this study.

The peritoneal exudate cells from Balb/c mice were pooled after washing with medium 199 and were suspended in the medium containing 10% foetal calf serum. 5×10^5 cells were put into each well for adherence for 2 hours. Non-adherent cells were removed by washing four times with the medium. The resulting monolayers of macrophages were cultured in basic maintenance medium.

Six-day old cultures of M. microti and BCG were prepared to give a 1 : 1 ratio of infecting bacteria to peritoneal macrophages. After a phagocytic period of 2 1/2 hours, the monolayers were washed four times to remove unattached mycobacteria and then overlaid with fresh medium containing the drug (day 0). The medium and the drug over the monolayers were replaced daily for 6 days. At 0, 48 and 144 hours the peritoneal macrophages were removed by scraping from the wells, sonicated, and dilutions were made and plated. After 6 weeks at 37°C, the colony forming units (CFU) were counted.

Cell free culture : After autoclaving, the 7H9 medium without Tween 80 was adjusted to pH 5.6 or 7.0 with sterile 0.1 N NaOH or HCl and a 6-day old culture of either **M. microti** or **M. bovis** BCG was

added. After incubation at 37°C for 3 days, the inoculated medium was divided into aliquots of 30 ml; drugs were added and the aliquots incubated at 37°C. Samples were taken at 0, 24 and 72 hours after drug addition, sonicated and the viable counts were set up.

All these experiments were repeated on another occasion and the results were similar.

The effect of PZA and PZC on the growth of **M. microti** is presented in the following table :

Culture	pH	Incubation period (hours)	CFU (x 10^4)of M. microti in the presence of pyrazinamide (mcg/ml)				pyrazinoic acid (mcg/ml)		
			0	5	10	30	5	10	30
Macro-phage culture		0	1.4	-	-	-	-	-	-
		48	4.0	4.1	3.5	2.9	3.8	3.5	3.0
		144	18.2	18.3	3.0	2.8	17.8	3.2	2.6
Cell free culture	5.6	0	38.4	-	-	-	-	-	-
		24	43.6	39.5	36.4	32.6	38.9	40.7	27.5
		72	42.9	38.0	30.2	26.6	24.6	20.9	25.2
	7.0	0	17.4	-	-	-	-	-	-
		24	20.1	20.4	20.4	18.2	15.2	18.0	12.9
		72	27.5	26.2	21.7	23.4	27.5	28.8	23.2

In mouse peritoneal macrophage system, the minimal inhibitory concentration (MIC) for **M. microti** is 10 mcg/ml for both PZA and PZC. The bactericidal effect was the same for PZA and PZC at concentrations of 10 or 30 mcg/ml.

In the cell-free system, the MIC for both PZA and PZC was around 10 mcg/ml at pH 5.6 and 7.0. The reduction of CFU at 72 hours at 30 mcg/ml of PZA and PZC at pH 5.6 was higher than that at pH 7.0.

To avoid the possible effect of drugs on pH, the drugs at different higher concentrations were added to the medium and autoclaved, adjusted to the required pH and inoculated with **M. microti**. The highest tested concentrations of PZA (150 mcg/ml) and PZC (750 mcg/ml) against **M. microti** at pH 5.6 showed that the reduction of CFU at 72 hrs were higher than that at pH 7.0 (data not presented). PZC, apart from lowering the pH, has some specific activity against **M. microti**.

The effect of PZA (5, 15 and 45 mcg/ml) and PZC (15, 45 and 135 mcg/ml) on the growth of BCG is presented in the following table :

Culture	pH	Incubation period (hours)	CFU (x 10^4)of M. bovis BCG in the presence of						
			pyrazinamide (mcg/ml)				pyrazinoic acid (mcg/ml)		
			0	5	15	45	15	45	135
Macro-phage culture		0	2.6	-	-	-	-	-	-
		48	13.8	16.2	19.9	15.9	17.4	15.9	15.3
		144	44.7	40.7	37.2	48.2	52.5	60.3	91.3
			Pyrazinamide (mcg/ml)				Pyrazinoic acid (mcg/ml)		
			0	5	10	30	5	10	30
Cell free culture	5.6	0	50.1	-	-	-	-	-	-
		48	87.1	67.6	72.4	74.1	78.7	50.1	60.2
		144	190.5	166.0	164.1	177.8	141.3	141.2	125.9
	7.0	0	30.2	-	-	-	-	-	-
		48	77.6	66.1	75.8	75.8	67.6	66.1	60.2
		144	169.8	166.0	158.5	138.6	118.8	128.8	117.5

Neither PZA at a concentration of 45 mcg/ml nor the PZC even at 135 mcg/ml exhibited any inhibitory effect on BCG in the mouse peritoneal macrophage culture; at lower concentrations of PZA and PZC, viz 5,

10 and 30 mcg/ml (data not presented), there was no inhibition. In cell-free medium at pH 5.6 in concentrations of 5, 10 and 30 mcg/ml there was slight inhibition with PZA and greater inhibition with PZC in the same concentrations. The same bacteriostatic effect was observed at pH 7.0 also for both the drugs.

Data (not presented) showed that after eliminating the drug effect on pH, both PZA and PZC were inhibitory (PZA 15, 75 and 150 mcg/ml and PZC 75, 375 and 750 mcg/ml) at both levels of pH (5.6 and 7.0). These findings suggest that PZA or PZC are not totally inactive at pH 7.0; they are, however, less inhibitory when compared to their activity at pH 5.6.

Results obtained in this study, showed that the MIC for both pyrazinamide and PZC against M. microti was 10 mcg/ml in mouse macrophage culture system whereas PZC did not have any inhibitory effect on BCG, even at a concentration of 135 mcg/ml. The inhibitory effect of PZA and PZC was the same in cell-free 7H9 liquid broth at pH 5.6 against both M. microti and BCG. Both PZA and PZC were not totally inactive at pH 7.0; however, they were less inhibitory when compared to their activity at pH 5.6. The action of PZC in an acid environment is more likely to be a combined effect of its specific activity and its ability to lower the pH, below the limits of tolerance of the target organism.

(started: 1989; completed: 1990).

Evaluation of an antigen from Dyna Gen (Boston,USA) for sero-diagnosis of pulmonary tuberculosis by ELISA

The diagnostic potential of an antigen (method of preparation not disclosed) referred to as Dyna Gen antigen was carried out at the Centre in collaboration with Dyna Gen, Boston, USA. The experiments were done on three consecutive days. On the first two days, assays were conducted without any controls. Dyna Gen had their own standard sera (sera R1 to R5) and on the basis of the O.D. values of these standards, they classified the results of the test specimens into three categories: Positive, Border-line (doubtful status) and Negative according to the criteria evolved by them earlier. A total of about 80 specimens (apart from standards) were assayed on each of the first two days. Since the reproducibility of the classification of the test specimens on the first two days was found to be encouraging, it was decided to evaluate the method under controlled conditions on the third day.

Two types of controls were introduced for the assays carried out on the third day. As the assays on the first two days were done with Alkaline Phosphatase as the marker, it was decided to carry out the assays

with Peroxidase method also (generally used at the Centre) for the purpose of comparison. A second type of control was the use of PPD antigen for coating the ELISA plates and to carry out assays by Phosphatase and Peroxidase methods in order to compare 'Dyna Gen' antigen against PPD antigen. With these two types of controls built into the design of the assay, a total of 40 specimens were selected and coded by statisticians before setting up the assays, to conceal the identity of specimens from the investigators.

Selection of specimens : Twenty specimens were obtained from pulmonary tuberculosis patients, diagnosed at the Centre. Sixteen of them were confirmed bacteriologically, while the other four patients were diagnosed radiologically. Of the other twenty specimens, ten were obtained from "cured" pulmonary tuberculosis patients, sera having been collected after a gap of at least 12 months after stopping anti-tuberculosis chemotherapy;

	Test result	True status		Total	Sensitivity %	Specificity %
		Positive	Negative			
Alkaline Phosphatase *						
Dyna Gen	Positive	8	9	17	40	45
	Borderline	3	2	5		
	Negative	9	9	18		
PPD	Positive	10	8	18	50	30
	Borderline	7	6	13		
	Negative	3	6	9		
Peroxidase *						
Dyna Gen	Positive	7	7	14	35	55
	Borderline	1	2	3		
	Negative	12	11	23		
PPD	Positive	11	4	15	55	50
	Borderline	2	6	8		
	Negative	7	10	17		
Total		20	20	40		

* Enzyme used as conjugate with second antibody in ELISA.

all these ten patients were free from active pulmonary tuberculosis. The remaining ten specimens were collected from healthy individuals.

True status of test specimens : The twenty specimens from tuberculosis patients are classified as 'True positives', while the other twenty specimens were classified as 'True negatives'.

The results of the comparison are presented in the table on page 82. A perusal of the estimates of sensitivity and specificity of Dyna Gen antigen shows that they are unsatisfactory and hence the antigen cannot provide a diagnostic test for pulmonary tuberculosis. Further, comparison of estimates of sensitivity and specificity of Dyna Gen antigen with PPD indicates that it is not superior to PPD. Therefore it is concluded that Dyna-Gen antigen is not useful for sero diagnosis and, even as a screening test, it has no advantage over PPD.

(started: 1990; completed: 1990).

STUDIES IN PROGRESS

Early bactericidal effect of pulsed exposure to RE and HZ on M.tuberculosis in vitro, in the murine model, and in tuberculosis patients.

In the ongoing randomised controlled trial of short course chemotherapy regimens of 6-month duration (see page 51), rifampicin and ethambutol are given on one day, and isoniazid and pyrazinamide on the next day; each combination is given thrice a week for the first 2 months in the first group of patients and for the first 3 months in the second group, followed by R and H twice a week for the next 4 months in the first group and next 3 months in the second group. These two groups are being compared with a third group given R, E, H and Z in a single dose thrice weekly for the initial 2 months, followed by R and H twice weekly for the next 4 months, in order to find out whether by giving RE and HZ on alternate days, adverse reactions could be minimised without compromising on efficacy.

It has been planned to study the early bactericidal effect of such pulsed exposures to RE and HZ on **M.tuberculosis** strains under in vitro conditions, in the murine model and in tuberculosis patients.

In vitro study : In the **in vitro** study, 7 strains of **M.tuberculosis** including **M.tuberculosis** H37Rv, two clinical isolates sensitive to all drugs and four isolates resistant to H, HR, SHR or SHRE have been studied following the method of Dickinson and Mitchison, (Tubercle 1987, 68: 183-193). Briefly, the log phase cultures of the **M.tuberculosis** strains in 7H9 liquid medium were treated either with RE and HZ on alternate days (RE/HZ) or with REHZ together (REHZ) over a seven-day period. Cultures which were not treated with any drugs were also included as controls. Viable counts were set up from all the cultures every day.

The overall results (mean + s.d.) for the 7 strains of **M.tuberculosis** studied so far are given in the top table on page 85.

In both the RE/HZ and REHZ treated cultures, a sharp fall in the log viable counts was observed over the six day period following the start of treatment with the drugs. As a result, starting from a log viable count of 7.58 on day 0, the counts fell to 1.41 in the cultures treated with RE/HZ compared to 0.99 in the cultures treated with REHZ, and 4.75 in the filtered control not treated with any drugs. These results indicate that treatment with both RE/HZ and REHZ in vitro had similar early bactericidal effect (a significant fall in log viable count by 6 th day) on **M.tuberculosis** strains.

Treatment	Log viable count on (day)						
	0	1	2	3	4	5	6
Control	7.58	5.14 ±0.72	4.75 ±0.75	4.66 ±0.92	4.71 ±1.01	4.56 ±0.83	4.75 ±1.03
RE/HZ	7.58	4.34 ±0.74	3.58 ±0.86	2.85 ±1.13	2.15 ±1.50	1.54 ±1.20	1.41 ±1.31
REHZ	7.58	4.04 ±0.86	3.31 ±0.93	2.59 ±1.46	1.93 ± 1.31	0.96 ± 1.39	0.99 ±1.22

The results (mean + s.d.) obtained with the three sensitive and four resistant strains of **M.tuberculosis** are given separately in the table below:

	Treatment	Log viable count on (day)					
		1	2	3	4	5	6
Sensitive strains	RE/HZ	4.16 ± 1.05	3.45 ± 0.70	2.45 ± 0.64	1.41 ± 1.58	0.66 ± 1.05	0.67 ±1.03
	REHZ	3.50 ± 0.92	3.06 ± 1.10	1.93 ±1.51	1.78 ± 1.39	0.0	0.33 ± 0.82
Resistant strains	RE/HZ	4.63 ± 0.19	4.20 +0.20	3.77 ± 0.23	3.33 ± 0.17	2.66 ± 0.33	2.63 ± 0.40
	REHZ	4.68 ± 0.38	3.85 +0.32	3.60 ± 0.35	2.72 ± 0.51	2.33 ± 1.27	1.98 ± 1.07

With both sensitive and resistant strains, there was a fall in the log viable count over the six-day period of treatment with RE/HZ or REHZ. However, the fall was greater in the sensitive strains and, while the early bactericidal effect of RE/HZ and REHZ was similar in the sensitive strains, REHZ treatment was slightly more effective in resistant strains.

Murine model study : For testing the bactericidal effect of pulsed exposure to RE and HZ in the murine model, BALB/C mice will be injected with 107 bacilli (total count) by the tail vein. In addition to **M. tuberculosis** H37Rv, the sensitive and resistant clinical isolates tested in the in vitro study will be included in this study. For each strain 68 mice will be used. After allowing 14 days for the infection to be established, the mice will be divided into 3 groups. The first group of mice will serve as control and will not be treated with any drugs. The second group will be administered RE on one day and HZ on the second day (RE/HZ) and so on for 2 weeks. The third group of mice will be administered REHZ

together on alternate days (REHZ). The drugs will be administered by gastric intubation and the doses will be those used by Grosset (Grosset, J. Tubercle. 1978, 59: 287-297; Grosset, J., et al., Bull. Int. Union Against Tuberculosis, 1983, 58: 90-96) that is, R 10mg/kg, E 100mg/Kg, H 25mg/kg and Z 150mg/kg.

From each group, 2 mice will be sacrificed on each day from the day of starting treatment. Viable counts will be set up using the spleen and lungs to study the course of infection during pulsed exposure to RE and HZ, and to REHZ. This study is in progress.

Study in tuberculosis patients: The early bactericidal effect of pulsed exposure to RE and HZ in tuberculosis patients included in the ongoing short course chemotherapy study will be examined by conducting serial sputum viable counts. Estimation of the viable units of tubercle bacilli will be made from single overnight collection specimens of sputum. The sputum specimens will be collected on days 0, 1, 2, 3, 4, 9 and 16 from patients receiving RE and HZ on alternate days, and on days 0, 2, 4, 9 and 16 from patients receiving REHZ twice a week.

In the laboratory, a smear of the sputum will be examined by fluorescence microscopy. The sputum will be processed for setting up viable count. It will be homogenised by adding large glass beads and shaking. To 2ml of homogenised sputum 4 ml of 10% dithiothreitol (sputolysin) will be added and mixed in a Vortex for 30 seconds. After standing at room temperature for 15 minutes, the mixture will be centrifuged and the deposit resuspended in sterile distilled water to make up a total volume of 4.8 ml. Five serial 10-fold dilutions will be prepared from this neat suspension. From the neat suspension and dilutions, 0.1 ml will be inoculated into selective 7H11 plates. The plates will be incubated at 37°C in the CO_2 incubator and colony counts will be taken after 4 and 6 weeks. From the colony count, viable count per ml of sputum will be calculated.

The intake to this study is in progress.

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

Standardisation of bioluminescence assay in estimating the viability and determining the drug susceptibility of M.tuberculosis cultures

Bioluminescence assay of adenosine triphosphate (ATP) was reported as a rapid method for the estimation of viable tubercle bacilli. For **M.tuberculosis** cultures stored at 4°C, the viable count done by ATP assay was comparable to that of CFU/ml by conventional method (1989 annual report). It was propsed to employ this assay for estimating drug susceptibility of **M. tuberculosis** cultures, isolated and purified on LJ medium.

About 100 cultures of M. tuberculosis with different patterns of susceptibility to various antimycobacterial agents and H37Rv as a control are being tested after coding. Single colony growth of these cultures are suspended in 7H9 broth and uniform suspensions from these are diluted to contain approximately 104 to 105 bacilli/ml. This culture suspension is added to equal volume of various dilutions of streptomycin, isoniazid, rifampicin and ethambutol and incubated at 37°C.

As a standardisation, ATP assay is done at 0, 2, 5, 7 and 10 days of incubation using biocounter (Lumac/3M) Model 2010A and reagents for ATP assay (Lumac/PM Kit). Three cultures have been tested so far using 2 different methods to release the mycobacterial ATP and, based on these results, the best suitable method to release ATP and the number and frequency of ATP assays during incubation will be determined. Single colony growth of these strains are tested for drug susceptibility by conventional method also for comparison. The main advantage of the ATP assay is the availability of drug susceptibility test results within a week, whereas by the conventional method the results will be available only at the end of 4 weeks. This rapid susceptibility testing by ATP assay may be helpful for the management of problematic cases, though not as a routine procedure. The study is in progress.

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

Susceptibility of mycobacteria to Cefadroxil

Cefadroxil is a semisynthetic cephalosporin antibiotic active against Gram-positive and Gram-negative bacteria. It is claimed to be resistant to inactivation by Beta lactamase. It has been reported to be highly bactericidal with low toxicity. It can be administered orally. The peak plasma concentrations attained after single doses of 500 mg and 1600 mg were 16 and 28 mg/l, respectively. Measurable levels were reported to be present up to 12 hours after the administration of the drug. About 90% of the drug was reported to be excreted unchanged in urine within 24 hours. Absorption was not affected by simultaneous intake of food. It is reported to be widely distributed in body tissues. The drug can be administered twice or even once a day.

The in vitro susceptibility of mycobacteria to cefadroxil employing the procedure described by Dickinson and Mitchison (Tubercle 1987, 68, 177) could furnish information regarding the suitability of the drug for further studies. Susceptibility tests will be set up for about 40 strains of tuberculous and 200 strains of non-tuberculous mycobacteria from consecutive clinical isolates.

(started: 1990; expected year of completion: 1991).

Use of vancomycin in selective Kirchner's medium

The use of multiple media and particularly the use of selective Kirchner's liquid medium (KL) has augmented the culture positives from extra pulmonary samples. But decontaminated deposits of gastric lavage cultured in KL resulted in a high degree of contamination (40-50%) mainly with aerobic spore bearers. Apparently the selective antibiotics in the KL are not able to control the multiplication of spore bearers. Preliminary experiments have shown that Vancomycin in MH Agar was able to prevent the growth of spore bearers from sputum deposits. It is proposed to study the usefulness of Vancomycin in the KL. For this, 200 sputum deposits are to be inoculated in KL with and without Vancomycin, and the rate of isolation of positives, the degree of growth and the incidence of contamination on subcultures compared. If Vancomycin effectively reduces the incidence of contamination without adversely affecting the positive isolations, the same may be tried for gastric lavage samples being collected from children.

(started: 1990; expected year of completion: 1991).

Bioavailability of anti-tuberculosis drugs from triple-drug and double-drug formulations

A number of triple-drug formulations containing isoniazid, rifampicin and pyrazinamide and double-drug formulations containing isoniazid and rifampicin suitable for daily or intermittent chemotherapy, are now available. It is believed that patient compliance would be better with these formulations than with the routinely administered combination of the 3 individual drugs as the bulk would be less. The bioavailability of the drugs from these formulations would depend not only upon the quality of the drugs employed but also on the pharmaceutical processes used for their manufacture. Investigations undertaken earlier had shown that even the order in which the drugs are mixed could profoundly affect the gastro-intestinal absorption of rifampicin. No study has so far been carried out to assess the bioavailability of these drugs from the formulations available in India. An investigation is therefore being undertaken to compare the bioavailability of these drugs from the different formulations with those obtained following administration of a control combination of the corresponding individual drugs. The subjects admitted to these studies are healthy volunteers with normal hepatic and renal function.

Triple-drug formulations : Six formulations (A to F) have been procured. The stated contents of isoniazid, rifampicin and pyrazinamide are 100, 150 and 500 mg, respectively in formulations A to D, 80, 120

and 250 mg in formulation E and 150, 225 and 500 mg in formulation F. The bio-availability studies will be undertaken in 2 phases.

During phase 1, formulations A, B and C will be investigated in 12 volunteers and the bioavailability of the 3 drugs from 3 tablets/capsules of each will be compared with those obtained following the administration of a control combination of isoniazid 300 mg plus rifampicin 450 mg plus pyrazinamide 1500 mg. Each volunteer will be investigated on 3 different occasions with at least 1 week between occasions. The 12 volunteers will be divided into 4 blocks of 3 each; each subject will be tested with the control combination and 2 of the 3 formulations. The choice of the formulations and the sequence of administering these and the control combination will be determined at random, ensuring however that each formulation is tested twice in each block. Thus, values for the control combination will be available from all 12 volunteers, and from 8 for each of the 3 formulations.

During phase 2, the bioavailability of the 3 drugs from 3 capsules of formulation D, 4 tablets of formulation E and 2 tablets of formulation F will be investigated in 12 volunteers as above. The control combination will consist of isoniazid 300 mg plus rifampicin 450 mg plus pyrazinamide 1000 mg.

During both phases, bioavailability of the 3 drugs will be assessed on the basis of concentrations of the drugs in plasma from blood collected at 1, 2, 3, 6, 9 and 12 hours, and excretion in urine of the drugs and their primary metabolites over the periods 0-8, 8-12 and 12-24 hours after drug administration (on an empty stomach).

Double-drug formulations : A total of 11 formulations have been procured. The stated contents of isoniazid and rifampicin are 300 mg and 450 mg, respectively, in all. The investigations will be undertaken in 3 phases; during each phase, the bioavailability of the 2 drugs from 1 tablet/capsule of 3 or 4 formulations will be investigated in 12 and 18 volunteers, respectively. The control combination during all 3 phases will be isoniazid 300 mg plus rifampicin 450 mg.

(started: 1990; expected year of completion: 1991).

Nitrite generation from the activated macrophages of pre and post BCG vaccinated individuals

M. tuberculosis, the etiological agent of tuberculosis, readily multiplies within macrophages of an infected host. Elimination of the bacteria and resolution of the disease must evoke extraordinary metabolic changes in the infected macrophages. Bacterial lipopeptides and lipopolysaccharides (LPS) are potent activators of macrophages. Once activated by these agents, macrophages produce cytotoxic and cytostatic compounds such as cytokines (tumour necrosis factor, gamma interferon, interleukin-1) and reactive oxygen species (superoxide anion and hydrogen peroxide). It was generally believed that activated macrophages kill the intracellular pathogens via the production of reactive oxygen intermediates. However, the elimination of **Leishmania major** by a murine macrophage cell line, IC-21, which is deficient in the production of oxygen metabolites, suggests that activated macrophages may kill intracellular parasites by a non-oxidative mechanism. Further, no differences were observed in the production of hydrogen peroxide of stimulated monocytes between tuberculous patients and healthy subjects.

It has recently been suggested that reactive nitrogen intermediates such as nitric oxide produced by activated macrophages may play a role in the intracellular killing of M. tuberculosis. Nitric oxide is a potent inhibitor of the respiratory chain and is formed from guanido nitrogen of L-arginine by a NADPH-dependent enzyme system present in the cytosol of stimulated macrophages. The release of nitrite is a good marker for the nitric oxide pathway in macrophages and is used to correlate the production of inorganic nitrogen oxides with macrophages effector activity. Normal macrophages are permissive for the growth of M. tuberculosis and this correlates with poor nitrite release as compared with highly tuberculostatic gamma interferon pulsed macrophages. Addition of N-monomethyl L-arginine or arginase to the medium cancels the effector activity of the activated macrophages and decreases the nitrite secretion. Both the effector activity and nitrite secretion are restored with the addition of excess L-arginine.

A study has been planned to compare the levels of nitrite in activated monocytes (activated with lipopolysaccharide and gamma interferon) from peripheral blood collected from 10 sputum culture-positive patients with pulmonary tuberculosis and 10 healthy individuals before and 8 weeks after vaccination with BCG. The generation of nitrite from activated monocytes will be correlated with the bactericidal activity against **M. tuberculosis** H37Rv. The intracellular nitrite-mediated killing will be confirmed by blocking nitrite generation by the addition of N-monomethyl L-arginine and restoring it with the addition of excess L-arginine.

The method for the estimation of nitrite production in murine and human macrophage culture system has been standardised.

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

Production of monoclonal antibodies against tuberculous antigens

Work on production and characterization of mouse monoclonal antibodies was continued. A number of hybrid cell lines were frozen and stored in liquid nitrogen. They included cell lines that were characterized earlier as well as cell lines with doubtful reactivity and cell lines yet to be characterized.

Fifty cell lines were thawed and tested for their reactivity. Clones 31-3, IC3, 5E9, 45-2 and IH12 were characterized earlier and they continued to make antibodies. Clones IF11, 2G8, 3F11 and 4C11 were initially of weak reactivity and subsequently lost the property of making antibodies. Cell lines 41-33, 41-10 and 44-2 were found to be reactive with M. tuberculosis H37Rv culture filtrate. These were cloned further by limiting dilution method. Monoclonal antibodies 41-10, 41-33, 1C3 and 44-2 along with 1H12 and 31-3 were tested in ELISA for their reactivity with Mycobacterial antigens other than Tuberculosis (MOTT) (see table below). The optical density (O.D.) presented in the table corresponds to the observed O.D. minus the O.D. for SP 2/0 control supernatant.

	IC3	IH12	31-3	41-10	41-33	44-2
M. avium	0.207	0.067	0.036	0.514	-0.007	-0.027
M. scrofulacem	0.189	0.149	0.167	0.824	0.573	-0.015
H37Rv	0.187	0.132	0.234	0.857	0.464	0.036
M.bovis	0.424	0.694	0.018	1.199	1.117	0
H37RV-cell wall	0.079	0.015	0.188	0.29	0.172	0.016
M.kansasii	0.047	0.015	0.144	0.508	0.211	0.027
M.flavescence	0.086	0.038	0.088	0.919	0.515	0.028
M.gordonae	0.132	0.055	0.028	0.379	0.154	0.028
M.terrae	0.203	0.075	1.164	0.707	0.328	0.039
M.fortuitum	0.038	0.007	0.024	0.034	-0.123	-0.031
M.chelonei	0.062	0.017	0.036	0.115	-0.039	-0.018
M.tb 7219	0.038	-0.014	-0.002	0.103	-0.128	-0.006
M.tb S.I.	0.076	0.12	0.598	0.534	-0.016	0.022
H37Ra-culture filtrate	0.245	0.445	1.811	1.017	0.078	-0.036
H37Rv-culture filtrate	1.665	1.665	1.665	1.208	0.487	0.229
M. xenopy	0.084	0.112	1.607	0.344	-0.053	-0.042
SeA	0.026	0.068	0.064	0.029	-0.069	-0.010
E.coli	0.02	0.034	0.029	0.015	-0.069	-0.017

Reactivity of antibodies 1H12 and 44-2 with M. tuberculosis H37Rv culture filtrate, M. tuberculosis H37Ra culture filtrate and S. digitata crude extract was studied by immunoblot. In ELISA, IC 3, 41-10 and 41-33 reacted with more than 5 species of atypical mycobacteria. Only one monoclonal antibody (44-2) showed no binding with H37Ra - culture filtrate and atypical mycobacteria, indicating specificity for M. tuberculosis H37Rv. However, in western blot, antibody 44-2 bound two bands in similar positions in both H37Rv-culture filtrate and H37Ra -culture filtrate. This antibody is being studied further.

Since conventional immunization methods have not yielded monoclonal antibodies of interest, attempts are also made to use different methods of immunization. Intraspleenic immunization is useful in immunizing mice with very small amounts (nanograms) of antigens. Procedures for intraspleenic immunization of mice were standardized.

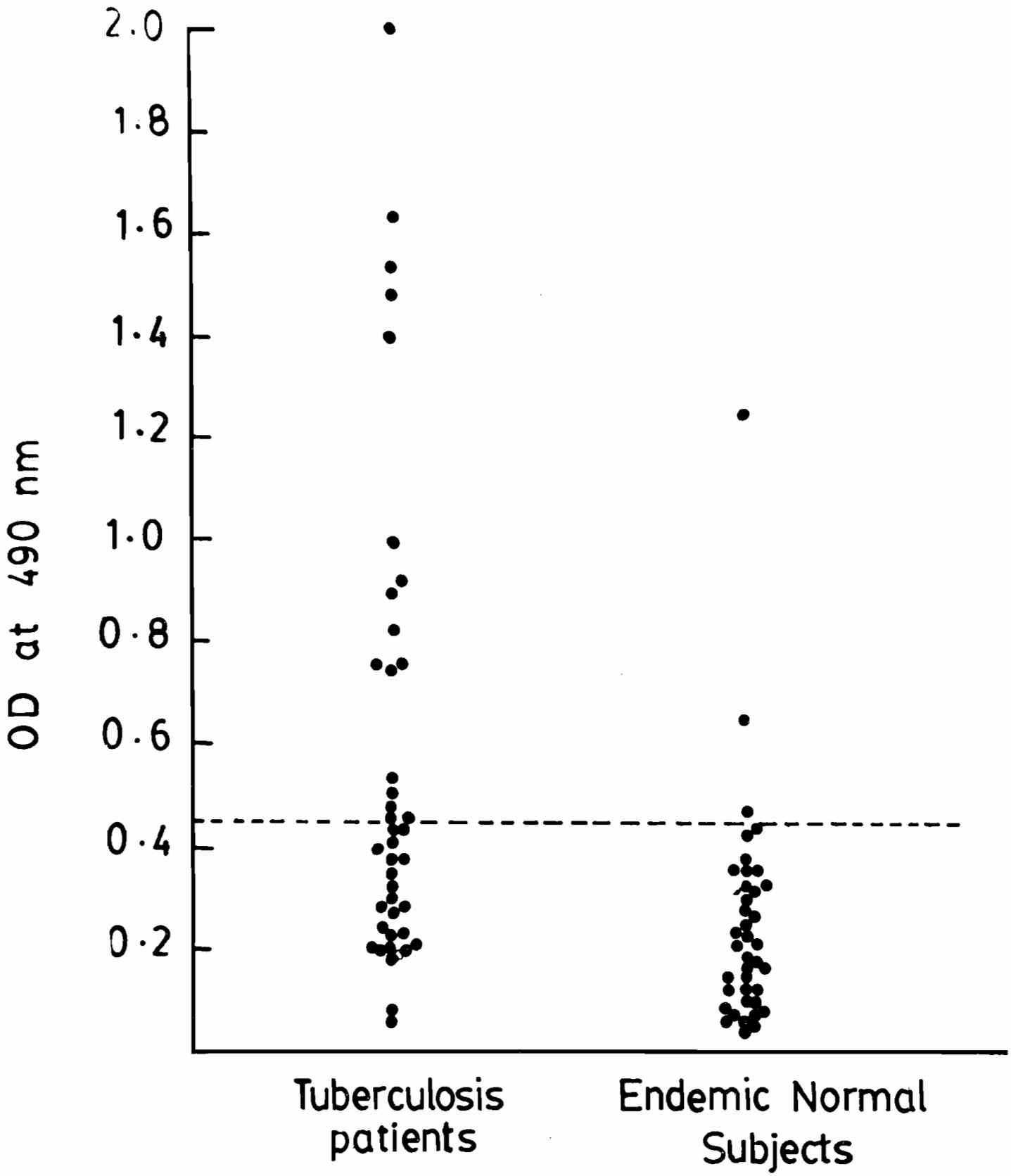
(started 1988; expected year of completion: 1991)

Characterization and purification of antigenic components of M. tuberculosis

Affinity chromatography purified antigens were prepared as described previously (annual report, 1989). Only about 10% of the bound antigens could be eluted. The eluted antigens were characterized by SDS-Polyacrylamide gel electrophoresis (SDS-PAGE), followed by immunoblotting with rabbit anti-BCG serum and pooled human tuberculous sera. Two bands of molecular weight 58 and 49 KDa were recognised by both the sera.

The purified antigen was evaluated in ELISA. The distributions of the optical density (O.D.) obtained at a serum dilution of 1:2000 in 40 smear and culture positive pulmonary tuberculosis patients and in 40 normal subjects in endemic area are shown in the figure on page 93. The figure indicates a high degree of overlapping in the two distributions and, for this reason, it is difficult to evolve an O.D. level as diagnostic criterion for tuberculosis. For example, if an O.D. level of 0.445 is arbitrarily chosen (see fig.) as the diagnostic criterion, it is found that 17 of the 40 patients and 3 of the 40 normal subjects would be classified as cases. Thus the expected values of sensitivity and specificity by this criterion would be 43% and 93% respectively. While the level of specificity is not unsatisfactory, the sensitivity is very low. It is hoped that evaluation of this antigen under different assay conditions like altered serum dilution, different conjugate system etc., might bring improvements in sensitivity and specificity.

Although our attempts have not been successful so far, efforts will be continued to design methods to elute the other antigens bound to gel.


2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
OD at 490 nm
Tuberculosis patients
Endemic Normal Subjects

Addition of a few more purified antigens (isolated by other affinity procedures or physico-chemical procedures) to the 58 and the 49 KDa might enhance the sensitivity and specificity.

Since the project cannot be completed in the anticipated time, it is being extended to 1993.

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

Development of DNA probes for M. tuberculosis

In the 1989 annual report, the steps involved in the construction of genomic library of M. tuberculosis in plasmid pBR - 322 and the possibility of evaluation of the recombinant clones as diagnostic probes was discussed. Construction of genomic libraries in two new vectors and their analysis is discussed in this report.

Due to technical problems faced with pBR-322, it was decided to construct two more libraries of M. tuberculosis in two new vectors, namely pGEM, a plasmid vector and mp 19, a phage vector. The cloning site for pGEM is the unique Hinc II and for mp 19 it is Pst 1 site.

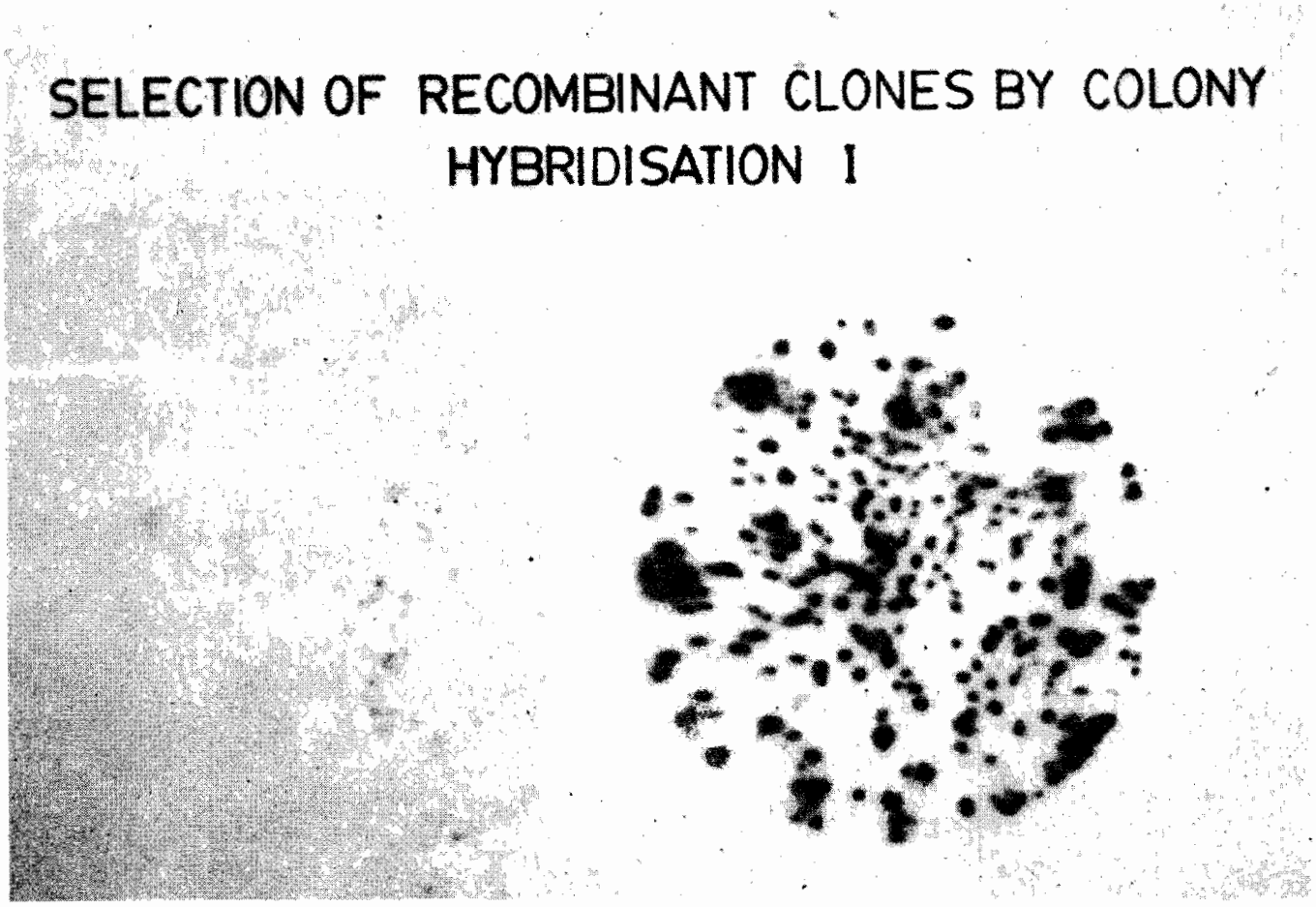
Screening of genomic library : The recombinant pGEM and mp 19 clones were analysed by hybridization with ^{32}P labelled M. tuberculosis DNA. In the hybridisation method, both colony hybridization and slot hybridization have been carried out.

The four figures on pages 95 - 98 show the colony hybridization of pGEM clones and slot blot hybridization of mp 19 clones. The strongly lighting clones have been chosen and reconfirmed again by alkaline lysis method. This was done by growing the chosen clones, preparing plasmid or phage DNA as the case may be, restriction digesting them and running the DNA in agarose gel.

The figure on page 100 show the sizes of the various fragments of M. tuberculosis cloned in pGEM and mp 19 respectively.

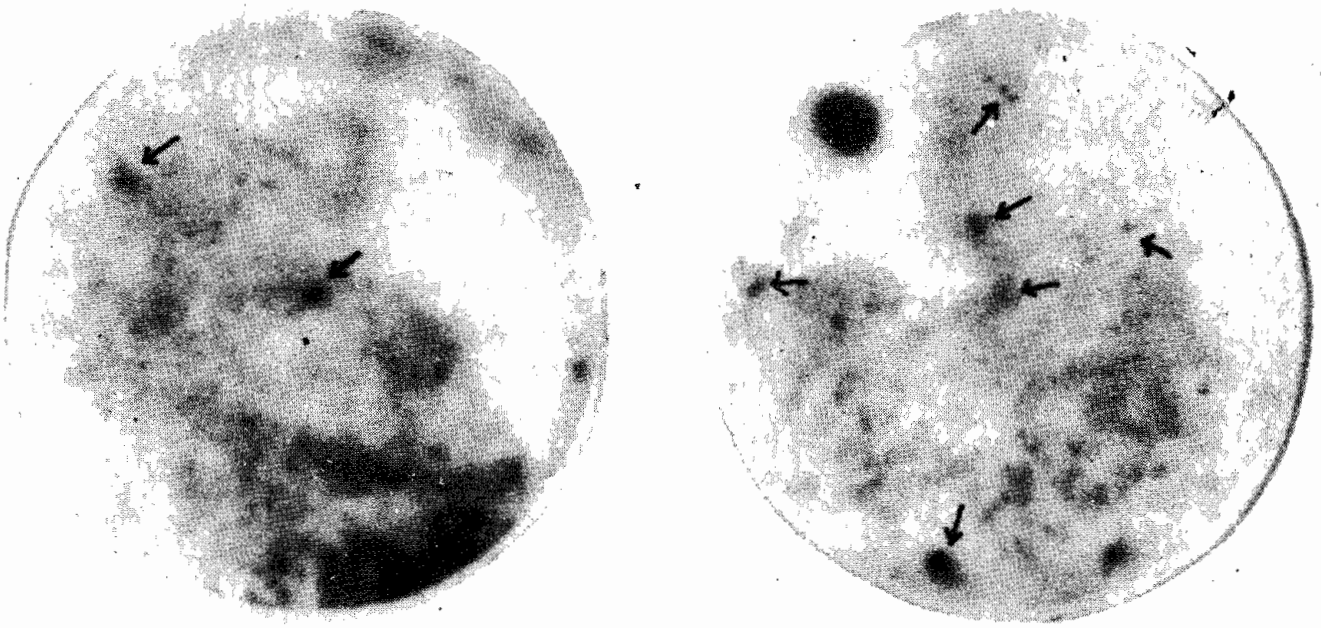
These 10 pGEM recombinant clones and 8 mp 19 recombinant clones have been purified by cesium chloride gradient centrifugation. These 18 clones were evaluated for specificity with ^{32}P labelled M. tuberculosis DNA and non-mycobacterial DNA (E.coli, Clostridium perfingens and human placenta). Among these 18 clones, only 4 appear to be worth investigating further and the work is in progress.

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

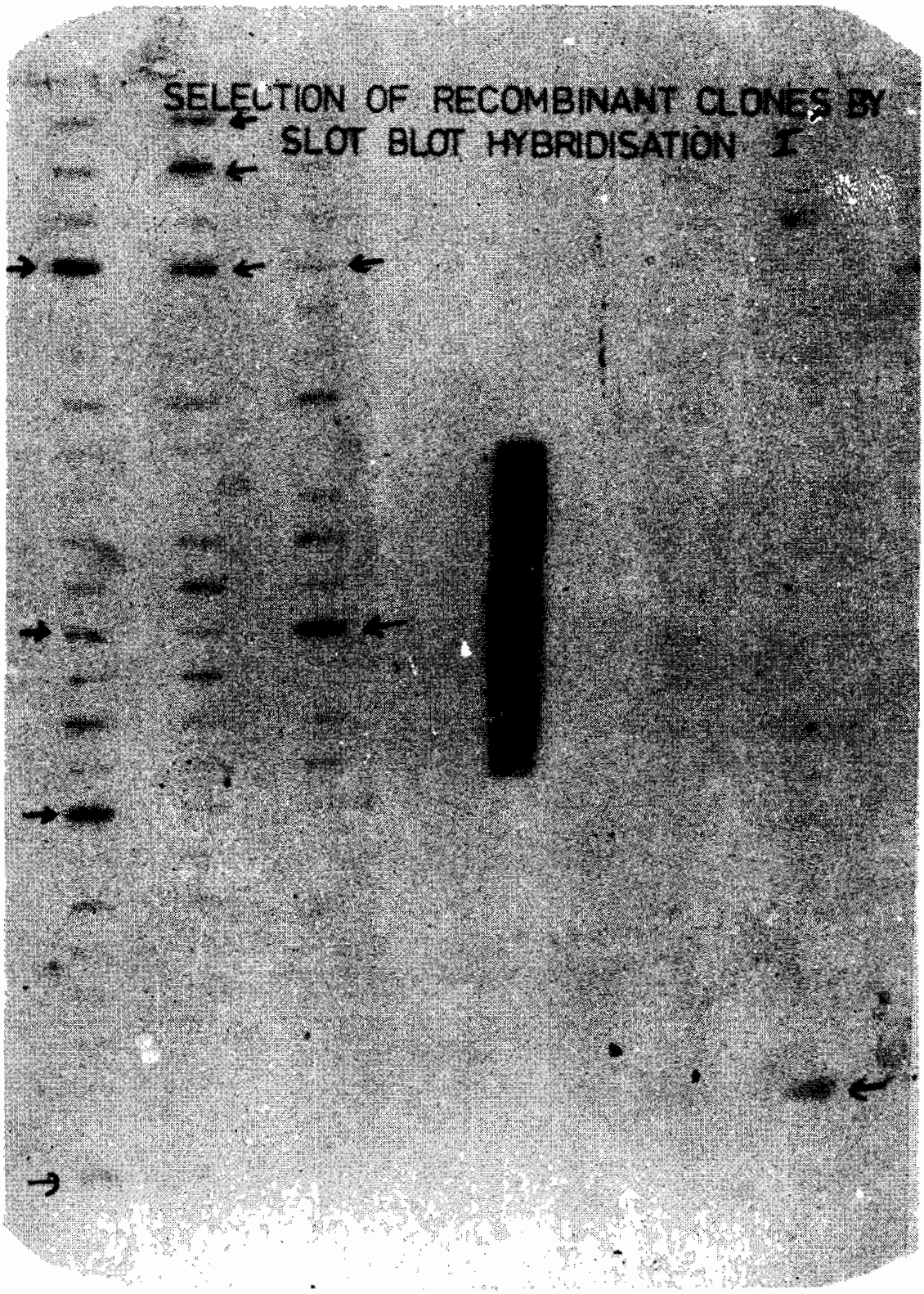


Individual colonies (transformants with pGEM plasmid - positive control) from Ampicillin containing LB Agar plate (right) were colony lifted on to a nitrocellulose paper (NCP) and probed with ^{32}P labelled pGEM. The left one is negative control (mp 18 plaques) probed with ^{32}P labelled pGEM.

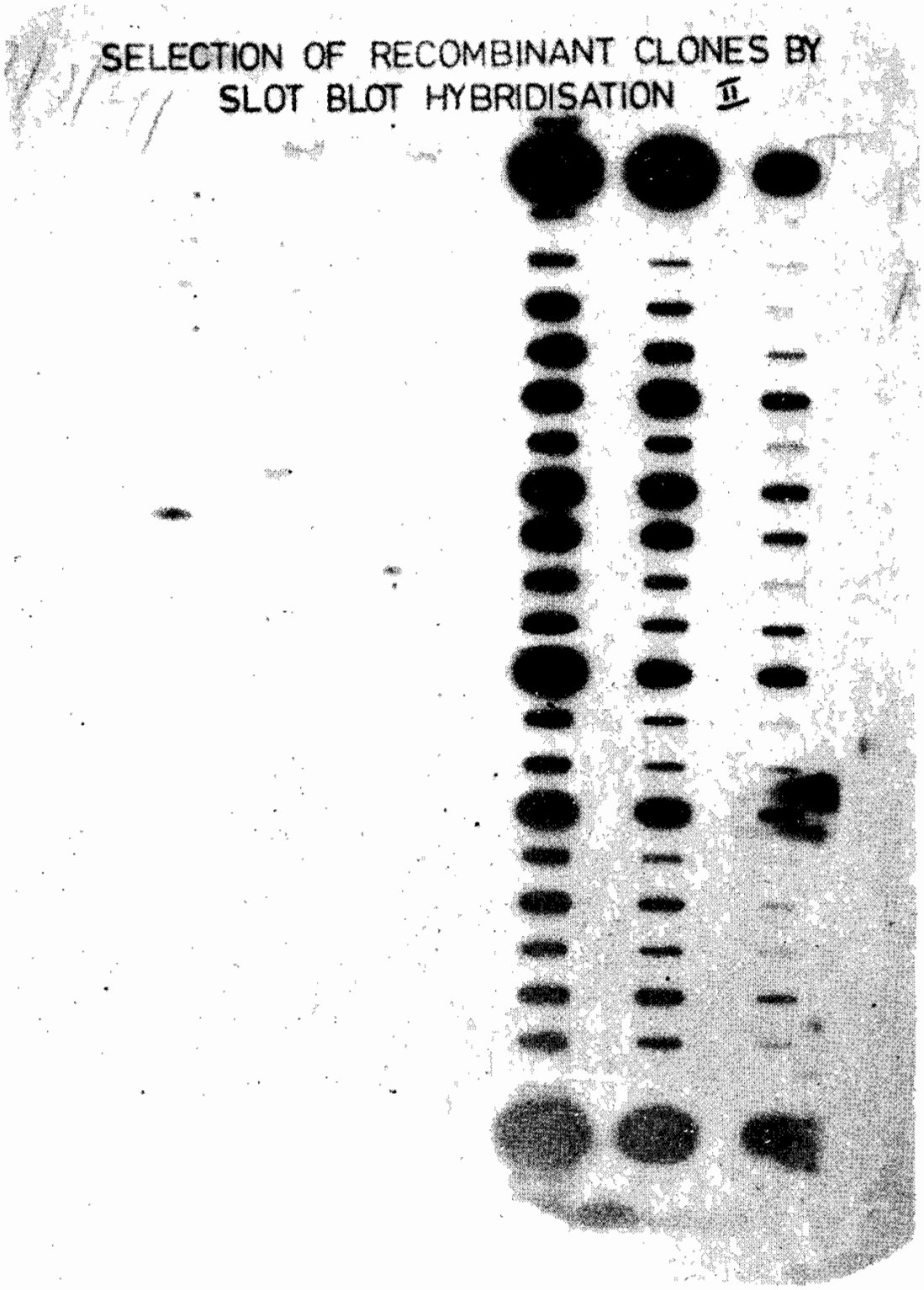
SELECTION OF RECOMBINANT CLONES BY COLONY HYBRIDISATION II



Individual colonies (recombinants with mycobacterial inserts) from LB AMP plates were colony lifted onto NCP and probed with ^{32}P labelled M. tuberculosis. The arrows indicate strongly lighting recombinant clones.



Fifty mcl of overnight cell suspension of individual colonies (recombinants) from LB (Luria Burtini) Amp Agar plate was spotted on a NCP and hybridised with ^{32}P labelled DNA of M. tuberculosis. The arrows indicate strongly lighting recombinants with mycobacterial insert.



1.0 mcg, 0.1 mcg and 0.01 mcg of the 18 recombinants were spotted on a NCP along with positive control (M. tuberculosis in the same concentration as above in the first and the last row) and hybridised with ^{32}P labelled DNA of M. tuberculosis.

HLA studies in tuberculosis

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

HLA -A, -B and -DR, -DQ serological typing was carried out in 14 volunteers. Total peripheral blood mononuclear cells were used for HLA - A, -B typing. Nylon wool column separated B-cells were used for HLA - DR and -DQ typing. For these two typings, the standard microlymphocyto-toxicity method was followed.

The "behaviour" of all the HLA anti-sera will be analysed with some more volunteers and "cell panel members" of other established laboratories. The serological determination of HLA phenotypes of the tuberculous individuals (individuals from whom tubercle bacilli were isolated at least once) will be carried out only after the 'behaviour' of the sera is established. Work is in progress.

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

Generation of T cell clones against mycobacterial antigens

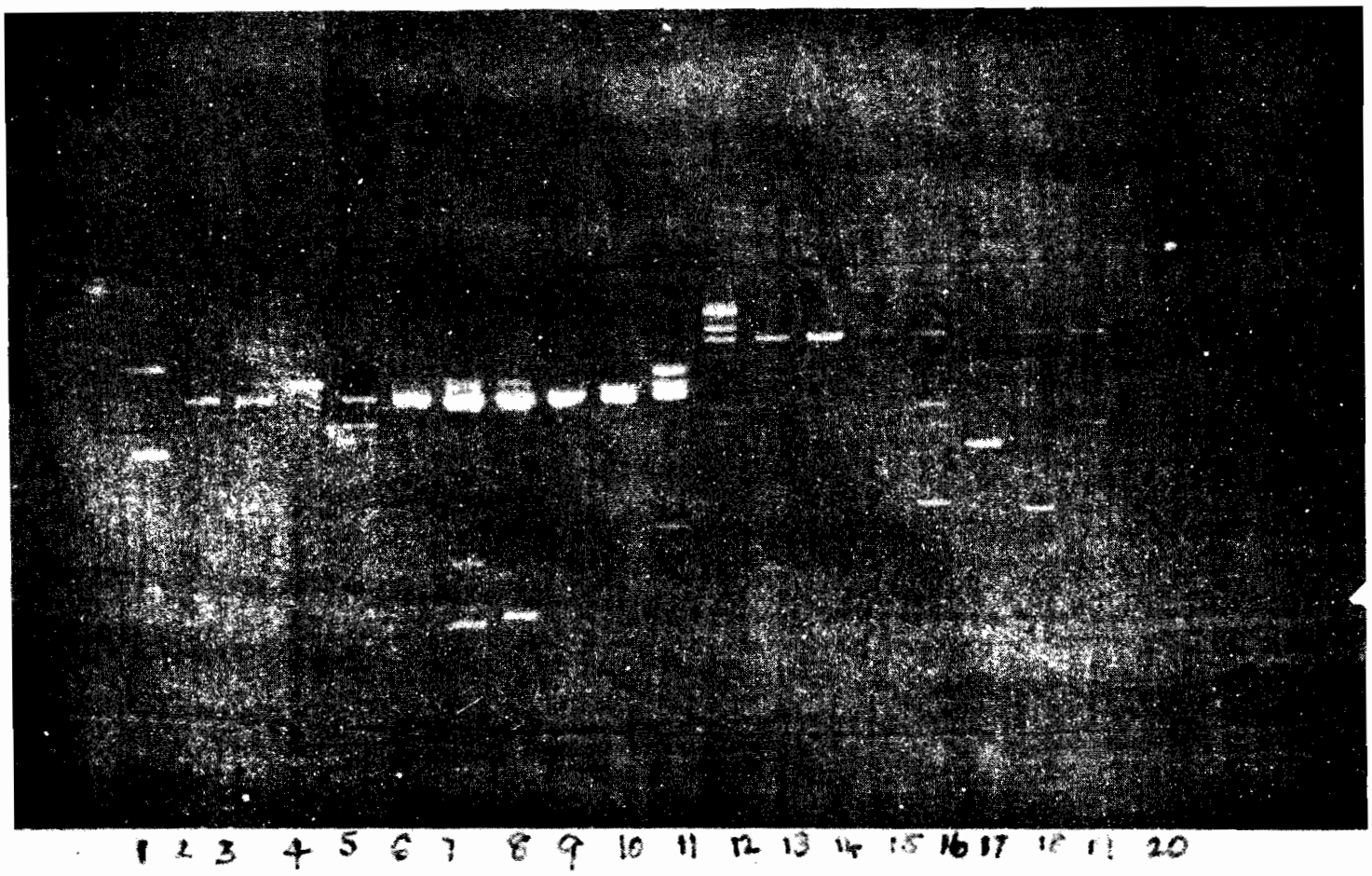
T lymphocytes play a crucial role in the host response against the bacteria that are capable of replication in mononuclear phagocytes and certain other host cells. Infection by **M. tuberculosis** causes expansion of several clones of T lymphocytes. The antigen specificity and functions of these T cell clones have not been fully studied. By cloning T lymphocytes which are responsive to **M. tuberculosis** antigens, it may become possible to characterize both types of antigenic determinants that are involved in pathogenesis and in protective immunity. The following strategy was adopted for the various experiments.

1. Two antigens, PPD and Sonicate extract of M. leprae (MLSON) were used for screening of volunteers for lymphocyte response to the antigens for which T cell clones were made.

2. T cell clones were generated by the method of limiting dilution from high responders.

3. The phenotype and the nature of the receptors of the clones were studied.

4. Assay of gamma interferon production by these clones was undertaken.



The DNA from the recombinant clones was restriction digested with ECORI & Hind III restriction enzymes and run on 1% agarose gel. Lane 1 represents pGEM vector alone (uncut). Lanes from 2 to 10 represent pGEM recombinants. Lane No. 11 shows M.wt. marker. Lane Nos. 12 to 19 show mp 19 recombinants.

Out of the six volunteers screened, successful cloning was achieved in two volunteers. Despite good proliferative response of the lymphocytes to MLSON, the cloning efficiency was poor for this antigen. Ten clones from volunteer 5 and eleven from volunteer 6 (against PPD) were raised. These clones were tested for their sensitivity for PPD and MLSON as well as two recombinant antigens -65kd (M. leprae) and 70kd (M. tuberculosis). The results of the proliferation assay are shown in the figures on pages 102 and 103. The stimulation index (SI) in this proliferation assay is defined as the ratio of the mean of CPMs in 3 stimulated cultures to the mean of CPMs in 3 control cultures.

The phenotype and the receptor of the T cell clones were studied by immunofluorescence staining. The cells were incubated with the corresponding monoclonal antibody (anti CD4 and anti CD8 for phenotype and anti beta and anti delta for receptor) in a multi-well slide. All the clones of volunteer 6 were CD4 positive and showed the presence of alpha beta receptor. All but two clones of volunteer 5 were CD4 positive; all were positive for alpha beta receptor. The clones 5D3 and 5F5 were CD8 positive.

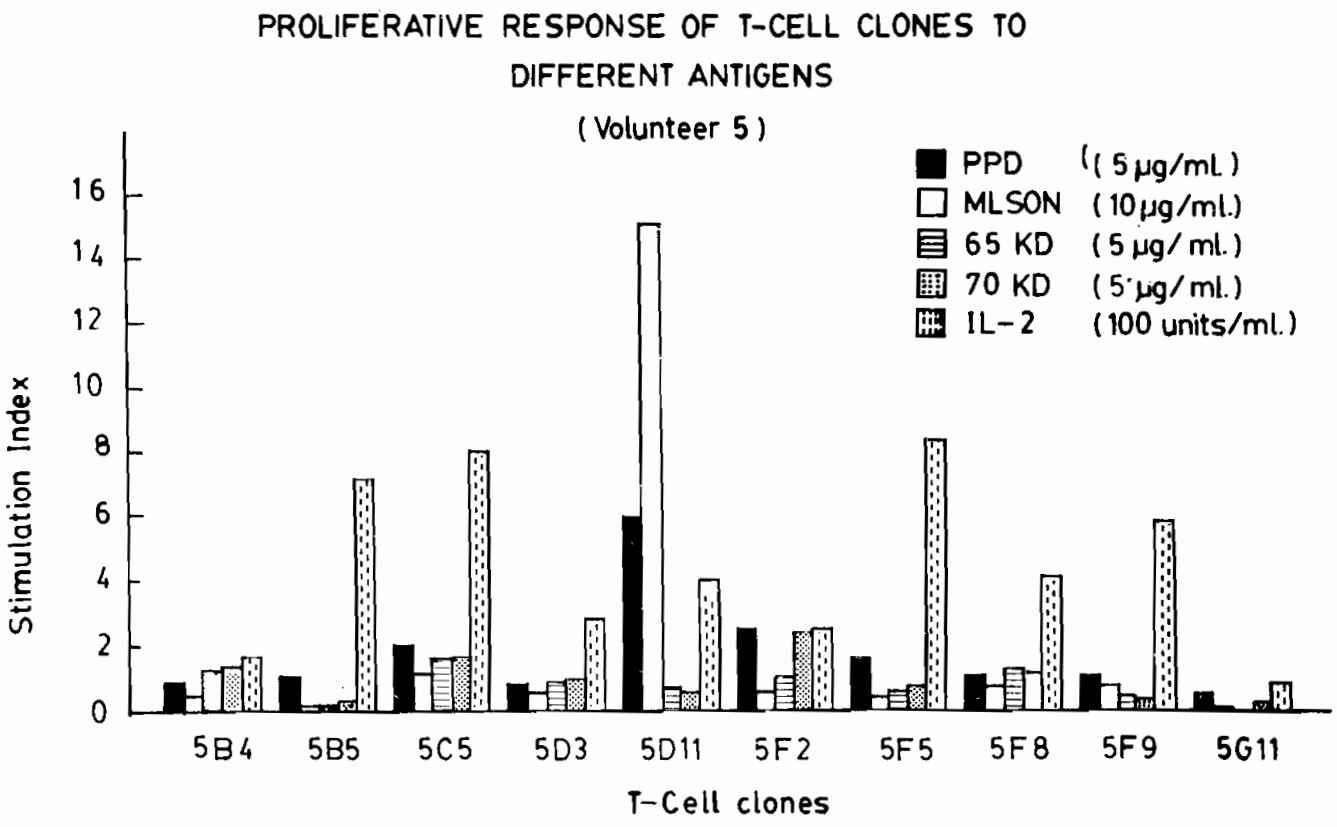
Gamma Interferon (IFN) assay: This was done by double antibody sandwich ELISA. Culture supernatants from antigen-stimulated T cell clones were tested for their IFN content. Only one clone of volunteer 6, 6G6 produced detectable amount of IFN (6 units) in response to stimulation by PPD. The results of the IFN assay are shown in the table below.

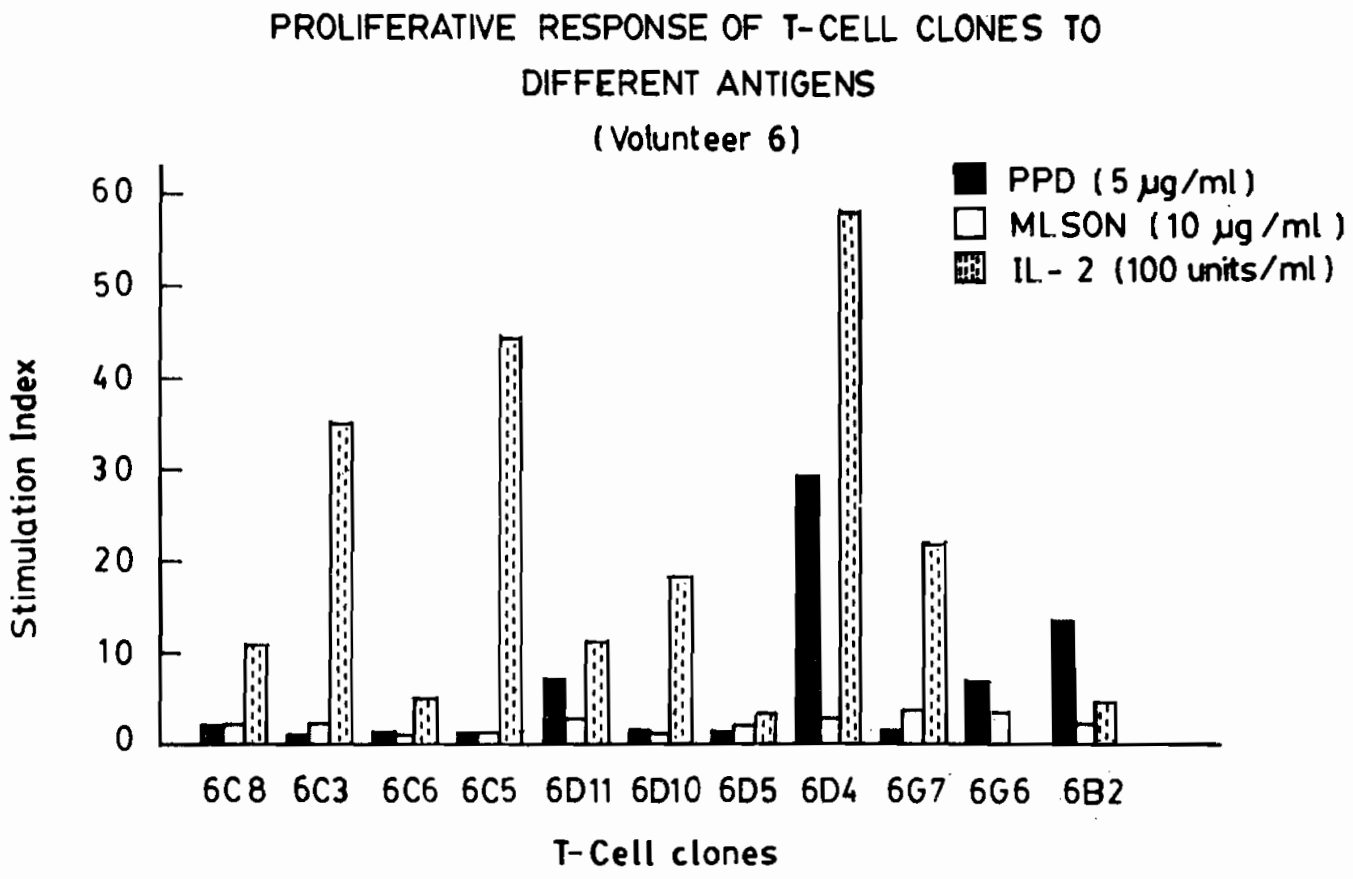
Clones	IFN-Gamma (units) produced by T-cell clones	
	PPD	MLSON
6G6	6	-
5D3	3	-
5D11	4	-
5F2	3	-
5F5	-	5
5B3	10	-

The reported work was carried out at the London School of Hygiene and Tropical Medicine between March and September 1990.

Since October 1990, 76 clones against PPD and 5 against streptomycin-killed whole M. tuberculosis have been generated from five volunteers at the Centre. These clones will be characterized in the following months.

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


PROLIFERATIVE RESPONSE OF T-CELL CLONES TO
DIFFERENT ANTIGENS
(Volunteer 5)
PPD (5 µg/ml)
MLSON (10 µg/ml.)
65 KD (5 µg/ml.)
70 KD (5 µg/ml.)
IL-2 (100 units/ml.)
Stimulation Index
0
2
4
6
8
10
12
14
16
5B4
5B5
5C5
5D3
5D11
5F2
5F5
5F8
5F9
5G11
T-Cell clones


PROLIFERATIVE RESPONSE OF T-CELL CLONES TO
DIFFERENT ANTIGENS
(Volunteer 6)
PPD (5 µg/ml)
MLSON (10 µg/ml)
IL-2 (100 units/ml)
Stimulation Index
60
50
40
30
20
10
0
6C8
6C3
6C6
6C5
6D11
6D10
6D5
6D4
6G7
6G6
6B2
T-Cell clones

Haematological profile of pulmonary tuberculosis patients

It has recently been shown in studies on pulmonary tuberculosis patients from Indonesia and South Africa that haematological parameters reflect the extent and activity of the disease. Further, using an experimental system, it has been demonstrated that in mycobacterial infections in mice, chronic neutrophilia is induced by T lymphocyte factor(s). The present investigation was started in order to study the relationship of haematological parameters with the clinical and bacteriological features of patients suffering from pulmonary tuberculosis in South India. The study involves a retrospective analysis of data available from three previous chemotherapy investigations.

In the first phase, data from 920 patients who were given intermittent short course (6 months) chemotherapy are being analysed. Haemoglobin, platelet count and total and differential leucocyte counts were estimated at intake and 2 and 6 months after starting chemotherapy.

It was found that while the haemoglobin levels were low, the platelet and total leucocyte counts were elevated at intake. Further, there was both a relative and absolute neutrophilia along with a relative lymphopenia. At 2 months after initiating chemotherapy, the haemoglobin and relative lymphocyte levels increased while the platelet, total leucocyte and neutrophil counts decreased. These differences were found to be statistically significant($p<0.0001$). The same trend was observed when the data at 6 months were compared with those at 0 month.

	0 month	2 months	6 months
Haemoglobin (g/dl)	9.84 ± 2.03	10.60 ± 1.92	11.02 ± 1.94
Platelets (10^9/l)	315 ± 70	288 ± 70	267 ± 70
Total leucocyte count (10^9/l)	9.48 ± 2.44	8.09 ± 2.14	7.23 ± 1.92
Neutrophil (%)	63.36 ± 10.80	55.82 ± 11.00	51.00 ± 11.60
Lymphocyte (%)	27.10 ± 9.30	32.90 ± 9.80	37.80 ± 11.10
Eosinophil (%)	6.97 ± 6.10	10.60 ± 7.30	10.60 ± 7.40

The levels of haemoglobin were significantly lower in females compared to males at all the 3 time points tested. There were no other differences between the sexes in the haematological parameters that were estimated. The patients were classified according to the extent of disease, and their haematological parameters on admission were compared. It was found that as the extent of the disease increased, there was more marked anaemia and lymphopenia along with thrombocytosis, leucocytosis and neurotrophilia (table on page 105). However, at 6 months, the haematological values in the different groups were comparable.

	Extent of disease			
	Slight	Limited	Moderate	Extensive
No.of patients	57	276	323	255
Haemoglobin(g/dl)	11.10 ± 2.03	10.20± 2.10	9.80 ± 1.90	9.40 ± 1.80
Platelets(10^9/l)	290 ± 60	310 ± 80	320 ± 70	320 ± 70
Total leucocyte count(10^9/l)	9.17 ± 2.49	9.03 ± 2.19	9.56 ± 2.44	9.91 ± 2.63
Neutrophils(%)	59.80±10.10	63.40+11.10	65.90±10.03	67.30+10.40
Lymphocytes(%)	30.80±10.10	27.80± 9.40	27.10± 9.03	25.80±8.80
Eosinophils(%)	8.70±6.10	8.40± 7.10	6.50± 5.03	5.70 ± 5.40

Similar results were observed when the patients were stratified according to the degree of cavitation or degree of smear grading (data not shown). The relationship of the haematological values with viable count of bacteria, tuberculin response, different regimens, response to treatment at 6 and 12 months and relapse is currently being evaluated.

(started: 1990; expected year of completion: 1991)

Histopathological classification of tuberculous lymphadenitis

The present study was initiated in 1988 in order to understand the pathogenesis of tuberculosis better using tuberculous lymphadenitis as a model. In addition, the study aims to define the diagnostic criteria for some of the unusual histological presentations of tuberculous lymphnodes. The study done so far reveals that tuberculous lymphadenitis can be categorised into four groups according to the nature and extent of granuloma, and type and number of the infiltrating cells.

During the year under review, a total of 227 lymphnodes were examined and 118 (52.0%) of these were found to show evidence of tuberculosis. As described in the 1989 annual report, tuberculous lesions were classified into reactive (60.0%), hyperplastic (25.5%), hyporeactive (10.3%) and nonreactive (4.2%) types. Since the study population comprises of both adults and children, the two groups are considered

separately. A detailed analyses of the clinical and bacteriological features in the histological subtypes will be undertaken when sufficient numbers of patients with less common presentations are included in the study.

(started: 1990; expected year of completion: 1991)

EPIDEMIOLOGICAL STUDIES

STUDIES COMPLETED

Tuberculosis prevalence survey in North Arcot District

A sample survey, similar to the one conducted in Raichur district (see 1989 annual report), was carried out in North Arcot district in Tamil Nadu with the objectives of obtaining an estimate of:

(1) the prevalence of tuberculous infection (using 1TU RT23) among children aged 0-9 years;

(2) the prevalence of bacteriologically positive (smear and/or culture) pulmonary tuberculosis in the district;

and (3) the prevalence of radiologically positive pulmonary tuberculosis.

In one-third of the selected villages and towns, x-ray examination was done for those aged 15 years and above, irrespective of symptoms. Sputum specimens were collected for x-ray abnormals also, in these villages.

For all examinations, the procedures were similar to those in the survey among tribals (see 1989 annual report).

Study population and coverages obtained : North Arcot district in Tamilnadu has a population of 48 lakhs spread over 13 taluks. There are 34 towns and 1872 villages. Short Course Chemotherapy was introduced in the DTP in 1983. The sample was selected using cluster sampling method.

The district was stratified talukwise and within each taluk, villages were selected at random, using probability proportional to population of the taluk. The towns were stratified according to population size. Within each stratum, a random sample of towns was selected, giving weightage to the total population within each stratum. Thus, 8 out of 34 towns were selected. Within each town, the street was treated as a cluster and the streets were covered in random order until the required sample size was obtained. Thus, the sample survey included 36 villages from the rural sector and 43 town streets from the urban sector comprising a sample of 1,05,339 persons.

High coverages were obtained for all examinations as shown below:

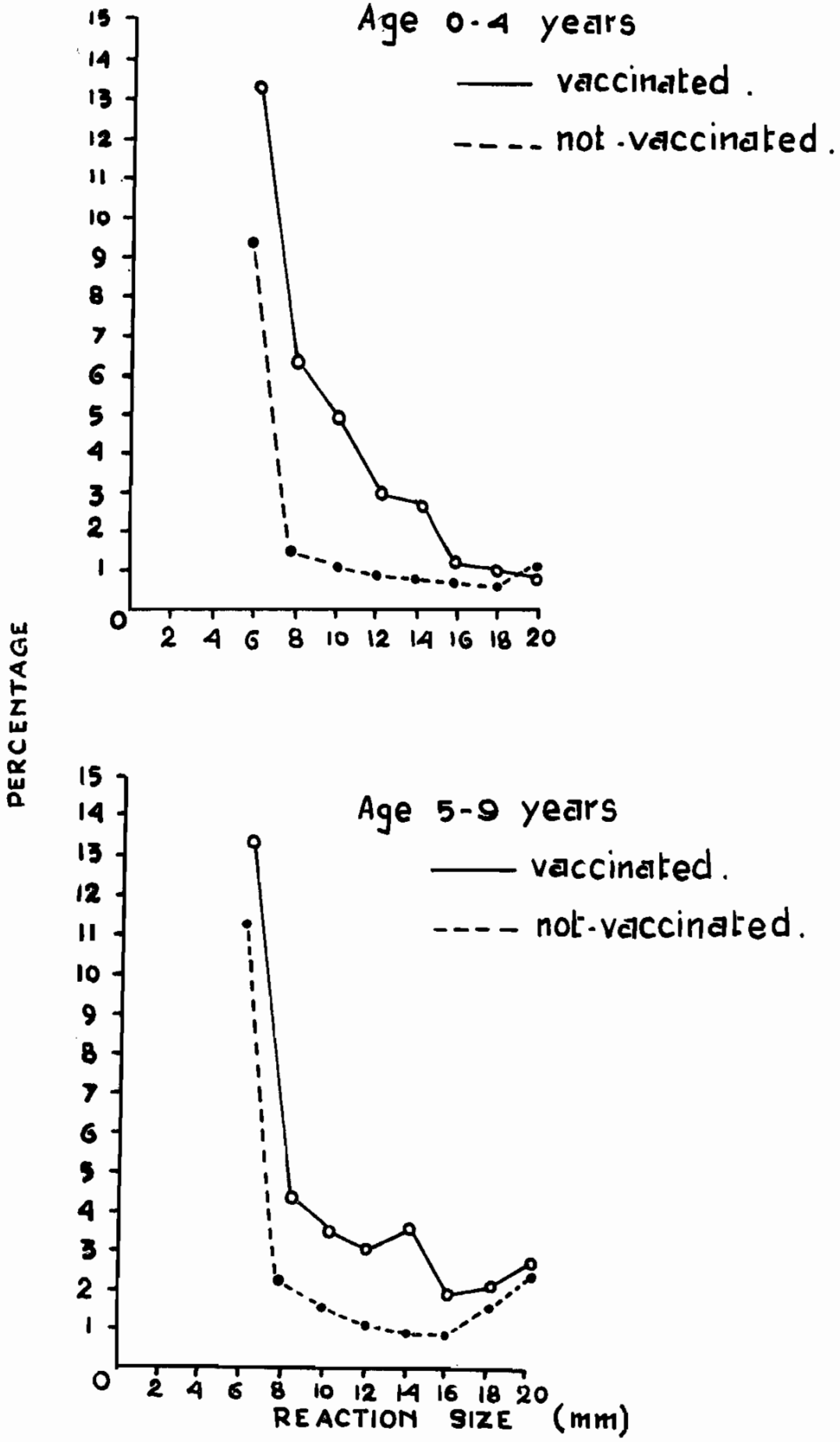
Examination	Eligible population	Population covered	Coverage %
Symptomatic exam.	64887	64274	99.1
X-ray exam. (1/3rd population)	28802	25812	89.6
Tuberculin test	24614	22219	90.3
Sputum	9827	9527	96.9

Infection rates : The presence of a scar was taken as evidence of vaccination. The graph (see page 109) shows the distribution of reaction sizes separately for those with and without a BCG scar. The antimode is not clearly seen among children aged 1 month to 4 years but is found to be 16 mm for children aged 5-9 years among the unvaccinated. The table below gives the infection rates using 12 mm and 16 mm as the cut-off points to define infection.

	Age (in years)	No. tested	≤12 mm No.	≤12 mm %	≥16 mm No.	≥16 mm %
Vaccinated	0-1	2029	189	9.3	37	1.8
	2	946	81	8.6	40	4.2
	3-4	1364	121	8.9	56	4.1
	5-9	3307	497	15.0	266	8.0
	Total	7646	888	11.6	399	5.2
Not vaccinated	0-1	1578	24	1.5	12	0.8
	2	1308	43	3.3	20	1.5
	3-4	3168	141	4.5	100	3.2
	5-9	8519	766	9.0	533	6.3
	Total	14573	974	6.7	665	4.6

It is seen that only about 5% of the children are found to be infected among the unvaccinated. Even among those vaccinated, only 5% of children show positive reactions if 16 mm and above is taken as the cut-off point.

FREQUENCY DISTRIBUTION OF CHILDREN BY SIZE OF TUBERCULIN REACTION (N.A.)



Bacillary disease : Bacillary disease was studied using two screening methods.

a) All individuals aged 15 years and above in the whole sample were screened for symptoms and sputum collected from symptomatics according to DTP definition and from those with a history of treatment.

b) In addition, radiographs were taken for all individuals aged 15 years and above in one-third of the selected villages and town streets. The radiographs were read by two independent readers, with umpire reading wherever there was disagreement. Sputum was collected from all individuals whose radiographs were read as abnormal by any one reader.

The overall proportion of individuals eligible for sputum examination increased with increase in age, and was higher among males than among females for both symptomatics and x-ray abnormals.

Yield of cases by method of screening: The table below refers to the one-third population where x-ray was also done. There were 25688 individuals out of whom sputum was collected from 6007, on the basis of symptomatic status or x-ray abnormality or both.

Method of screening	Examined for sputum	No.of sputum positives			
		S+C+	S+C-	S-C+	All
Symptomatics	3828	6	20	35	61
Symptomatics with abnormal x-ray	684	44	3	27	74
Abnormal x-ray	1495	18	8	44	70
Total	6007	68	31	106	205

In all, two hundred and five sputum positives cases were detected and the prevalence of bacillary cases in this population was 8 per thousand. If only x-rays were used, only 144 (70+74) cases would have been detected giving a prevalence of 5.6 per thousand. If only symptoms were used for screening 135 (74+61) cases would have been detected giving a prevalence of 5.2 per thousand. Thus either method of screening when used individually will underestimate the prevalence by 3 per thousand.

Bacteriological examinations : The table below gives the prevalence of sputum positivity by age and sex for symptomatics. The overall prevalence was 4.3 per 1000 among persons aged 15 years or more. The prevalence increased with age and was about 2 times higher among males (6.0 per thousand) as compared to that .among females (2.7 per thousand)

Age group (yrs)	Sex	Examined for		Number sputum positive						Rate per thousand
		symptoms	sputum	ZN (spot)	ZN (ov)	FL (spot)	FL (ov)	Sm.-Cul+	All pos	
15-24	M	8712	835	2	3	4	1	1	11	1.3
	F	9653	619	3	1	1	1	6	12	1.2
	T	18365	1454	5	4	5	2	7	23	1.3
25-44	M	11982	1655	13	8	4	5	22	52	4.3
	F	13372	1652	9	3	2	4	19	37	2.8
	T	25354	3307	22	11	6	9	'41	89	3.5
45+	M	9705	1932	40	15	8	9	47	119	12.3
	F	10653	1339	12	3	7	3	18	43	4.0
	T	20358	3271	52	18	15	12	65	162	8.0
Total	M	30399	4422	55	26	16	15	70	182	6.0
	F	33678	3610	24	7	10	8	43	92	2.7
	T	64077	8032	79	33	26	23	113	274	4.3

Under the District Tuberculosis Programme (DTP), the procedure is to examine a spot specimen for AFB using ZN technique. Considering only sputum spot specimens examined for AFB at the Field Camp, the prevalence of sputum positive disease was 1.2 per 1000 (1.8 among males and 0.7 among females). Examination of an 'overnight' specimen for AFB, which is not feasible under DTP conditions, increased the prevalence rate to 1.7 per 1000. Repeating the smear examination of the spot and overnight specimens at the Central Laboratory using Fluorescence Microscopy (FL) yielded an additional prevalence of 0.8 cases per 1000. However, subjecting the sputum specimens for culture examination gave a substantial yield of 113 additional cases which worked out to a prevalence rate of 1.8 per 1000. Thus, nearly half (41%) the cases are positive

only on culture, and will not be detected by the District Tuberculosis Program.

Distribution of sputum positive cases by symptom status : The table below gives the distribution of sputum positive cases by symptom status. It can be seen that of the 8032 symptomatics as many as 4932 (61%) had cough of 14 days or more and contributed 211 (77%) of the 274 sputum positive cases. There were 2790 (35%) symptomatics without cough but with chest pain of one month or more and contributed 56 (20%) sputum positive cases. There were only 69 (0.9%) symptomatics with fever alone with 2 (0.7%) sputum positives. Others, comprising 241 (3%), yielded 5 (1.8%) sputum positive cases.

Symptom	Examined for sputum		Sputum positive				
	No.	%	S+C+	S-C+	S+C-	Total No.	Total %
C (all)	4932	61.4	103	71	37	211	77.5
P (without C)	2790	34.7	4	36	16	56	20.4
F (without C,P)	69	0.9	0	2	0	2	0.7
Others	241	3.0	1	4	0	5	1.8
Total	8032	100	108	113	53	274	100

Abacillary x-ray cases : Of the 25812 individuals examined by x-ray, there were 541 individuals whose x-ray was read as abnormal (probable-'D' or possible-'C' by at least 2 of the three readers) and of these, 108 were sputum positive. Among the 1462 x-rays read as C or D by one reader and sputum examined, 26 were smear or culture positive.

X-ray results	Examined by sputum	Positives No.	Positives %
C or D by at least two readers	541	108	20.0
C or D by one reader	1462	26	1.8
Other abnormals	176	10	5.7
Total	2179	144	6.6

Radiological disease was defined as an x-ray shadow read as probable (D) or possible (C) tuberculosis by any two readers with negative sputum. The age-sex distribution of radiological cases is shown in the table below. The overall prevalence of abacillary x-ray cases was 17.0 per 1000 among persons aged 15 years and above. The prevalence increased with age and was higher among males (22.8 per 1000) than females (11.8 per 1000).

Age (years)	Sex	No. x-rayed	Cases No.	Cases per thousand
15-24	M	3408	7	2.1
	F	3842	15	3.9
	T	7250	22	3.0
25-44	M	4768	60	12.6
	F	5460	54	9.9
	T	10228	114	11.1
45+	M	3841	207	53.9
	F	4164	90	21.6
	T	8005	297	37.1
15+	M	12017	274	22.8
	F	13466	159	11.8
	T	25483	433	17.0

Distribution of culture positive cases by drug sensitivity status: The table on page 114 gives the distribution of culture positive cases by drug sensitivity to isoniazid, streptomycin and rifampicin. .Of the 278 patients for whom drug sensitivity results were available, 216 (77.7%) were sensitive to all three drugs, 9 (3.2%) were resistant to both INH and rifampicin and another 45 (16.2%) to INH; 6 (2.2%) patients were resistant to streptomycin alone and another 2 (0.7%) patients to rifampicin alone. Among males 22.8% (45/197) and and females 13.5% (11/81) were resistant to INH or rifampicin or both. Twenty-three (62.2%) of the 37 cases with a history of Rx and 33 (13.7%) of the 241 cases without a history of treatment were resistant INH or rifampicin or both.

Age group (years)	Sex	No. culture positive	Sens. to S,H,R	Resistant to					
				S	H	R	SH	HR	SHR
15-24	M	7	6	-	1	-	-	-	-
	F	10	8	-	-	-	1	1	-
	T	17	14	-	1	-	1	1	-
25-44	M	50	33	2	3	1	8	2	1
	F	30	23	2	5	-	-	-	-
	T	80	56	4	8	1	8	2	1
45+	M	140	109	2	15	-	11	1	2
	F	41	37	-	1	1	-	-	2
	T	181	146	2	16	1	11	1	4
Total	M	197	148	4	19	1	19	3	3
	F	81	68	2	6	1	1	1	2
	T	278	216	6	25	2	20	4	5
History of treatment	No	241	202	6	17	2	10	2	2
	Yes	37	14	-	8	-	10	2	3

Note : The 283 culture positives include 221 obtained from symptomatic screening and 62 from asymptomatic x-ray abnormals. Sensitivity results are not available for 5 cases.

Sputum positivity and history of treatment (among symptomatics): The table on page 115 gives the distribution of patients by sputum positivity and history of treatment. Nearly one-fourth (23%) of the cases who were positive on both smear and culture gave a history of treatment for tuberculosis, compared with 447 (5.8%) of the 7758 symptomatics who were sputum negative.

Action taken by symptomatics: Sixty-eight percent of individuals had taken some action for symptoms. It was seen that 41% of the

Sputum positivity	History of treatment for TB		
	No	Yes	Total
Sm+, Cul+	83	25	108
Sm+, Cul-	49	4	53
Sm-, Cul+	101	12	113
Sm-, Cul-	7311	447	7758
Total	7544	488	8032

symptomatics preferred to go to a private doctor and 24% to the nearest PHI or Taluk Hospital. As many as 32% did not take any action for symptoms.

(started: 1989; completed: 1990).

Tuberculin survey at 20 years for tuberculous infection rates

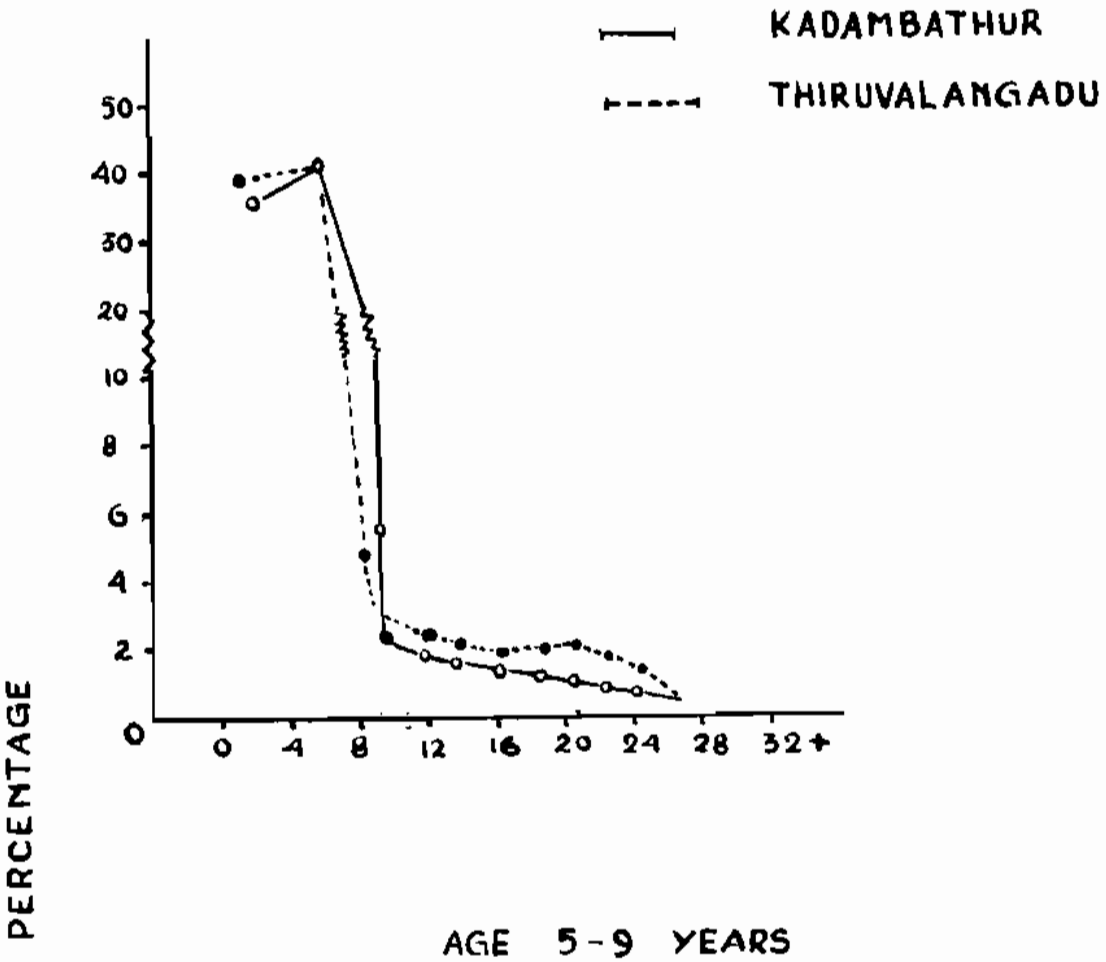
In the BCG trial area, tuberculin surveys were conducted at 0, 10 and 15 years, in order to study the trend of infection in the randomly selected villages of Kadambathur and Tiruvalangadu Panchayat Unions. At 20 years, a similar survey was conducted in the same villages. Children aged 0-12 years were tested with 3 IU of PPD-S and reactions were read after 72 to 96 hours. The coverage in each survey was over 90%.

In this survey, 29 villages (14 villages in Kadambathur Panchayat Union and 15 villages in Tiruvalangadu Panchayat Union) were included. For comparison purposes the analysis were done only for children aged 1-9 years. Among 10915 children aged 1-9 years registered, 9839 (90%) children were tested from these villages. UIP had been introduced in this area during 1988, but BCG scars were seen only in 1358 (14%) of 9827 children examined for scar. The distribution of tuberculin reactions among unvaccinated children is shown in the figure (see page 116).

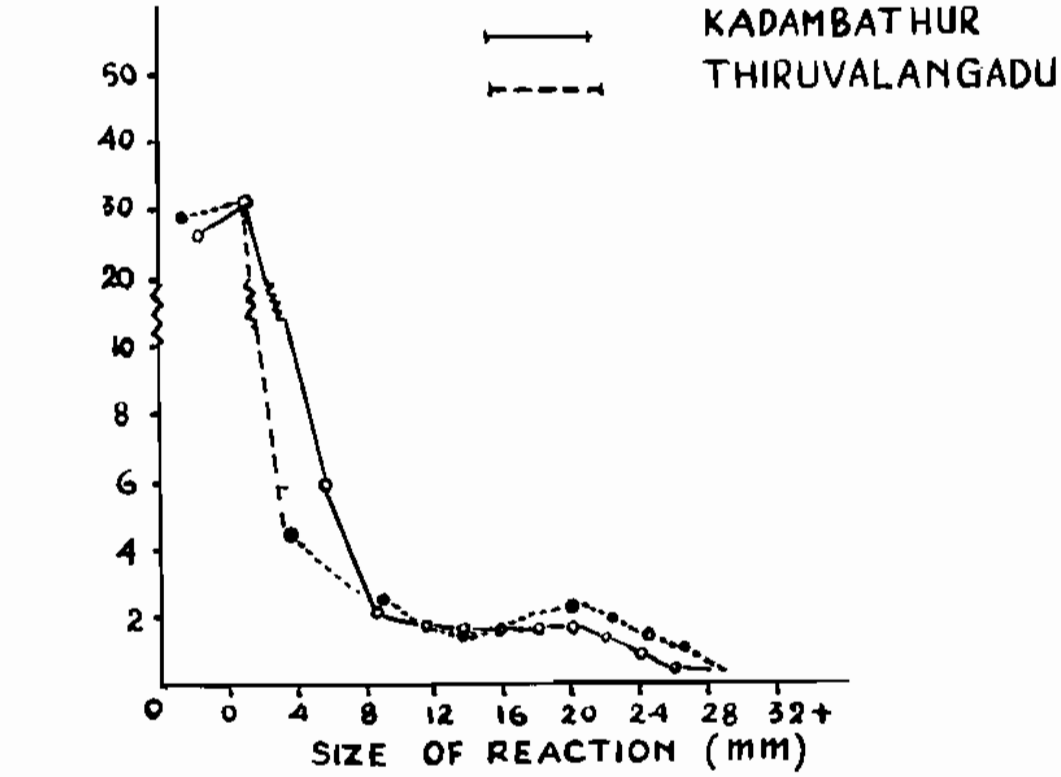
The trend of tuberculin infection over 20 years in this area, as seen from the data available at 0 years, 10 years and 20 years, are shown in the top table on page 117. (At 15 years only 16 villages out of 29 were covered. Hence the data are not included for the comparison).

ISTRIBUTION OF CHILDREN ACCORDING TO SIZE OF REACTION TO PPD-S (3IU)

AGE 1-4 YEARS



AGE 5-9 YEARS



Age (in yrs.)	0 years		10 years		20 years	
	No. tested	Percentage infected (≥12mm)	No. tested	Percentage infected (≥12mm)	No. tested	Percentage infected (≥12mm)
			Kadambathur			
1-4	2145	4.1	2509	4.2	1918	4.9
5-9	2792	11.6	3128	12.3	2569	12.1
1-9	4937	8.3	5637	8.17	4487	9.0
			Tiruvalangadu			
1-4	1615	5.2	1774	6.0	1507	6.6
5-9	2151	16.0	2298	17.2	2475	14.1
1-9	3766	11.4	4072	12.3	3982	11.2

There is no significant difference in the infection rates within each Panchayat Union in each age group over 20 years, except in the 5-9 year age group of Tiruvalangadu Panchayat Union.

The antimode is not clear in the 1-4 year age group. The distribution in the 5-9 year age group shows an antimode at 14 mm. If the antimode is taken as defining infection, at 20 years it has shifted to 14 mm. Accordingly the percentage infected (≥ 14 mm) in the age groups 1-4 and 5-9 is shown in the table below, separately for Kadambathur and Tiruvalangadu Panchayat Unions.

Age (in yrs.)	Kadambathur			Tiruvalangadu			Both		
	No. Examined	Reactors No.	Reactors %	No. Examined	Reactors No.	Reactors %	No. Examined	Reactors No.	Reactors %
1-4	1918	81	4.2	1507	85	5.6	3425	166	4.8
5-9	2569	249	9.7	2475	301	12.2	5044	550	10.9
1-9	4487	330	7.4	3982	386	9.7	8469	716	8.5

The higher infection rates in both age groups in Tiruvalangadu Panchayat Union as compared to Kadambathur Panchayat Union is maintained even in this round. The difference in the overall prevalence is highly significant.

(started: 1989; completed: 1990).

Assessment of health workers in screening for Childhood Tuberculosis

Standard methods of examination are a pre-requisite for any study involving large populations and several investigations, such as a study of the epidemiology of tuberculosis in children. An earlier study showed that field workers of this Centre can be trained to screen children and identify symptoms and signs attributable to tuberculosis in children (1988 annual report). The present study aims to standardise the method of examination. The methodology for this study is described in detail in the 1989 annual report. The general aim is for the health worker (HW) to pick out for examination or investigations, all cases that would have been picked out by the paediatrician.

Two thousand children in 5 villages were screened by four health workers, of whom 337 were labelled as abnormal by them. All these with suitable controls were examined by the paediatrician. Thus, 436 were examined by the paediatrician, of whom 91, 150, 113 and 82 had been examined earlier by the 4 health workers, respectively. The levels of agreement reached in eliciting the principal signs and symptoms are given in the table below:

Signs and symptoms	HW-A n = 91		HW-B n = 150		HW-C n = 113		HW-D n = 82	
	Prev.	Kappa	Prev.	Kappa	Prev.	Kappa	Prev.	Kappa
Fever	0.14	0.3063	0.15	0.4595	0.12	0.2926	0.11	0.2576
Cough/ Repeated Respiratory Episode	0.26	0.5078	0.29	0.5234	0.29	0.5206	0.27	0.4980
Swelling of gland	0.19	0.3938	0.17	0.4040	0.31	0.4247	0.24	0.4646
Abdominal pain	0.14	0.3884	0.15	0.1540	0.12	0.3979	0.16	0.3867

Other symptoms like Swelling of Joint, Back Pain, Difficulty in walking, Gibbus, Meningeal signs and phlyctenule were very rare and hence not included.

An algorithm for health worker screening had been developed based on the signs and symptoms that were found most frequently, in those children who were found to be tuberculous as confirmed bacteriologically.

The efficiency of the health workers in picking out the signs and symptoms that would result in such referral was examined using the same data and is presented in the table below

Health Worker		Investigations		
		Gastric Lavage/ Sputum	Clinical Exam.	Either GL/Sp. or Clinical Examination
A (N = 91)	Prevalence	0.26	0.08	0.28
	Sensitivity	0.56	0.29	0.68
	Specificity	0.92	0.93	0.88
B (N = 150)	Prevalence	0.29	0.08	0.33
	Sensitivity	0.58	0.15	0.61
	Specificity	0.92	0.76	0.76
C (N = 113)	Prevalence	0.29	0.07	0.22
	Sensitivity	0.64	0.09	0.62
	Specificity	0.88	0.92	0.81
D (N = 82)	Prevalence	0.28	0.07	0.30
	Sensitivity	0.52	0.33	0.56
	Specificity	0.92	0.94	0.86

Prevalence : Proportion considered as eligibles by the paediatrician.

Sensitivity : Proportion of referrals picked out by the health workers among referrals by the paediatrician.

Specificity : Proportion of those defined as normals by the health workers among normals defined by the paediatrician.

It is seen that the specificity is high; therefore the number of cases wrongly referred by the HWs is small. However, the sensitivity is low, suggesting that cases needing referral are being missed. This is a reflection of the agreement levels and suggests that the definitions of the signs and symptoms should be more objective. Methods of correcting this will be examined in the pilot study (see page 124).

(started : 1989; completed : 1990).

STUDIES IN PROGRESS

Longitudinal study of factors associated with bacteriological quiescence and relapse under programme conditions

An earlier study from this Centre has shown that about 31% of smear positive pulmonary tuberculosis patients remained bacteriologically positive 6-30 months after starting chemotherapy (1989 annual report). Among those remaining positive, 66% had resistance to INH and 12% to rifampicin including 5-7% who had resistance to more than one drug. However, in that study, a retrospective cohort of patients had been assembled, and hence information on pre-treatment specimens was not available. A longitudinal study has been undertaken to have a better understanding of sputum conversion and relapse and to identify the socio-economic factors (other than chemotherapy and pre-treatment drug resistance) if any, influencing these.

The methodology for this study has been described in detail in the 1989 annual report.

Over a one-year period (November 89 to November 90), 628 patients have been registered. House visits within 10 days of registration showed that 17 (2.7%) were dead and another 89 (14.2%) were not available at the address given. Among the remaining 522 cases, sputum could be collected from 514 patients. Culture was positive in 461 (90%) patients. Of these, 140 (30%) showed resistance to streptomycin, 74 (16%) showed resistance to INH and 19 (4%) to rifampicin.

All these individuals are being followed up in their homes every three months and 2 specimens of sputum are collected. The status obtained at each round of follow up is given below:

Month of follow-up	No. eligible	Dead	Migrated	Available
Intake	628	17	89	522
3 months	522	68	114	340
6 months	340	48	38	254
9 months	254	9	12	233

It is observed that the maximum loss due to migration is within the first three months.

The study is in progress.

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

Development of surveillance methodology for tuberculosis

The burden of illness due to tuberculosis in a community is the cumulative effect of exposures to tuberculosis several years previously, and of quality of treatment for cases and re-exposure to tuberculosis infection at the present time. Since cases of tuberculosis remain infective over highly variable periods of time, the prevalence of infection in a community is also a composite of exposure over several years. Thus the epidemiology of tuberculosis is a complex multifactorial problem in which changes in prevalence or incidence are apparent only over long periods of time.

Annual risk of infection to tuberculosis is widely accepted as the best tool for epidemiological surveillance. However, this has been developed in areas with low prevalence of Non-Specific Sensitivity (NSS) and the usefulness of this tool in areas with high prevalence of NSS and high coverages with BCG Vaccination needs to be established. Therefore, there is still a need to develop a simple tool that is capable of wide application and is sensitive to changes in the parameters being measured.

A long term community-based epidemiological study has been undertaken with the general objective of identifying a simple inexpensive tool for the surveillance of the tuberculosis situation in a community. The parameter(s) to be used can be related to infection or disease or both.

The following parameters are being studied:

a) Prevalence and trend in the age - specific infection rates in the community.

b) Age-sex specific distribution of adult bacillary cases and the trend of this distribution separately for prevalence and incidence cases during follow up.

c) The proportion of chronic excretors among prevalence cases and their nature in terms of drug sensitivity, at each round.

Methodology: The following activities are being carried out.

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

2. Comprehensive survey for detecting tuberculosis disease will be undertaken in the same area as the infection surveys, and will cover a population of about 100,000. All individuals aged 10 years and above will be screened by means of both x-rays and symptomatic status. Sputum will be collected from symptomatics and those with an abnormal x-ray shadow, and examined for smear, culture and drug sensitivity status at the Centre's Laboratory. This survey will also be carried out once in two years.

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

Selective follow-up: Those found to be symptomatic, or to have an abnormal chest x-ray and are sputum negative will be treated as suspects and followed up every 6 months. Such selective follow-up rounds will be undertaken every 6 months. Thus, if the surveillance interval is 2 years, there will be three rounds of selective follow-up in between surveillance rounds. The suspects will be x-rayed, screened for symptoms and sputum collected from eligibles.

Passive case finding: In addition to the above methods of active case finding, passive case finding centres will function from the peripheral health institutions, normally once a week. Patients reporting with symptoms will be attended to at these centres. Facilities for x-ray and sputum collection will be provided.

Treatment: Those found to be sputum positive either by smear or by culture will be offered treatment with short course regimens immediately through the passive case finding centres in the study area. Individuals whose x-rays are read as definite or doubtful tuberculosis by two readers will be treated as suspects. Sputum will be repeated and if still negative, they will be put on standard regimens. Short course regimens, either daily unsupervised (2EHRZ/6EH) or supervised twice weekly ($2EHRZ_2$/ $4RH_2$), will be offered depending on acceptability to patient. Treatment activities will be undertaken by the staff of the Epidemiology Unit.

Cases put on treatment will be followed up every three months, and sputum collected. Those found to have resistance to INH and/or rifampicin will be offered more intensive treatment. Defaulter action will be as per the District Tuberculosis Program procedures.

This study will be undertaken initially in the 78 groups of Thiruvallur taluk where complete coverage in the BCG trial was carried out. The sample sizes and surveillance interval mentioned are tentative. The data collected in the first few months will be reviewed by the Epidemiology Sub-Committee (see page 9), which will decide upon these, as well as the duration of the study.

The study has been started in four villages, and a population of 4632 has been registered up to 31.12.1990.

(started: 1990)

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

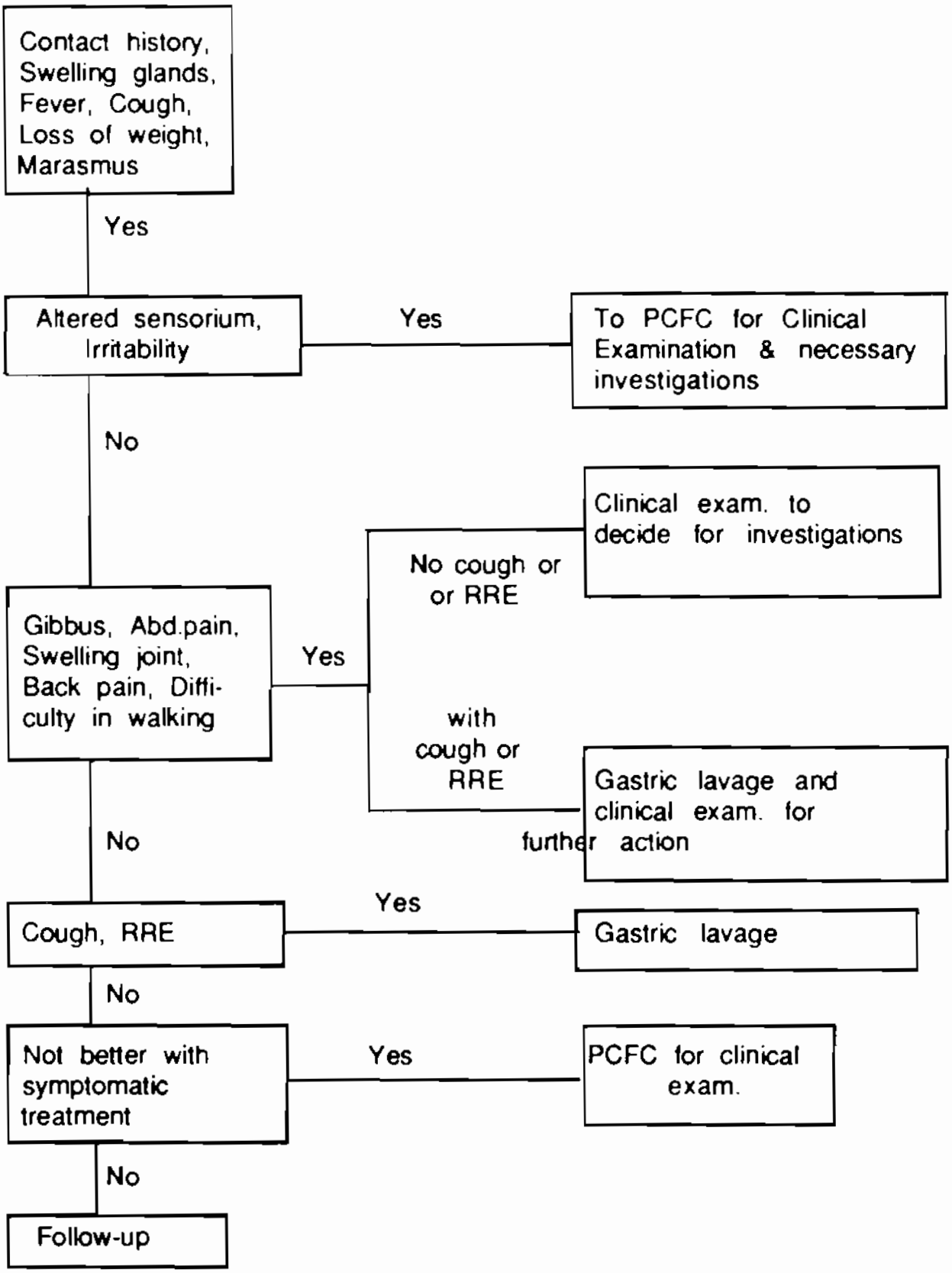
A study on the burden of illness due to tuberculosis in the community requires that all cases of all different manifestations of tuberculosis in children in the community be documented. This involves the collection and processing of several specimens like sputum, gastric lavage and gland biopsy; taking special x-rays to document skeletal tuberculosis; and special investigations like CSF or peritoneal fluid examination for rare forms like meningeal and abdominal tuberculosis. These investigations are traditionally carried out in a hospital. An earlier study carried out in this Centre (1988 annual report) showed that:

a) it is possible to carry out these investigations under field conditions with reasonably good coverages;

b) the criteria used in the selection of cases for carrying out these investigations yielded only a small proportion of positives in relation to the number of specimens processed.

Based on the findings of that study, an algorithm for use by health workers to screen and refer children for investigations and/or clinical examination was developed (see figure on page 125). This algorithm is expected to reduce the number of children investigated without affecting the number of cases detected.

ALGORITHM FOR HEALTH WORKER - SCREENING FOR TUBERCULOSIS IN CHILDREN BELOW 10 YEARS



Note : Any child with a History of anti-TB Treatment or X-ray abnormality, irrespective of the algorithm, will be examined by the M.O.

The pilot study is undertaken to explore the feasibility of the health workers using the algorithm, the applicability of the algorithm in referring children for investigations and to define the logistic problems associated with documenting cases of tuberculosis in children in the field.

The study is being carried out in the same villages where surveillance is undertaken and involves the following aspects:

1. Complete census (the cards from the surveillance team will be used).

2. Screening of all children by health workers for abnormality suggestive of TB using the algorithm presented in page 125.

3. Tuberculin testing with 1TU RT23 for children aged 2 months to 9 years and reading of reactions after 72 hours.

4. Full-plate chest radiograph for all children of age two months and above will be taken and read by two independent readers. Children whose radiographs are read as abnormal by either reader will be re-x-rayed at 3 months.

Children will be referred for clinical examination and investigations according to the algorithm.

Screening will be repeated for all children at 6 months and 1 year.

Children will be referred for treatment if they are found to be bacteriologically positive, or if there is strong clinical or radiological suspicion.

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

Surveillance of individuals infected with the human immunodeficiency virus for tuberculosis

The existence of a relationship between infection with Human Immunodeficiency Virus (HIV) and the development of tuberculosis is now well established. There is evidence in the literature of the occurrence of different forms of tuberculosis in HIV-infected individuals, especially in countries with a high prevalence of tuberculosis. An intensive surveillance of HIV infected individuals has been undertaken since June 1989 by the Epidemiology Unit of the Centre, to monitor the occurrence and nature of tuberculosis in these individuals and also to study the pattern of transmission of HIV infection in close contacts.

Methodology: Addresses of patients identified to be positive for HIV infection on routine ELISA testing are obtained from the various surveillance centres in the state of Tamil Nadu. These individuals are contacted in their houses and asked to report to the Centre, where they are registered for the study. At intake and at 6-monthly intervals, they are examined clinically by a medical officer. Weight is recorded and a radiograph of the chest is taken. Two to four samples of sputa are collected from those individuals having an abnormal radiograph or with symptoms referable to tuberculosis. Those individuals found to be positive on smear or culture are started on anti-tuberculosis chemotherapy. A nine-month daily regimen with 2 months of EHRZ followed by 7 months of HR is now being offered. Tuberculin testing (1TU RT23) is being done at intake and at yearly intervals. Finger prints are taken at the time of registration and at every follow-up. These are being compared by trained persons to ensure correct identification. Sociological assessment is made regarding aberrant sexual behaviour of the patient.

The family members including the spouse and other sexual partners are also being registered and followed up at 6-monthly intervals to study the pattern of transmission of HIV infection.

A centre had been established in Pondicherry from June 1990 in the STD Clinic of the Govt. Hospital where the same procedures are being carried out.

Up to the period of reporting (Dec. 1990), addresses of 328 individuals had been obtained in both the centres. It has been possible to trace 97 (30%) of these individuals and register them along with their contacts. Twenty-five of these individuals are from Madras city, twenty-eight from Pondicherry and the rest from other districts. Eight individuals had died. Subsequently, 6 contacts have become HIV positive, making the total number of positive individuals 103. The age ranged from 15 years to 55 years. There were forty-nine males and fifty-four females. Forty-five (44%) were prostitutes.

The profile of the infected individuals is given in the top table on page 130.

Up to December 1990, seven HIV infected individuals with sputum positive on culture had been started on anti-tuberculosis chemotherapy. Only forty-three out of 97 individuals showed a positive reaction (≥12 mm) to 1TU RT23. All seven tuberculosis patients showed a positive reaction.

		Madras Centre Male	Madras Centre Female	Pondicherry Centre Male	Pondicherry Centre Female	Total
Registered		33	20	16	34	103*
Abnormal radiograph		14	5	5	7	31
Sputum positive	MTb**	5	1	1	-	7
	NTM	9@	2	-	-	11@
Mantoux (1TU RT23) positive (≥12 mm)		24	3	5	11	43

* Includes 6 contacts who became ELISA positive.
** MTb : M. tuberculosis; NTM - Non-tuberculous mycobacteria
@ Including 3 subjects with NTM on more than one occasion.

The table below gives the coverages obtained at each follow-up.

Follow up (months)	Madras centre			
	No. eligible	No. examined	Not examined	
			Traceable	Not traceable
3	34	30	3	1
6	24	20	4	-
9	18	13	3	2
12	15	13	1	1
15	4	3	-	1
18	1	1	-	-

The study is continuing.

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

STATISTICAL STUDIES

STUDIES COMPLETED

An attempt to develop a mathematical model for evolving diagnostic criteria for tuberculosis in children

Diagnosis of tuberculosis in children poses a difficult problem as there are no universally acceptable clinical or radiological diagnostic criteria, and isolation of **Mycobacterium tuberculosis** from specimens in children is difficult. Further, lack of bacteriological confirmation may not be an indication of the absence of tuberculosis in children. Empirical schemes are not able to discriminate between children with and without bacteriological confirmation of disease. The aim of this study is to make an attempt to develop a model for evolving diagnostic criteria using data from a validation study (Yash, Symposium on recent advances in Tb Research, Bombay, 1980) conducted by the Epidemiology Unit.

This study was conducted at the Institute of Child Health and Hospital for Children, Madras and included 1285 children with different manifestations of tuberculosis. Of these, 753 children were identified as having respiratory type of tuberculosis, with bacteriological confirmation in 145 (19%) cases.

There are five different types of information available for each child, namely,

(i) general information (age, sex, nutritional status etc.),

(ii) signs and symptoms referable to systemic illness,

(iii) signs and symptoms referable to the respiratory system,

(iv) different types of radiographic shadows along with their interpretation by both individual readers and by a panel, and

(v) bacteriological results.

A preliminary analysis was done for finding the association of history, signs, symptoms and radiographic shadows with bacteriological confirmation. The sensitivity, specificity and predictive values for these variables were calculated. This approach is deterministic and may not be very helpful in identifying the influence of a group of variables in the presence of other variables. Hence a multivariate (probabilistic) approach was adopted. As the data consisted of a large number of qualitative variables,

stepwise multiple logistic regression was employed for statistical analysis. In view of the complexity involved in testing a number of models, analysis was done using BMDP statistical package in the VAX 11/750 computer at the Centre.

For the purpose of analysis, a case is defined as positive if (i) bacteriological confirmation is available and/or (ii) if radiographic abnormality of the respiratory system is observed. There were mainly two approaches: (a) using bacteriology (positive or negative) as standard (response variable), and (b) using radiology (abnormal or normal) as standard (response variable). The different types of information available for each child were considered as predictor variables.

Models of form $P = 1 / [1 + \exp\{-(\beta_0 + \beta_1 x_1 + \ldots + \beta_p x_p)\}]$ were fit separately according to different types of information. Here x_1, x_2, ... x_p are predictor variables; β_0, β_1, ... β_p are coefficients estimated by maximum likelihood approach and P is the probability of a child being a case/non-case. Variables selected by each of these models were considered together and an overall model was fit. Using this model, probabilities were computed, and the table below illustrates the sensitivities and specificities calculated by comparing what was observed (bacteriological result as standard) with that predicted by the model using different cut-off probabilities.

Respiratory tuberculosis cut-off probability	Observed according to bacteriology vs predicted by model	
	Sensitivity (%)	Specificity (%)
0.23	78	45
0.27	38	80
0.31	34	82
0.35	31	84

The table on page 131 gives the sensitivity and specificity estimates obtained by considering radiology as standard and comparing with that predicted by the model using different cut-off probabilities.

Respiratory tuberculosis cut-off probability	Observed according to radiology (among bacteriological negatives) vs predicted by model	
	Sensitivity (%)	Specificity (%)
0.21	100	3
0.41	98	15
0.61	84	43
0.81	37	85

It is observed from the above tables that the sensitivity and specificity are far below satisfactory levels for any of the cut-off probabilities indicating that these models are not suitable for evolving diagnostic criteria for tuberculosis in children.

(started:1990; completed: 1990).

STUDIES IN PROGRESS

Regression models in the prediction of lung function

The measurement of lung function has gained wide acceptance in the past four decades as a simple method of monitoring for, and screening against chronic respiratory disease. Chronic respiratory disease is the collective name given to a number of chronic lung diseases including bronchitis, emphysema and asthma. The two most commonly used indirect indices to quantify lung function are Forced Vital Capacity (FVC) and Forced Expiratory Volume (FEV), both derived from the volume of air in litres expired in a single forced expiration following a full inspiration. FVC is the total volume expired and FEV is the volume expired in a shorter measured interval (1 sec). The other indices considered are the Functional Residual Capacity (FRC) and diffusing capacity measurements (Effective Alveolar Volume (VA), Carbon-monoxide Transfer Factor (TLCO) and Transfer Co-efficient (KCO)).

Lung function indices provide a sensitive, repeatable and rapidly obtained measure of general respiratory health in the individuals and so are invaluable in the epidemiological study of chronic respiratory disease.

When it is used for screening, either in clinical diagnosis or epidemiologically, the aim is to identify individuals or groups "at risk" as a result of, for example, their smoking habits or occupation. This is done by assessing the individual's lung function relative to the (known) distribution of such values in a healthy but otherwise suitably matched population. The values are usually known as "normal" or "reference values".

Selection of co-variates: It has been long known that ventilatory function declines with age after about age 25, even among individuals free of chronic respiratory disease; the presence of such a disease increases the rate of decline. Many recent studies have shown that in large cross sectional samples, the relation with age is closer to linear, so that the annual 'rate of decline' can be estimated by linear regression, giving an age co-efficient between 20 and 40 ml/year typically. However, it is unjustified to infer from this that an individual's ventilatory function declines linearly over life; but the observed age co-efficient provides a simple and convenient method of correcting for age within the sample, and indeed for comparing the sample with other ones.

The strong correlation between ventilatory function and body size can be easily quantified. Numerous indices of body size have been considered in the past including body surface area, height, sitting height, weight, chest circumference and chest expansion, but most workers now settle for height, which is not only highly correlated with the FEV and

the FVC, but is also a very repeatable measurement. Sitting height, although better correlated with chest size, is far less repeatable.

Other factors known to affect ventilatory function are sex and ethnic variation. The ventilatory capacity of women is about 75% that of men from the same population and North American blacks have long been known to have FEV averaging between 10 and 15% less than whites (Coates, 1975). The nature of these sex/ethnic group differences is discussed by many authors, but our discussion is confined to the effects of age and height in ethnic South Indian population.

Both the FEV and FVC are known to decline in adult life as part of the normal aging process and considerable attention in the pulmonary epidemiology literature has been devoted to identify risk factors which accelerate this aging process and ultimately lead to chronic airflow obstruction (CAO), defined in population studies by an abnormally low FEV_1, either based on cross sectional data or longitudinal data. In order to distinguish relatively low from abnormally low lung function, it is necessary to develop prediction expirations to describe 'normal' lung function both cross-sectionally and longitudinally.

Data: Data considered here are from a study conducted at the Centre in the normal ethnic South Indians living in Madras, aged 15-40 years (males 130, females 117). Along with the pulmonary function measurements, sex, ethnic identity, standing height to the nearest centimetre, weight in kilo- grams, age, smoking habit, occupation and food habits were also recorded.

At least three forced vital capacity manoeuvres were obtained for each subject; for FVC and FEV_1, the best two of the three tracings had to be within + 5%. The largest of the three FVC and FEV_1 values was accepted even if the three volumes did not come from the same curve. FRC, RV and TLC were measured by the closed circuit helium dilution method. Duplicate measurements of FRC were made for each subject and the mean value was used for analysis. The TLCO was measured with a gas mixture containing helium 14%, carbon monoxide 0.28% and oxygen 18%, the rest being nitrogen. VA was obtained during the single breath TLCO measurements. KCO was derived as the ratio of TLCO to VA. TLCO and KCO were also connected to the standard haemoglobin (14.6 g/dl).

Regression methods: No consensus has yet developed as to how best to model pulmonary function data. Many investigators use cross sectional models. For example, for FEV_1, the model used is:

$$FEV_1 = a + b.Ht + c.Age$$

This model tacitly implies that the rate of change of FEV_1, over time is constant and equal to c. The inclusion of age-height and age^2 terms in the model would further allow the rate of decline to depend on height and age respectively.

As an alternative, some investigators propose division of the dependent variable by a power of height as a more suitable outcome variable. This approach has intuitive appeal because it seeks to express spirometric measurements in terms of liters by unit size. Further, for cross-sectional data, the correlations of both FEV_1/Ht^2, and FEV_1/Ht^3 with height are much less than the correlation of FEV_1 with height. Despite these apparent advantages some questions remain. First how well do the various size adjustments work? Second, is it possible to measure the goodness of fit of one approach versus another? Third, even if division by a power of height works well for the cross sectional data, does it follow that rates of decline over time as measured from longitudinal data should also be divided by a power of height?

Any regression model for FEV_1/Ht^n with age implicitly assumes a certain structure for the relationship between untransformed measurement FEV_1 and age and height. For example, if we were to model the dependence of FEV_1/Ht on age as

$$FEV_1/Ht = l + m.Age,$$

then we would be tacitly assuming that

$$FEV_1 = a + b.Ht + c.Age\ .Ht \qquad (2)$$

This is at variance with models usually considered such as

$$FEV_1 = a + b.Ht + c.Age \qquad (3)$$

or $$FEV_1 = a + b.Ht + c.Age + d.Age\ .\ Ht \qquad (4)$$

Even these models are incomplete, however, since they imply that the rate of decline of FEV_1 over time does not depend on age. The addition of an age term in the above models would imply such an age dependency for the slope. Similarly, the inclusion of age-height interaction terms tests whether the rate of decline of FEV_1 depends on height. The difference between models (2) and (4) is that model (2) assumes lung function decline is absolutely proportional to height while model (4) does not. In practice, the distinction is less clear, since both models carry the same message, i.e., lung volume declines with age and the magnitude of this decline varies as a function of size.

This paper uses data from a study conducted at the Centre to attempt to resolve some of these issues by presenting an unified approach to the problem of analysing pulmonary function data with special reference to FEV_1.

Results: The baseline characteristics of the subjects are presented in the table below.

Covariate	Male	Female
Age (yrs)		
mean ± SD	26± 7	27±6
Height (cm)		
mean ± SD	166± 8	152±5
Weight (kg)		
mean ± SD	53±12	45±9
Haemoglobin(gl/dl)|		
mean ± SD	14.1±2.1	10.7±1.6
Smoking		
Yes	28%	0%
No	65%	100%
Formerly	7%	0%
Food habit		
Veg	9%	8%
Non-veg	91%	92%

The division of FEV_1 by successively higher powers of height results in successively smaller correlations between the resulting measures and height as seen in the table on page 136.

FEV_1/HT^n	Males		Females	
	Age	Height	Age	Height
FEV_1	-0.0432	0.7053**	-0.0763	0.3613
FEV_1/HT	-0.0632	0.5528**	-0.0882	0.1440
$FEV_1/HT^{1.605}$	---	---	-0.0939	-0.0005
FEV_1/HT^2	-0.0911	0.3222	---	---
FEV_1/HT^3	-0.1223	0.0268	---	---
$FEV_1/HT^{3.085}$	-0.1248	0.0006	---	---

** $P < 0.001$

The correlation coefficients become almost zero for n=3.085 in males and for n=1.605 in females. The likelihood ratio statistic may be used to test the goodness of fit of the hierarchical models. We use the deviance and R^2 to compare the various models. Further comparisons are made using residual analysis. Each regression model assumes that the standardized residuals (observed-predicted)/SD are normally distributed with mean 0 and variance 1. If the models adequately describe the dependence of FEV_1 on age and height, then these measures should be uncorrelated with the residuals. We tested each of these assumptions for various models.

On the basis of cross-sectional analysis we found little to distinguish the various models. In summary we have presented a method for comparing competing models for spirometric data. Model (1) or its slight variation (age replaced by age^2) seems to be adequate for modelling our data. Further work is under progress with other data sets.

(started: 1990; expected year of completion: 1991).

ELECTRONIC DATA PROCESSING

The Electronic Data Processing unit is undertaking data entry and verification, data processing, and statistical analysis for a few studies carried out by the Centre. However the main support till the year end was provided for the TB Prevention Trial and Leprosy Prevention Trial. Apart from data entry and verification, the DTP monitoring system received increasing attention in data processing and report generation.

The SPSS-X statistical package was installed on the VAX computer system during the year, in addition to the BMDP statistical package which was installed the year before. Several application computer programs were developed mostly in VAX-FORTRAN and some programs in other languages such as VAX-BASIC and VAX-C. Some of the tabulations for the TB Prevention and Leprosy Prevention Trials needed specialized programmes due to the special nature of the community-based study data.

The VAX computer system is being used for analysing the TB Prevention Trial data. In all, about 21 lakh punched cards were read through the card_reader and the files thus created were checked, verified and corrected. The entire data that was collected under the survey conducted in 209 villages and a town over 15 years of follow-up period have been copied to the magnetic tapes from punch cards and complete data backup is being maintained. The TB Prevention Trial data file, consisting of about 4 million records, has been split up into 28 subfiles for ease of processing.

Tabulations have been done to facilitate complete data analysis. Tables showing the bacteriological and radiological status of individuals by age and sex have been taken out separately for various factors such as dosage of vaccine, strain and different batches of vaccine, size of induration to PPD-S and PPD-B, and 7 rounds of two-and-a-half year periods of follow-up. Tabulations for studying the incidence of bacteriological and radiological cases have been done separately for these factors.

For the Leprosy Prevention Trial, data consisting of about 14 lakh punch cards (4th and 5th round of Leprosy examination) were read through the card-reader and transferred to magnetic tapes and were merged with 15 lakh records of the first three rounds, which had previously been transferred to magnetic tapes.

All these records have been checked for various types of data errors and corrections effected, wherever necessary. This file also has been organised into 28 subfiles. Software has been developed for the analyses of the data.

Computerisation of the District Tuberculosis Programme (DTP) data is being done. Routine data entry and verification of monthly and quarterly returns received from 18 districts has been undertaken. Software to check data for completeness and consistency have been developed. Attempts are being made to develop a time-bound reporting system, to high-light salient features of the monthly and quarterly reports received, which would help those involved in the monitoring of DTP.

System analysis approach is being adopted for the Surveillance Study of Tuberculosis in Chingleput district. Data entry and verification procedures have been set up. Procedures have been finalised for data entry and verification of data generated from the Surveillance Study of HIV infected individuals. Development of software and procedures for data management are underway for this study.

APPENDICES

TRAINING PROGRAMMES

WHO fellows

Mr. Salem Omar Abdulla Ba Kwairi and Mr. Ja'afer Salem Ba Rabaa, Democratic Yemen, for 1 month from 1.3.90.

Mr. Santhosh Kumar Shrestha, Nepal from 23.4.90 to 18.5.90.

Trainees

The following underwent training in different departments as follows:

Bacteriology

Eighteen students of Diploma Course in Medical Laboratory Technology, Voluntary Health Services, Adyar, from 2.5.90 to 14.5.90.

Immunology and Cardio-Pulmonary Medicine

Dr. G.Jayaraj from Neyveli Lignite Corporation Hospital, Neyveli, from 1.10.90 to 10.10.90.

Cardio-Pulmonary Medicine

Dr. S.Ramesh, Asst. Prof. of Medicine from K.C.General Hospital, Bangalore from 18.10.90 to 24.10.90.

Dr. S.Ramakrishnan, Consultant Physician from K.S.Hospital, Madras from 15.11.90 to 7.12.90.

General

Miss V.Radhika, B.Sc. (Nutrition and Dietetics) from Women's Christian College, Madras.

Others

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

Post-graduate students

Dr. Maitreyee Roy Chowdhary and Dr.Priye Abraham, MD (Microbiology) students and Mr. Ravichandran, Mr. Umapathy, Miss Padmashree and Miss Sheeba, M.Sc. (Microbiology) students from Christian Medical College, Vellore.

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

Three M.D. (TB & CD) students from Andhra Medical College, Visakhapatnam, Andhra Pradesh.

Two M.D.(TB) students from S.V. Medical College, Tirupathy, Andhra Pradesh.

One M.D. (TB) student from Kakatiya Medical College, Warangal, Andhra Pradesh.

Two M.D. (TB) students from Kurnool Medical College, Kurnool, Andhra Pradesh.

Nursing and para-medical students

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

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

Students of Sanitary Inspector and Diploma in Sanitary Science courses, Faculty of Rural Health and Sanitation, Gandhigram Rural Institute, Ambathurai - 1 batch.

Multi-purpose Health Workers from Durgabai Deshmukh Hospital, Madras - 2 batches.

ICMR-WHO WORKSHOPS

Two 2-day workshops were organised at Ujjain, Madhya Pradesh (covering Ujjain, Vidisha, Sagar, Baroda and Nagpur districts) and Pondicherry Union Territory (covering Pondicherry and North Arcot districts), as mentioned on page 24. The subjects covered, the speakers and the numbers of participants are given below:

UJJAIN on 11th and 12th October 1990
Lectures for Medical Officers (22)

Subject	Speaker
Over-view of the programme	Dr.N.M.Sudarsanam
Case finding	Dr. Rani Balasubramanian
Bacteriology	Dr.C.N.Paramasivan
Chemotherapy	Dr.R.Prabhakar
Case holding	Mrs.Sudha Ganapathy
Documentation	Mr.P.R.Somasundaram
Community participation	Mrs.Sudha Ganapathy

Panel on "Case Finding"

Moderator : Dr.G.P.Saxena

Members : Dr.R.Prabhakar, Dr.C.N.Paramasivan, Dr.N.M.Sudarsanam, Dr.A.K.Jain, Dr.A.N.Banerjee and Dr.Joshi

Panel on "Case Holding"

Moderator : Dr.R.Prabhakar

Members : Dr.N.K.Nayak, Dr.Rani Balasubramanian, Mrs.Sudha Ganapathy, Dr.C.B.Varma, Dr.S.G.S.Khare and Dr.S.N.Gupta

Panel on "Documentation"

Moderator : Dr.S.Radhakrishna

Members : Mr.P.R. Somasundaram, Mr.S. Sivasubramanian, Dr.J.N.Khadse, Dr.J.B.Phaye, Dr.A.K.Jain and Mr.Aravind Kavishwar

Panel on "Community Participation"

Moderator : Dr.S.C.Khasgivala

Members : Dr.Rani Balasubramanian, Mrs.Sudha Ganapatny, Mr.K.C.Valsarajan, Dr.B.M.Soni, Dr.C.K.Bal Pande

Lectures for Lab. Technicians(30), Treatment Organizers(5) and Drug Distributors(49)

Overview of DTP and role of paramedicals	Dr.N.M.Sudarsanam
Case finding	Dr.C.N.Paramasivan
Chemotherapy in DTP	Dr.Rani Balasubramanian
Case holding	Mrs. Sudha Ganapathy
Documentation	Mr.S.Sivasubramanian

PONDICHERRY on 14th and 15th December 1990
Lectures for Medical Officers(18)

Subject	**Speaker**
District TB Programme	Dr.N.M.Sudarsanam
Review of 18 districts	Dr.R.Prabhakar
Chemotherapy	Dr.Rajeswari Ramachandran
Case finding	Dr.K.Rajaram
Bacteriology	Dr.N.Selvakumar
Case holding	Mrs. Geetha Ramani Shanmugam
Documentation	Mr. A.S.L.Narayana
Community participation	Mr.S.A.Rajagopal
Management of Programme (health) Services	Prof.Basu Ghosh

Group Discussion (on all the components of DTP)

Group I

Moderator	:	Dr.S.Radhakrishna
Rapporteur	:	Dr.N.M.Sudarsanam
Members	:	Dr.Rajeswari Ramachandran, Dr.N.Selvakumar, Mr.P.Venkataraman, Mr.A.S.L.Narayana, Mrs. Geetha Ramani Shanmugam and Mr.V.Chandrasekaran

Group II

Moderator	:	Prof.Basu Ghosh
Rapporteur	:	Dr.K.Rajaram
Members	:	Mr.S.Sivasubramanian, Mr.Victor Mohan, Mr.Shripad Bhat and Mr.K.Chandrasekaran

Lectures for Lab. Technicians(16), Treatment Organizers(2) and Drug Distributors(22)

Overview of DTP/role of paramedicals	Dr.N.M.Sudarsanam
Case finding	Mr.P.Venkataraman
Chemotherapy in DTP	Dr.Rajeswari Ramachandran
Case holding	Mrs.Geetha Ramani Shanmugam
Documentation	Mr.S.Sivasubramanian

STAFF DEVELOPMENT PROGRAMME

1. Dr.N.Selvakumar attended a workshop on Enzymatic Techniques in Diagnostic Microbiology at Defence Research and Development Establishment, Gwalior from 12-16 February, 1990.

2. Dr.Rajiswamy underwent a 6-month training in T cell immunology under TCTP Colombo Plan at London School of Hygiene and Tropical Medicine, U.K from March 1990.

3. Dr.Ramesh Paranjape was awarded a 3-month WHO fellowship for training in Human Hybridoma technology at Division of Infectious Diseases, Medical School, Case Western Reserve University, Cleveland, U.S.A. from April, 1990.

4. Mrs.S.Vijayalakshmi underwent practical training on "General Basic Laboratory Techniques" at Department of Bio-technology, National Institute of Nutrition, Hyderabad from 5.3.90 to 31.5.90.

5. Dr.M.Nazeema was awarded a one year British Council - TCTP fellowship to undergo M.Sc. degree course in laboratory animal husbandry and care training at Royal Veterinary College, London, U.K from October, 1990.

6. Dr.V.K.Vijayan was awarded Ph.D. in Medicine by the University of Madras, Madras during 1990.

7. Dr.K.V.Kuppurao was awarded Ph.D. in Physiology by the University of Madras, Madras during 1990.

8. Mrs.S.Vijayalakshmi was awarded M.Sc.(Chemistry) degree by the Annamalai University, Annamalainagar, during 1990.

PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

Name of conference, venue and date	Title of paper	Name of staff member
International Clinical Epidemiology (INCLEN) - VIII Meeting, Puebla, Mexico, 20-26 January, 1990	Diagnostic criteria for childhood tuberculosis	Mr.P.V.Krishnamurthy
VII Annual Convention of Indian Society for Medical Statistics, Varanasi, 1-3 February, 1990	Evolution of effective and practicable regimens of chemotherapy for pulmonary tuberculosis by controlled clinical trials over a 30-year period	Mr.P.R.Somasundaram
- do -	Comparison of Cox's and logistic regression models under fixed period outcomes	Mr.P.Venkatesan
- do -	An application of Ridit analysis using EM algorithm	- do -
- do -	Estimation of error rates in medical screening tests	- do -
- do -	Statistical and methodological aspects in designing vaccine trials	Mr.R.S.Vallishayee
Conference of the Madhya Pradesh Anti-TB Association, Bhopal, April, 1990	Short Course Chemotherapy for pulmonary tuberculosis (Dr.N.L.Bordia Memorial Oration)	Dr.R.Prabhakar
CME Programme: Apollo Hospital, Madras, 1 July,1990	Interstitial lung diseases	Dr.V.K.Vijayan
IMA, Badagara, 22 August, 1990	Tropical eosinophilia (Guest lecture)	- do -

Name of conference, venue and date	Title of paper	Name of staff member
XIV All India Medical Microbiology Congress, Vellore, 25-27 October, 1990	Evaluation of a cold staining method for tubercle bacilli	Mrs.Sara Mathew
VIII Annual Convention of Indian Society for Medical Statistics, Vellore, 1-3, November, 1990	A comparative study of non-parametric censored rank tests in the analysis of tuberculosis survival data	Mr.P.Venkatesan
-do -	A semi parametric approach for model selection in survival data analysis	- do -
- do -	A comparison of Markov model to life table in the analysis of medical survival data	- do -
- do -	Sampling procedure followed for a tuberculosis prevalence survey in North Arcot district	Mr.P.G.Gopi
Respiratory Disease update, Medical Education and Research Trust and Indian Academy of Medical Sciences, Bangalore, 4 November, 1990	Simple lung function tests	Dr.V.K.Vijayan
National Conference on Paediatric Pulmonology, Madras, 10 November, 1990	Interstitial pneumonia (Guest lecture)	- do -
CME Programme: National conference on Paediatric Pulmonology, Madras, 11 November, 1990	Pulmonary functions interpretations	- do -

Name of conference, venue and date	Title of paper	Name of staff member
X National Congress on Respiratory Diseases, Bombay, 15 December,1990	Lung epithelial lining fluid antifilarial antibodies in tropical eosinophilia	Dr.V.K.Vijayan
Update in Chest Medicine TB Association of Pondicherry, 16 December, 1990	Basic pulmonary function tests	- do -
IMA, Badagara, 23 December, 1990	Bronchial asthma (Guest lecture)	- do -

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

Name of the event, venue and date	Name of staff member	Title of paper
Meeting of post-graduate students of statistics, PSG College of Arts and Science, Coimbatore, 26-27 February, 1990	Mr.R.Selvaraj (Guest lecture)	Epidemiology and statistics
World Conference on Lung Health, American Lung Association, American Thoracic Society and International Union against Tuberculosis and Lung Diseases, Boston, 22-24 May, 1990	Dr.R.Prabhakar	--
Symposium on Neurotuberculosis, 40th Anniversary Celebrations, Institute of Neurology, Government General Hospital, Madras, 3 June, 1990	Dr.Manjula Datta	Epidemiology of tuberculosis
- do -	Dr.Alamelu Raja	Immunology of tuberculosis
- do -	Dr.R.Prabhakar	Chemotherapy of tuberculosis
- do -	Dr.T.Santha Devi	Adverse reactions to anti-tuberculous drugs
- do -	Dr.C.N.Paramasivan	Laboratory diagnosis of TBM
- do -	Dr.Padma Ramachandran	Management of TBM
- do -	Dr.Rajeswari Ramachandran	Short course chemotherapy for Pott's paraplegia and for tuberculoma
- do -	Dr.T.Santha Devi & Dr.P.Selvaraj (Panel discussion)	

Name of the event, venue and date	Name of staff member	Title of paper
Symposium on Respiratory Medicine, Apollo Hospital, Madras, 1 July, 1990	Dr.V.Kumaraswami	Pulmonary eosinophilia
WHO Workshop on Quality control in Clinical Micro-biology, JIPMER, Pondicherry, 19-21 July, 1990	Dr.C.N.Paramasivan	Quality control in isolation and identification of myco-bacteria from clinical specimens
Refresher course on DTP including SCC for PHI Medical Officers, Members of the Indian Medical Association and Private Practitioners organised by District TB Association,Vellore, CHAD Hospital, Vellore, 4 August,1990	Dr.R.Prabhakar	Chemotherapy of tuberculosis
-do-	Dr.C.N.Paramasivan	Sputum microscopy
-do-	Dr.N.M.Sudarsanam	District TB Programme-Rationale and concept
Ivermectin in lymphatic filariasis - investigator meeting, Paris, France, 24-25 August, 1990	Dr.V.Kumaraswami	Ivermectin trials in India
National Symposium on tuberculosis, Rajan Babu TB Hospital, New Delhi, 22-23 September, 1990	Dr.V.K.Vijayan	Chemotherapy of tuberculosis
V National Symposium on Flexible Fibreoptic Bronchoscopy, New Delhi, 28 October, 1990	- do -	Bronchoalveolar lavage in Tropical Eosinophilia

Name of the event, venue and date	Name of staff member	Title of paper
Silver Jubilee Symposium of the Bio-statistics department, Christian Medical College, Vellore, 31 October, 1990	Mr.P.R.Somasundaram	
National Symposium on current Trends in Bio-technology, Cochin University of Science and Technology, Cochin, 28-30 November, 1990	Dr.N.Selvakumar	Strategies for the development of BCG as a polyvalent vaccine
- do -	Dr.Vanaja Kumar	Mycobacterial plasmid profiles as epidemiological markers
Update in Chest Medicine, TB Association of Pondicherry, Pondicherry, 16 December, 1990	Dr.R.Prabhakar	Chemotherapy of tuberculosis
- do -	Dr.V.K.Vijayan	Pulmonary Function Tests
- do -	Dr.N.M.Sudarsanam	District Tuberculosis Programme
National Symposium on Behavioural Sciences & XIX Annual Meeting of the Ethological Society of India, American College, Madurai, 27-28 December, 1990	Dr.V.D.Ramanathan	Current concepts in behavioural immunology

LIST OF PUBLICATIONS

Papers published

1. Sudarsanam, N.M. Supervision aspects in District Tuberculosis Programme with special reference to Short Course Chemotherapy. NTI Newsletter, 1989, 25, 10.

2. Sudarsanam, N.M. and Prabhakar, R. Programme implementation. NTI Newsletter, 1989, 25, 67.

3. Prema Gurumurthy, Rajeswari Ramachandran, Rani Balasubramanian, Fathima Rahman, Lalitha Victor, Narayana, A.S.L. and Raghupati Sarma, G. Gastro-intestinal absorption of isoniazid and rifampicin in patients with intestinal tuberculosis. Indian Journal of Tuberculosis, 1990, 37, 5-10.

4. Weir, Jerry P. and Narayanan, P.R. Expression of the herpes simplex virus type 1 glycoprotein C gene requires sequences in the 5' noncoding region of the gene. Journal of Virology, 1990, 64, 445-449.

5. Renu B. Lal, Kumaraswami, V., Cathy Steel and Nutman, T.B. Phosphocholine -containing antigens of Brugia malayi non-specifically suppress lymphocyte function. American Journal of Tropical Medicine and Hygiene, 1990, 42, 56-64.

6. Raghupati Sarma, G., Kailasam, S., Datta, M., Loganathan, G.K., Rahman, F. and Narayana, A.S.L. Classification of children as slow or rapid acetylators based on concentrations of isoniazid in saliva following oral administration of body-weight and surface-area-related dosages of the drug. Indian Pediatrics, 1990, 27, 134-142.

7. Prema Gurumurthy, Fathima Rahman, Narayana, A.S.L. and Raghupati Sarma, G. Salivary levels of isoniazid and rifampicin in tuberculous patients. Tubercle, 1990, 71, 29-33.

8. Chandra Immanuel, Acharyulu, G.S., Kannapiran, M., Segaran, R. and Raghupati Sarma, G. Acute phase proteins in tuberculous patients. Indian Journal of Chest Diseases and Allied Sciences, 1990, 32, 15-23.

9. Vijayan, V.K., Jawahar, M.S., Reetha, A.M. and Prabhakar, R. Persisting alveolitis in miliary tuberculosis despite treatment with short-course chemotherapy. Indian Journal of Chest Diseases and Allied Sciences, 1990, 32, 49-53.

10. Ottesen, E.A., Vijayasekaran, V., Kumaraswami, V., Perumal Pillai, S.V., Sadanandam, A., Sheila Frederick, Prabhakar, R. and Tripathy, S.P. A controlled trial of ivermectin and diethylcarbamazine in lymphatic filariasis. The New England Journal of Medicine, 1990, 322, 1113-1117.

11. Thilakavathy Subramanian. Sample survey of awareness of symptoms and utilisation of health facilities by chest symptomatics. Indian Journal of Tuberculosis, 1990, 37, 69-71.

12. Padma Ramachandran. Long-term follow-up (4 1/2 - 8 years) of children treated for tuberculosis meningitis in South India (Summary). Indian Journal of Tuberculosis, 1990, 37, 102.

13. Vijayan, V.K. Single-breath diffusing capacity in healthy young adult South Indians (Summary). Indian Journal of Tuberculosis, 1990, 37, 105.

14. Reetha, A.M. Controlled clinical trial in the treatment of tuberculosis of spine without paraplegia (Summary). Indian Journal of Tuberculosis, 1990, 37, 110.

15. Kailasam, S. Determination of the isoniazid acetylator phenotype of adults and children based on salivary concentrations of the drug (Summary). Indian Journal of Tuberculosis, 1990, 37, 111.

16. Chandra Immanuel. Adrenocortical function in patients with pulmonary tuberculosis (Summary). Indian Journal of Tuberculosis, 1990, 37, 113.

17. Gupte, M.D., Anantharaman, D.S., Nagaraju, B., Kannan, S. and Vallishayee, R.S. Experiments with Mycobacterium leprae soluble antigens in a leprosy endemic population. Leprosy Review, 1990, 61, 132-134.

18. Thomas, A., Balakrishnan, A., Nagarajan, M., Prabhakar, R., Tripathy, S.P., Christian, M. and Somasundaram, P.R. Controlled clinical trial of two multidrug regimens with and without rifampin in highly bacilliferous BL/LL South Indian patients: a five-year report. International Journal of Leprosy, 1990, 58, 273-280.

19. Vijayan, V.K., Kuppu Rao, K.V., Venkatesan, P., Sankaran, K. and Prabhakar, R. Pulmonary membrane diffusing capacity and capillary blood volume in Tropical Eosinophilia. Chest, 1990, 97, 1386-1389.

20. Raji Swamy. Macrophage microbicidal mechanism. (Letter) International Journal of Leprosy, 1990, 58, 389.

21. Vijayan V.K., Kuppu Rao, K.V., Venkatesan, P., Sankaran, K. and Prabhakar, R. Pulmonary function in healthy young adult Indians in Madras. Thorax, 1990, 45, 611-615.

22. Jawahar, M.S., Sivasubramanian, S., Vijayan, V.K., Ramakrishnan, C.V., Paramasivan, C.N., Vanaja Selvakumar, Sare Paul, Tripathy, S.P. and Prabhakar, R. Short course chemotherapy for tuberculous lymphadenitis in children. British Medical Journal, 1990, 301, 359-362.

23. Eric A. Ottesen, Weller, P.A. and Kumaraswami, V. Ivermectin in lymphatic filariasis. The New England Journal of Medicine, 1990, 323, 918.

24. Rajiswamy. Macrophage functions in tuberculosis. Tropical Medicine and Parasitology, 1990, 41, 327.

25. Paranjape, R. Immunodiagnosis in tuberculosis. Tropical Medicine and Parasitology, 1990, 41, 366.

26. Deivanayagam, N., Vasudevan, S., Krishnamurthy, P.V., Jagadish Shankar, V., Ashok, T.P., Nedunchelian, K., Mala, N. and Shaffi Ahmed, S. Prevalence of placentally transmitted antibodies for measles in infants 3 to 11 months old in an urban slum community. Indian Pediatrics, 1990, 27, 919-923.

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29. Vijayan, V.K. How to conduct an interview? A guide to professional interviews for medical institutions. Lung India, 1990, 8, 213-214.

30. Raghupati Sarma, G., Chandra Immanuel, Geetha Ramachandran, Krishnamurthy, P.V., Kumaraswami, V. and Prabhakar, R. Adrenocortical function in patients with pulmonary tuberculosis. Tubercle, 1990, 71, 277-282.

31. Rani Balasubramanian, Sivasubramanian, S., Vijayan, V.K. Rajeswari Ramachandran, Jawahar, M.S., Paramasivan, C.N., Selvakumar, N. and Somasundaram, P.R. Five year results of a 3-month and two 5-month regimens for the treatment of sputum-positive pulmonary tuberculosis in South India. Tubercle, 1990, 71, 253-258.

32. Rajajee, S., Pushpa, V. and Narayanan, P.R. Cell-mediated immunity in childhood malaria. Indian Journal of Pediatrics, 1990, 57, 209-211.

33. Menon, P., Jayaraman, G. and Paramasivan, C.N. DNA relatedness among the strains of Mycobacterium tuberculosis. Proceedings of National Symposium on Microbial Gene Technology, Osmania University, Hyderabad. Ed: H.Polasa, South Asian Publishers, New Delhi, 1990, 134-145.

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35. Jawahar, M.S. Problems in the management of drug resistant tuberculosis. In: Proceedings of Continuing Medical Education Programme, IX National Congress on Respiratory Diseaes, Hyderabad, 1989, Ed: Murthi, K.J.R. Indian Chest Society, 1990, 41-45.

36. Venkatesan, P., Viswanathan, K. and Prabhakar, R. A model for the analysis of repeated specimens in clinical trials: In: Statistics in Health and Nutrition, Proceedings of the Sixth Annual Convention of the Indian Society for Medical Statistics and the National Seminar on Statistics in Health and Nutrition, Hyderabad, 1988. Ed: Visweswararao, K., Radhaiah G. and Narayana, V. National Institute of Nutrition, Hyderabad, 1990, 336-342.

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Papers accepted for publication

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

2. Mayurnath, S., Vallishayee, R.S., Radhamani, M.P. and Prabhakar, R. Prevalence of tuberculous infection, over a period of fifteen years, in a rural population in Chingleput district, South India. Indian Journal of Medical Research.

3. Sulochana Das, Sujatha Narayanan, Paramasivan, C.N., Lowrie, D.B. and Narayanan, P.R. Human tuberculosis sera show prominent antibody responses to particulate fractions of Mycobacterium tuberculosis. Journal of Clinical Immunology.

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

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

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

7. Vijayan, V.K., Kuppu Rao, K.V., Sankaran, K., Venkatesan, P. and Prabhakar, R. Tropical Eosinophilia: clinical and physiological response to diethyl carbamazine. Respiratory Medicine.

8. Brahmajothi, V., Pitchappan, R.M., Kakkanaiah, V., Sashidar, M., Rajaram, K., Ramu, V., Palanimurugan, V., Paramasivan, C.N. and Prabhakar, R. HLA association of pulmonary tuberculosis in South India. Tubercle.

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

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

11. Rajeswari Ramachandran, Balambal, R., Ramanathan, V.D. and Prabhakar, R. Cutaneous vasculitis during anti-tuberculosis treatment in tuberculoma brain - a report of 2 cases. Indian Journal of Tuberculosis.

JOURNAL CLUB

Journal club meetings were held each week, at which published scientific articles covering different areas of research were reviewed by staff members of various departments in turn. A synopsis of the papers to be presented and the reference details were circulated in advance, to facilitate better participation of the audience in the discussion that followed the presentation. In all, 37 such meetings were conducted.

Research discussions were held periodically for presenting the current activities of different departments. During the year, the staff members of Bacteriology and Biochemistry laboratories presented their research activities in 3 and 5 sessions, respectively.

LECTURES BY VISITING SCIENTISTS

Subject	Speaker
Diagnosis of leprosy using specific serology assay	Dr.Utpal Sengupta, Deputy Director, JALMA, Agra.
Malnutrition and tuberculosis	Dr.R.Bhaskaram, Asst. Director, National Institute of Nutrition, Hyderabad.
On the importance of estimation (rather than hypothesis testing) and confidence intervals in presenting study findings	Dr.S.Radhakrishna, Director, Institute for Research in Medical Statistics (Madras Chapter), Madras.
Determination of sample size	- do -
Analysis of the Leprosy Prevention Trial, based on provisional data	Dr.M.D.Gupte, Officer-in-charge, CJIL Field Unit, Avadi.
A simple technique in tackling tuberculosis in economically poor countries	Mrs.J.L.Monga, Former Nursing Officer, Tuberculosis Research Centre, Madras.

DISTINGUISHED VISITORS

1. Dr.Ian Simms, Head, Projects Unit, British Council Division, New Delhi, Dr.T.Shearer, First Secretary (Cultural Affairs), British Council Division, Madras and Mr.M.S.Shanthiah, Sr. Project Officer, British Council Division, Madras.

2. Dr.David Catty, Department of Immunology, University of Burmingham, U.K. and Dr. Marcel Hommel, Department of Tropical Medicine and Infectious Diseases, Liverpool School of Tropical Medicine, U.K.

3. Dr.Astrid Brundin, Dept. of Chest Diseases, University Hospital, Uppsala, Sweden.

4. Dr.S.D.Vachharajani, Consultant Chest Physician, Vadodara.

5. Brig. M.S.Dash, Officer-in-charge, RMRC, Bhubaneshwar.

6. Dr.Olavi Elo, WHO Representative to India, New Delhi.

7. Mr.S.Sathyam, Principal Secretary, Gas Relief, Govt. of Madhya Pradesh, Bhopal.

8. Dr.R.C.Jain, Medical Superintendent, Institute of TB & Allied Diseases, Mehrauli, New Delhi.

9. Prof.M.P.S.Menon, Professor of Chest Diseases, V.P.Chest Institute, New Delhi.

10. Prof.K.P.Govindan, Professor of Chest Diseases, Calicut Medical College, Calicut.

11. Dr.Vollmer, Kaiser Permanente, Portland, U.S.A.

12. Dr.S.Roy, Research Scientist, Dept. of Paediatrics, All India Institute of Medical Sciences, New Delhi.

STAFF MEMBERS ON ADVISORY COMMITTEES OF OTHER INSTITUTIONS

Staff member	Name of committee
Dr. R. Prabhakar	Standing Technical Committee, Tuberculosis Association of India, New Delhi.
- do -	Fellow, International Academy of Chest Physicians and Surgeons of the American College of Chest Physicians, Illinois, USA.
- do -	Planning Board, Dr. M.G.R. University of Medical Sciences, Madras.
- do -	Member, Senate, Dr. M.G.R. University of Medical Sciences, Madras
- do -	Planning and Research - Medical Research Committee of the University of Health Sciences, Vijayawada.
- do -	Board of Management, Vision Research Foundation, Madras.
- do -	Research Sub-Committee, Vision Research Foundation, Madras.
- do -	Scientific Advisory Committee of the Regional Medical Research Centre, ICMR, Port Blair, Andamans.
- do -	Editorial Advisory Committee, Lung India, Madras.
- do -	Project Review Committee for Tuberculosis, ICMR, New Delhi.
- do -	Steering Committee, Advanced Centre for Clinical Epidemiological Research and Training, Madras.
- do -	Research Advisory Panel, Schieffelin Leprosy Research and Training Centre, Karigiri.

Dr.G.Raghupati Sarma	Research Committee of the Drug Addiction Research Centre, Madras
- do -	Editorial Board, Indian Journal of Chest Diseases and Allied Sciences, New Delhi.
Dr.V.K.Vijayan	Project Advisory Committee on Clinical studies and Broncho-alveolar Lavage studies on MIC-exposed people at Bhopal, Bhopal Gas Disaster Research Centre, ICMR, Bhopal.
- do -	Central Crisis Group for Chemical Disasters, Ministry of Environment and Forest, Government of India, New Delhi.
- do -	Assistant Editor, Lung India, Madras.
- do -	Respiratory Medicine Specialists panel, Institute of Integral Health Studies, Madras.
- do -	Treasurer, International Academy of Chest Physicians and Surgeons, South India Chapter.
- do -	Expert member, Centre for Pulmonary Medicine, Madhya Pradesh Government, Bhopal.
Dr.V.Kumaraswami	Temporary Adviser, WHO Filariasis Research cum Training Centre, Wardha, Maharashtra.
Dr.Manjula Datta, Mr.P.R.Somasundaram Mr.P.V.Krishnamurthy	Steering Committee, Advanced Centre for Clinical Epidemiological Research and Training, Madras.
Dr.Manjula Datta	Task Force for the National ARI Control Programme, Govt. of India, New Delhi.
- do -	Task Force for the ARI Control Programme in Tamil Nadu, Govt. of Tamil Nadu, Madras.
Dr.Rajeswari Ramachandran	Convenor, Symposium of Neurotuberculosis, Institute of Neurology, Government General Hospital, Madras.

PRIZES AND AWARDS RECEIVED BY STAFF MEMBERS

Dr.R.Prabhakar was elected as a Fellow of the International Academy of Chest Physicians and Surgeons of the American College of Chest Physicians.

Dr.R.Prabhakar was awarded the Dr.N.L.Bordia gold medal by the Madhya Pradesh Anti TB Association.

ACKNOWLEDGEMENT

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