TUBERCULOSIS RESEARCH CENTRE

CHETPUT MADRAS-600 031

REPORT ON RESEARCH ACTIVITIES DURING 1989

INDIAN COUNCIL OF MEDICAL RESEARCH

NEW DELHI

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C O N T E N T S

PREFACE

The Centre with its broadened objectives is continuing to contribute towards operational research in the District Tuberculosis Programme, while pursuing research by controlled clinical studies to evolve highly effective regimens of short course chemotherapy for tuberculosis, and basic studies in bacteriology, biochemistry, pharmacology, immunology and pathology of tuberculosis. In addition, epidemiological studies are in progress to estimate the prevalence and infection rate of the disease in selected districts, including tribal areas.

The operational studies are focussed more towards effective community participation in the Programme, which has been identified as an important strategy for successful implementation and operation of health programmes. Apart from operational research studies, in-service training programmes for the district level staff were conducted to improve the managerial and technical skills of the personnel, and to motivate the staff for better performance.

Surveys were conducted to study the awareness of the disease in the community in different socio-economic strata and situations such as rural, urban and metropolitan areas, to improve the health care delivery system in the community.

A controlled clinical study with fully oral 6- and 8- month regimens with varying rhythms of administration of drugs is in progress at Madras and at the Centre's Unit at Madurai. The outcome of this study will be helpful in framing policies for treatment with short course regimens of pulmonary tuberculosis in patients with drug-sensitive and drug-resistant organisms, since the experience gained from the monitoring of the District Tuberculosis Programme has shown that a substantial proportion of patients reported as "newly diagnosed" have initially drug-resistant organisms.

Extra-pulmonary forms of tuberculosis are also important as some of them are associated with high levels of morbidity and mortality, and are commonly encountered in areas of high prevalence of the disease such as India and other developing countries. Hence, short course chemotherapy, which is highly encouraging in the treatment of pulmonary tuberculosis, is also logically applied for the treatment of extra-pulmonary forms of tuberculosis; studies of its efficacy in tuberculoma of the brain, tuberculous meningitis, abdominal tuberculosis and tuberculous lymphadenitis are underway.

Detailed bacteriological studies are being carried out for biological characterisation of mycobacteria. Pharmacokinetics of anti-tuberculosis drugs in patients with renal impairment is being studied, which will be useful to derive optimum dosages of these drugs. Studies of adrenocortical function in tuberculosis patients are being conducted using salivary estimation of cortisol. Salivary estimations of anti-tuberculosis drugs are also being undertaken as this procedure is non-invasive and most suited for pediatric practice.

Attempts at deriving histo-pathological grading of tuberculous lymph-adenopathy using histochemical techniques are being made in an effort to obtain more objective and precise grading of the lesions. Metabolism of iron in mycobacteria and its relation to virulence and pathogenesis are being studied in detail. Hybridoma and gene cloning techniques are being progressively used in attempts at developing simple and reliable immuno-diagnostics. The latest technologies in HLA typing are being used in studies of human genetics with respect to susceptibility to tuberculosis and morbidity due to the disease with reference to families and the community. Epidemiological studies to estimate the prevalence of the disease in the community and infection rates in children have been initiated in two districts, including one with tribal populations.

Detailed bacteriological investigations are being carried out in districts with different policies of treatment with short course chemotherapy under the District Tuberculosis Programme, in order to assess the efficacy of the regimens under field conditions.

Group Education Activities were conducted under the joint auspices of the ICMR and WHO to acquaint academicians in the medical colleges with recent trends in the treatment and management of tuberculosis. The activities were also extended to the statisticians and laboratory technicians of the districts, in an attempt at augmenting their knowledge and improving their attitude, for better performance.

Studies to standardise methodologies for screening children for tuberculosis by health workers are in progress, which will be helpful in the diagnosis of childhood tuberculosis.

Long-term studies to assess cardio-pulmonary functions are in progress in patients with pulmonary or spinal tuberculosis. Broncho-alveolar procedure is being adopted to study the immuno-pathology of pulmonary tuberculosis.

Dr. R. Parthasarathy, Deputy Director (retired) of the Division of Chemotherapy in the Centre was appointed as an Emeritus Medical Scientist at the Centre during the year by the Council.

The Centre continued to receive excellent guidance and useful suggestions for future research from the Scientific Advisory Committee, which met on 17.6.1989 under the chairmanship of Prof. J. S. Guleria. The protocols of clinical studies were meticulously screened and approved by the Ethical Committee.

The research activities of the Centre continue to be focussed towards the main objectives and aims of contributing to the National Tuberculosis Programme through research and developmental activities in operational, clinical and laboratory aspects, keeping in mind the major constraints in the programme.

Dr. R. Prabhakar
Director.

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AS ON 31.12.1989

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Emeritus Medical Scientist

R. Parthasarathy, B.Sc., M.B.B.S., T.D.D.

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Prof. S.K. Jain	Head, Department of Cardio-respiratory Physiology, Vallabai Patel Chest Institute, New Delhi
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Prof. R.N. Srivatsava	Principal, B.R.D.Medical College, Gorakhpur
Prof. K.V. Thiruvengadam	Professor of Medicine (Retd.), Madras Medical College, Madras

Dr. S.P. Tripathy	Additional Director-General, Indian Council of Medical Research, New Delhi
Dr. B.T. Uke	Adviser-in-Tuberculosis, Government of India, New Delhi
Dr. R. Prabhakar (member-secretary)	Director, Tuberculosis Research Centre, Madras

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Name of consultant	Field of specialisation	Designation and Institution
Prof. K. V. Thiruvengadam	Medicine	Former Professor of Medicine, Madras Medical College, Madras
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Dr. S. Radhakrishna	Statistics	Director, Institute for Research in Medical Statistics (Madras Chapter), Madras
Prof. K. V. Krishnaswamy	Tuberculosis	Former Director, Institute of Thoracic Medicine, Madras
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Dr. I. Kandaswamy	Radiology	Professor of Vascular Radiology, Government General Hospital, Madras
Dr.N.S.Venugopal	Ophthalmology	Former Superintendent, Government Ophthalmic Hospital, Madras
Dr.S.Thyagarajan	Ophthalmology	Retired Professor of Ophthalmology, Government Rajaji Hospital, Madurai
Dr.G.Ramu	Leprosy	Former Deputy Director, Central JALMA Institute for Leprosy, Agra

OPERATIONAL RESEARCH STUDIES

STUDIES COMPLETED

Address card system in North Arcot district

In the sample of 'lost' cases, for whom home visits were attempted in North Arcot and Raichur districts, addresses were found to be inadequate or incorrect in 16% and 9.5% of the patients, respectively. Recording of the correct addresses is vital for effective defaulter action. The "Address Card System" has proved to be an efficient and inexpensive means of obtaining accurate addresses of patients, in large towns in Tamil Nadu. In order to try out the effectiveness of this system in small towns and villages, a study was undertaken in North Arcot District.

Two large hospitals (GH) and six primary health centres (PHC) including one upgraded Panchayat Union Dispensary were chosen for this study and the local staff of the selected health facilities were briefed regarding the procedure. In all, 394 patients were given the address cards at their respective treatment centres and were requested to get their complete postal address written on the address cards by the postman or a neighbour or friend. The patients were instructed to post the completed address cards to the Centre's field unit at Vellore. The findings are given in the table below:

		GHs	PHCs	Total
No. of address cards given to patient		198	196	394
Cards received from patient with address	No.	185	189	374
	%	93	96	95
No. of Type A letters posted		184*	189	373
Reply cards received for Type A letters	No.	150	156	306
	%	82	83	82
Letters probably not received by patient	No.	29	29	58
	%	16	15	16
Letters returned undelivered	No.	5	4	9
	%	3	2	2

* For one patient the address card was to a school, hence letter was not posted.

In all, 374 (95%) of the address cards were returned to the field unit with completed address. Hence the rate of acceptability of the address cards is 95%; reply-paid letters were posted to 373 of the patients to the addresses obtained through the address cards (Type A letter). Of these 373 Type A letters posted, 306 reply cards were received, which shows that the accuracy of these address cards is at least 82%. Of the remaining 67 Type A letters posted, 9 were returned undelivered and the other 58 letters were probably not received by the patients.

The addresses of these patients as recorded on the treatment cards were compared with the addresses obtained through the address cards. Where there was a discrepancy, another letter was posted to the address recorded on the treatment card (Type B letter). The addresses entered on the address card and treatment card were found to be the same for 140 patients; for the remaining 233 patients, for whom there was discrepancy, Type B letters were posted to the address entered on the treatment cards. The outcome of the posting of Type B letters is compared with that of Type A letter and presented in the 2-way table below:

Type B letters (treatment card address)	Type A letters (address card address)						Total	
	Received		Probably not received		Definitely not received		No.	%
Received	140		18		3		161	*69*
Probably not received	45		16		1		62	*27*
Definitely not received	8		0		2		10	*4*
Total	193	*83*	34	*15*	6	*5*	233	*100*

It was found that among these 233 patients, 140 had received both Type A and Type B letters, 16 patients had probably not received either type of letter, and 2 patients had definitely not received either type of letter. In 8 cases, the Type B letter was not received while the Type A letter was received, compared with 3 cases where the Type A letter was not received but the Type B letter was received - a difference which is not statistically significant (McNemar's test, $P > 0.2$).

Home visits were attempted for 104 patients who had probably not received either or both types of letters and the data are presented in the table on page 13.

Home visit findings	A-R, B-PNR	A-PNR, B-PNR	A-N, B-PNR	A-PNR, B-Not posted	A-PNR, B-R	Total
Posted back(R)	31	7	1	13	17	69
Received	7	5	0	3	1	16
Definitely not received	0	2	0	3	0	5
Information not available	4	1	0	5	0	10
Visit not made	3	1	0	0	0	4
Total	45	16	1	24	18	104

R = Received; PNR = Probably not received; N = Definitely not received.

Of the 104, a visit could not be made for 4 cases and no information could be obtained from 10 others. Of the remaining 90, 85 patients said that they had received the letter, including 69 who said that they had posted back the reply card. The other 5 said that they had not received any letter. It was not possible to ascertain which type of letter(s) had been received. However, it may be concluded that in the great majority of the cases, the letters had been received.

It would therefore appear that the "Address Card" has not added significantly to the accuracy of the address recorded in the treatment card, at least in the 2 small towns and 6 villages in North Arcot District in Tamil Nadu where the study was conducted. However, since there is likely to be wide variation in the efficiency of the postal system from state to state, it would be worthwhile conducting similar studies in other states, especially those with a high proportion of "lost cases".

(started:1988; completed:1989).

Influence of initial and repeated motivation on case-holding in North Arcot

Treatment default and premature discontinuation of treatment continue to be major constraints for the successful implementation of the National Tuberculosis Programme. In order to assess the influence of motivation in overcoming this problem and improving patient compliance, a study was undertaken in North Arcot District. Three of the major centres, namely District Tuberculosis Centre (DTC), Vellore and General Hospitals at Gudiyattam and Vaniyambadi were selected for the conduct of the study. All new smear positive patients initiated on treatment between October 1987 and April 1989 were admitted to the study.

At the DTC, the patients were imparted health education by a Medical Social Worker at the start of treatment regarding the disease in general, its cause, spread, symptoms and diagnosis. The patients were then motivated regarding the need for a complete course of treatment, laying stress on the need for continuing chemotherapy even after disappearance of symptoms. The harmful effect of premature discontinuation of treatment was also mentioned. Flash cards were used as an aid in ;motivating patients. Some of the patients were subsequently transferred out to other Peripheral Health Institutions (PHIs) from the DTC after initial motivation. At Gudiyattam, in addition to the initial motivation, repeat motivation was done at 1, 2 and 5 months after the start of treatment. Patients at Vaniyambadi served as a control group (no motivation).

In all, 278 patients, 170 from the DTC and 108 from Gudiyattam have been preliminary analysis regarding the influence of motivation with regard to treatment completion compared with patients started on treatment prior to this study was done. The findings are presented in the table below:

	Period before motivation			Period after motivation		
Centre	No. of patients	≥ 80% Rx. received No.	%	No. of patients	≥ 80% Rx. received No.	%
DTC,Vellore	108	56	*52*	92	58	*63*
DTC then PHI	71	30	*42*	78	40	*51*
Gudiyattam	79	28	*35*	108	59	*55*
Vaniyambadi	37	13	*35*	50	15	*30*
Total	295	127	*43*	328	172	*52*

As can been seen, the percentage of patients completing treatment at the DTC as well as those transferred out to PHIs has increased after motivation. At Gudiyattam, where motivation was repeated periodically, this difference is statistically significant ($P = 0.01$). No such increase is observed in the centre where there was no motivation.

(started:1987; completed:1989).

Sample survey of awareness of symptoms and utilisation of health facilities by chest symptomatics

In the National TB Programme, case finding is 'passive' and persons with chest symptoms are expected to seek diagnosis and treatment from the available health facilities. It is therefore essential that chest symptomatics in the community are aware of the illness and motivated enough to get diagnosed and treated.

Sample surveys were undertaken in rural, urban and metropolitan areas in Tamil Nadu to (1) identify the chest symptomatics as defined in the National TB Programme, (2) to find out their knowledge about TB, and (3) to know about the action taken by them to get relief. The methodology of the survey and sample sizes have already been described (see annual reports of 1986-87, 1987 and 1988). Some of the main findings of the surveys are presented here.

The following table gives the population registered, number of chest symptomatics identified, and the coverages for interview and sputum examination. As can be seen, the coverages are high.

	Rural		Urban		Metropolitan	
	No.	%	No.	%	No.	%
Population registered	18257		17396		38084	
Population >15 years	11841	*64.9*	10795	*62.0*	26389	*69.3*
Chest symptomatics	1042	*8.8*	1009	*9.3*	1747	*6.6*
Interviewed	987	*94.7*	956	*94.7*	1592	*91.1*
Sputum examination	967	*98.0*	868	*92.6*	1436	*90.2*
Positive by						
(a) smear	39	*4.0*	51	*5.9*	50	*3.6*
(b) culture	25	*2.6*	36	*4.1*	36	*2.6*
(c) smear and/ or culture	47	*4.9*	66	*7.6*	60	*4.2*

Among the symptomatics from the rural area, 88% mentioned that they had heard of tuberculosis, while 97% of the symptomatics from urban area and 93% from the metropolitan area had heard of tuberculosis.

The source from which the symptomatics came to know about the illness is shown in the table on page 16. Some of the symptomatics had mentioned more than one source.

Source	Rural		Urban		Metropolitan	
	No.	%	No.	%	No.	%
Relatives, friends or neighbours	694	*79.9*	415	*44.9*	735	*49.5*
TB patients	396	*45.6*	210	*22.7*	139	*9.4*
Health institutions	92	*10.6*	282	*30.5*	405	*27.3*
Mass media (Books/ Cinema/Radio/TV)	28	*3.2*	73	*7.9*	323	*21.8*
Others	55	*6.3*	49	*5.3*	103	*6.9*
Heard of TB	869		925		1484	

Friends, relatives and neighbours were the main source of information in all the three areas viz., 80% from rural, 45% from urban and 50% from metropolitan areas. The role played by mass media seems to be minimal among symptomatics from rural and urban areas. Person to person communication was the main source of information in the areas studied.

Though more than 80% had heard of tuberculosis, 41% from rural, and 30% from urban and 48% from metropolitan areas had 'no idea' about the cause of the illness. The various causes attributed for the disease are given in the table below. Some of the respondents had mentioned more than one cause. Only 4%, 14% and 8% respectively knew that the disease was caused by a germ. It is noteworthy that superstition was given as a cause by a very small proportion even in rural areas.

Cause	Rural		Urban		Metropolitan	
	No.	%	No.	%	No.	%
Smoking/alcohol	182	*20.9*	151	*16.3*	137	*9.2*
Lack of proper food	157	*18.1*	108	*11.7*	100	*6.7*
Overwork/worries	67	*7.7*	38	*4.1*	36	*2.4*
Germs	37	*4.3*	131	*14.2*	119	*8.0*
Superstitions	15	*1.7*	11	*1.2*	7	*0.5*
Others	286	*32.9*	325	*35.1*	519	*35.0*
No idea	354	*40.7*	278	*30.1*	709	*47.8*
Heard of TB	869		925		1484	

As regards the knowledge about the spread of tuberculosis, 64% of the symptomatics from rural, 68% from urban and 55% from metropolitan areas considered the disease to be infectious, while 6%, 7% and 12% respectively expressed the view that the illness was hereditary. The following table gives the symptomatics' knowledge on methods of diagnosis.

Diagnosis	Rural		Urban		Metropolitan	
	No.	%	No.	%	No.	%
Sputum	385	*44.3*	481	*52.0*	617	*41.6*
X-Ray	331	*38.1*	476	*51.5*	530	*35.7*
Blood test	159	*18.3*	261	*28.2*	454	*30.6*
Physical exam	408	*47.0*	268	*29.0*	111	*7.5*
Others	198	*22.8*	53	*5.7*	187	*12.6*
No idea	230	*26.5*	127	*13.7*	404	*27.2*
Heard of TB	869		925		1484	

Some of the symptomatics had mentioned more than one method. It is interesting to note that 44% from rural, 52% from urban and 42% from metropolitan areas mentioned sputum examination as a diagnostic method. X-Ray was mentioned by 38%, 52% and 36% respectively; 27% of the symptomatics from rural area, 14% from urban area and 27% from metropolitan area did not have any idea about the methods of diagnosis.

Of the sympotomatics interviewed, 75% from rural, 81% from urban and 81% from the metropolitan areas had sought medical advice to get relief from their symptoms. The type of health facilities utilised by the symptomatics who had sought medical advice is given below.

Health facility	Rural %	Urban %	Metropolitan %
Government	54.3	46.6	52.7
Private	61.8	50.6	52.3
Indigenous	1.0	0.9	1.6
Homeopathic	7.1	5.4	0.9
Others	2.3	1.2	2.1
Sought medical advice	736	772	1286

The reasons for not seeking medical advice were elicited and are presented in following table.

Reasons	Rural		Urban		Metropolitan	
	No.	%	No.	%	No.	%
Symptoms not severe	83	33	87	47	118	39
Pressure of work	96	38	37	20	90	29
Dissatisfaction	7	3	10	5	13	4
Lack of money	134	53	16	9	22	7
Family obligation	30	12	11	6	19	6
Transport problem	51	20	3	2	2	1
Others	24	10	26	14	84	28
Medical advice not sought	251		184		306	

In all, 33% from rural, 47% from urban and 39% from metropolitan areas said that they did not seek medical advice as their symptoms were not severe. Pressure of work was mentioned as a reason by 38%, 20% and 29% respectively.

More detailed analysis with respect to sputum positivity in relation to the symptoms and their duration and other factors are being carried out.

(started:1986; completed:1988).

Bacteriological investigations for short course chemotherapy under DTP in two districts

Examination of sputum by smear and culture are the standard methods for the diagnosis of pulmonary tuberculosis and for the assessment of progress during treatment. Culture examination is more sensitive than smear and is also more reliable since the viability of tubercle bacilli is measured. Although voluminous data are available on culture results of sputum from patients admitted to various controlled clinical studies in India, such information on patients treated under programme conditions is not so far available. Even to-day, under the District Tuberculosis Programme (DTP) in India, case finding is undertaken only by means of smear examination of sputum from the symptomatics at Peripheral Health Institutions.

The Government of India has introduced Short Course Chemotherapeutic (SCC) regimens of 6-8 months' duration in a phased manner on a national level, and at present 174 districts in India come under this programme. Earlier, this Centre had been assigned the job of monitoring the implementation under programme conditions of 3 SCC regimens in 18 districts all over India

so as to provide information on the acceptability of these regimens in both urban and rural communities.

To assess the efficacy of these SCC regimens under programme conditions, it is important to obtain reliable information on the culture and sensitivity results on admission, at the end of treatment and during follow-up. This Centre had taken the responsibility of obtaining this information in two neighboring districts, North Arcot and Pondicherry, on admission, at the end of treatment and at 9 and 12 months, wherever possible. The sputum specimens were collected from the patients attending the District Tuberculosis Centre (DTC) and other major centres contributing a high proportion of cases in North Arcot, and mostly from those attending DTC at Pondicherry. The specimens were transported to the Centre's laboratory at Madras through messengers for examination by smear, culture, sensitivity tests and identification tests. An analysis of the results obtained so far are presented here.

Of the 3625 pretreatment sputum specimens processed from North Arcot, 77% were positive by culture for *M.tuberculosis*, 16% were negative and 6% of specimens were lost due to contamination. Non Tuberculous Mycobacteria (NTM) was isolated from 1% of the specimens (see table below).

Culture	% of specimens	
	North Arcot	Pondicherry
Positive	*77*	*92*
Negative	*16*	*3*
Contaminated	*6*	*3*
NTM	1	2
Total No. of specimens	3625	1992

Of the 1992 specimens from Pondicherry, 92% were culture positive, 3% were culture negative, 3% were lost due to contamination and NTM were isolated from 2% of the specimens.

All positive cultures were tested for their sensitivity to streptomycin (S), isoniazid (H) and rifampicin (R). The findings among the pretreatment specimens from North Arcot is given in the top table on page 20.

Of the 2779 cultures from North Arcot, 2082 (75%) were fully sensitive to streptomycin, isoniazid and rifampicin; 102 (4%) were resistant to streptomycin, 352 (13%) to isoniazid and 195 (7%) to both streptomycin and isoniazid. Besides, there were 48 strains (2%) resistant to rifampicin, either alone or in combination with other drugs.

Sensitivity result		No.	%
Sensitive to	S, H, & R	2082	75
Resistant to	S	102	4
	H	352	13
	SH	195	7
	R	2	2
	SR	1	
	HR	19	
	SHR	26	
Total		2779	100

The drug sensitivity test results of Pondicherry cultures (see table below) show that, out of 1761 cultures tested, 1526 (87%) were sensitive to all 3 drugs tested. Resistance to streptomycin was seen in 70 (4%), to INAH in 101 (6%) and to both drugs in 53 (3%). The incidence of rifampicin resistance was very low (0.6%), one culture being resistant to rifampicin alone, five to INAH and rifampicin and five to all three drugs.

Sensitivity result		No.	%
Sensitive to	S, H & R	1526	87
Resistant to	S	70	4
	H	101	6
	SH	53	3
	R	1	0.6
	HR	5	
	SHR	5	
Total		1761	100

Bacteriological status at 6 months, of patients who had received 80% or more of the prescribed chemotherapy, was compared to their initial bacteriological status. The table on page 21 shows the results obtained with North Arcot patients.

On admission	Total	Results at 6 months		
		Culture positive		Resistant to
		No.	%	S, H or SH
Sensitive	318	42	*13*	34
Resistant	90	38	*42*	34
Total	408	80	*20*	68

Culture results were available on admission for 408 patients. Of these, 80 patients had a positive culture at the end of treatment; 42 had initially drug sensitive organisms and 38 had initial resistance to 1 or more drugs. Of the former, 34 had developed resistance to streptomycin, isoniazid or both drugs. Of the latter 38 patients, 34 continued to excrete drug resistant organisms. Of these, 12 were resistant to rifampicin, including 2 who had shown resistance to rifampicin on admission also.

The table below shows the corresponding results from Pondicherry. Of the 725 patients who had received 80% or more of their treatment, and from whom culture results were available on admission, 673 (93%) were culture negative at 6 months. Of the 52 patients with a positive culture, 36 had had drug sensitive organisms initially. Of these, 13 had acquired resistance to one or more drugs but none had developed resistance to rifampicin. Of the 16 patients who had initial resistance to one or more drugs, 14 continued to excrete organisms resistant to 1 or more drugs; 11 of these had resistance to rifampicin, including 2 who had shown resistance to rifampicin on admission also.

On admission	Total	Culture positive		Resistant to
		No.	%	S, H or SH
Sensitive	632	36	*6*	13
Resistant	93	16	*17*	14
	725	52	7	27

To conclude, of the patients who had received at least 80% of their scheduled treatment, 80% from North Arcot and 93% from Pondicherry became sputum culture negative at the end of treatment, which is a very encouraging finding under programme condition. Even among the patients with drug resistance on admission, a high proportion had a favourable response. However, the overall efficacy of these regimens will depend upon the relapse rates in these patients, and specimens are being collected at different periods of follow up for bacteriological investigations.

(started:1983; completed:1989)

STUDIES IN PROGRESS

Short Course Chemotherapy under District Tuberculosis Programme

Short Course Chemotherapy has been 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. The work is being reported in annual reports (1983 onwards). Periodic analysis is undertaken based on the returns received from these districts and the data presented in each year's annual report.

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. $2RHZ_7/6TH$: Rifampicin 450 mg plus isoniazid 300 mg plus pyrazinamide 1.5 g daily for 2 months followed by thioacetazone 150 mg and isoniazid 300 mg daily for the next 6 months, the drugs being collected by patients once in 15 days for self administration.

3. $2RHZ_7/4RH_2$: 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 2 months, 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.

Policies: 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 Centre and the PHI. The Centre's staff make periodic monitoring visits to the districts; during 1989, all districts have been visited atleast by one team consisting

of a medical officer, bacteriologist, statistician and social worker. Wherever any lacunae is identified during the visits, the team discusses it with the staff concerned and follows it up with letters.

District	Population (00,000s)	No. of PHIs	Mean new smears examined per month	Average positive No.	%	Mean eligible for SCC No.	Mean put on SCC No.	%	I reg* %
Policy A									
N.Arcot	45.0	95	3366	176	5	173	100	58	–
Puri	29.2	81	1269	47	4	41	30	73	62
Baroda	25.6	79	1491	207	14	194	89	46	25
Thane	33.5	92	2376	125	5	122	85	70	1
Ujjain	11.2	56	813	73	9	61	29	45	52
Dehra Dun	7.6	27	814	69	8	61	43	70	3
Policy B									
Karnal	13.2	24	809	59	7	54	23	43	–
Kanpur	37.4	28	1660	125	8	98	24	24	–
Nagpur	25.9	43	2247	164	7	156	60	38	–
Rajkot	20.9	75	829	75	9	54	33	61	–
Raichur	17.8	72	1233	78	6	66	33	50	–
Sagar	13.2	42	634	57	9	34	25	74	–
Policy C									
Pondicherry	4.4	59	1604	75	5	70	30	43	99
Vidisha	7.8	26	568	46	8	39	30	77	26
Aurangabad	24.3	47	914	92	10	88	45	51	7
Varanasi	37.0	33	1400	73	5	55	36	65	74
Sabarkantha	15.0	69	1594	112	7	106	71	67	2
W.Godavari	28.7	58	1641	94	6	88	39	44	98

* Proportion prescribed the main regimen, out of total put on SCC, in policies A and C districts.

Implementation of the programme: In 1989, 12 of 18 districts had implemented DTP in 100% of PHIs, 4 in 90-99% and 2 in 70-79% of the

PHIs. Nine districts had implemented SCC in 100% of the DTP implemented PHIs, 2 in 90-99%, 4 in 70-89% and 3 in 50-69% of the PHIs.

Sputum examination and intake to SCC: The average sputum examination per month ranged from 813 to 3366 and the percentage of sputum positivity ranged from 4 to 14% in the 6 districts with policy A, 634 to 2247 and 6 to 9% in policy B, and 568 to 1641 and 5 to 10% in policy C districts (see table on page 23). The percentage of eligible patients put on SCC regimens ranged from 45 to 73% in policy A districts, 24 to 74% in policy B, and 43 to 77% in policy C districts. Considering all 18 districts, the admissions to SCC ranged from 24%- 39% in 2 districts, 40-59% in 8 districts and 60-77% in the remaining 8 districts.

Year by year comparison: By March 1985, SCC was implemented in all the 18 districts. The table below gives a comparison of smear positivity rates and intake to SCC year by year since 1985 in each of the 18 districts. There has been no significant variation in any of the districts as far as smear positivity is concerned. Considering intake to SCC there seems to be an improvement in most of the districts over the years.

District	Percentage sputum positive					Percentage put on SCC				
	1985	1986	1987	1988	1989	1985	1986	1987	1988	1989
North Arcot	6	5	5	4	4	66	61	68	55	51
Puri	3	4	3	3	5	88	64	79	67	68
Baroda	15	15	13	12	12	64	49	44	32	59
Thane	6	7	6	4	4	72	64	64	72	82
Ujjain	8	9	8	10	11	46	43	47	44	48
Dehra Dun	10	7	8	9	11	43	49	86	85	89
Karnal	8	8	6	6	7	36	38	31	50	73
Kanpur	10	9	6	5	5	26	12	18	36	30
Nagpur	9	8	7	6	8	16	32	33	64	53
Rajkot	11	10	10	8	8	75	59	51	53	68
Raichur	4	3	7	7	12	27	30	39	54	76
Sagar	13	9	7	7	11	44	37	79	87	91
Pondicherry	4	5	5	5	4	45	52	48	50	36
Vidisha	10	9	7	7	8	82	80	76	81	70
Aurangabad	16	10	7	12	8	39	46	48	33	76
Varanasi	5	5	4	6	6	58	64	62	61	81
Sabarkantha	10	7	7	5	7	60	60	59	70	83
W. Godavari	4	6	6	5	9	24	30	40	44	80

Cohort analysis of treatment completion rate: The proportion of patients completing 80% or more of chemotherapy according to the regimen is given in the following table.

Regimen		No.of pts.	Rx ≥ 80% recd. No.	%	No. due sputum exam.	Sputum examined No.	%	Sputum positive No.	%
$2RHZ_2/$ $4RH_2$	Total	8915	4381	*49*	3602	2393	*66*	130	*5.4*
	Median	754	425	*58*	370	306	*85*	10	*1.3*
	Range	75- 6195	45- 2796	*45- 71*	45- 2215	5- 1190	*8- 100*	0- 105	*0- 8.8*
2RHZ/ 6TH	Total	19520	10332	*53*	9728	7581	*78*	82	*1.1*
	Median	946	409	*50*	393	248	*76*	4	*1.4*
	Range	57- 2874	51- 1929	*23- 89*	49- 1774	44- 1586	*12- 100*	0- 14	*0- 4.9*
2RHZ/ $4RH_2$	Total	3983	2461	*62*	2354	2018	*86*	23	*1.1*
	Median	564	304	*69*	281	220	*83*	2	*1.5*
	Range	36- 1638	31- 1138	*41- 86*	31- 1094	30- 953	*73- 97*	1- 13	*0.5- 6.7*

The average treatment completion rate with regimen 1 in 6 districts with policy A is 58%, 50% in 16 districts with regimen 2 and 69% with regimen 3 in 6 policy C districts.

The proportion of patients for whom sputum was examined at the end of treatment also given in the table above. Eighty-five percent in regimen 1, 76% in regimen 2 and 83% in regimen 3 had their sputum examined and the positivity was 1.3% (0-8.8%) in regimen 1, 1.4% (0-4.9%) in regimen 2 and 1.5% (0.5-6.7%) in regimen 3.

The table on page 26 gives the treatment completion rate for 4 different cohort periods for each policy. The average completion rates for policy A districts were 46%, 57%, 51% and 54% for the 4 cohort periods, 48, 42, 52 and 48% for policy B districts and 52, 46, 52 and 51% for policy C districts. Thus the average completion rate has been fairly constant over the 4 cohort periods. Comparing the treatment completion rate between cohort 3 and 4, 5 districts have shown an increase of 5% or above and 5 districts have shown a decrease of 5% or above. In the remaining 8 districts, the completion rate has been fairly constant.

District	Cohort I*			Cohort II			Cohort III			Cohort IV		
	Total pts.	Rx≥80% No.	%	Total pts.	Rx≥80% No.	%	Total pts.	Rx≥80% No.	%	Total pts.	Rx≥80% No.	%
Policy A												
Total pts.	4239	2069	*53*	4100	2220	*58*	4482	2646	*57*	3298	1796	*58*
Median	666	457	*46*	334	228	*57*	787	479	*51*	500	338	*54*
Range	282-	131-	*45-*	89-	55-	*47-*	270-	118-	*44-*	256-	139-	*44-*
	3291	1481	*69*	2347	1231	*69*	1215	828	*75*	915	401	*94*
Policy B												
Total pts.	1775	995	*48*	1244	643	*43*	2391	1415	*53*	2160	1100	*49*
Median	266	128	*48*	160	78	*42*	306	184	*52*	324	122	*48*
Range	73-	27-	*28-*	82-	4-	*5-*	196-	48-	*24-*	171-	79-	*25-*
	635	456	*72*	434	300	*70*	902	655	*75*	792	518	*76*
Policy C												
Total pts.	1580	811	*54*	2231	1028	*48*	2395	1108	*50*	2545	1357	*54*
Median	376	172	*52*	364	176	*46*	342	182	*52*	400	236	*51*
Range	145-	86-	*32-*	245-	114-	*34-*	200-	92-	*26-*	304-	78-	*22-*
	683	381	*81*	539	218	*66*	837	281	*68*	677	357	*86*

* Cohort I - From inception of SCC programme up to October 1985; Cohort II - November 1985 to June 1986; Cohort III - July 1986 to June 1987; Cohort IV - July 1987 to June 1988.

Various operational studies are being undertaking at the district level to find out methods by which case finding and case holding can be improved; these studies are reported elsewhere.

(started:1983).

Training programmes and monitoring

The Centre's activities continued, giving priority for Short Course Chemotherapy under the District Tuberculosis Programme (DTP). Some more staff members were given orientation in DTP activities this year by visits to the adjoining North Arcot District.

Training programmes for the DTC and PHI staff in various districts were continued by the Centre. Eight districts were covered during the year. The

training session were held in 21 venues, imparting training to 349 Medical Officers and 575 paramedical staff.

Monitoring activities were continued throughout the year. A monitoring committee was formed and 15 meetings were held. At these meetings, members who had toured the district presented their reports for discussion and necessary follow-up action.

As part of the monitoring activities, the DTC and a sample of PHIs were visited in each district (table below).

	DTC	XC	MC	RC
Total available	18	128	488	459
Visited	18	85	140	65

XC = X-ray centre; MC = Microscopy centre; RC = Referral centre

By suitable programming, all the 18 districts were visited during the year. Computerised data of the district concerned were given to the team before departure, to enable them to plan the PHI visit programme. Salient features requiring attention were also indicated.

The monthly and quarterly reports on tuberculosis from the 18 districts were scrutinised on receipt by 'desk monitoring' teams, each consisting of a medical officer, a bacteriologist, a social worker and a statistician. Based on their reports, follow-up action in the concerned district was effected. Supplies by way of standard forms or replenishing drugs were made, based on scrutiny of these reports. Stream-lining the MRT by allotment of code numbers to the various PHIs in each district has been effected.

Revised editions of 3 booklets entitled

(a) District TB Programme - Salient Features,

(b) Treatment Aspects in District TB Programme, and

(c) Sputum microscopy: Instructions to Laboratory Technicians

were printed and distributed to the PHI's in the 18 districts. Handbills were printed in Tamil and Telugu with important points regarding tuberculosis, and distributed primarily to the public in North Arcot and West Godavari Districts.

(started: 1988).

A study of 'lost' cases under Short Course Chemotherapy in Raichur District

It has been observed that nearly 45% of the patients started on treatment under the District Tuberculosis Programme discontinue treatment. In order to find out why such a large proportion of patients do not complete treatment, a study of the 'lost' cases had been undertaken in North Arcot district (see 1985-86 annual report). A similar investigation was undertaken in Raichur

District in Karnataka, in order to find out whether the reasons given by patients for getting 'lost' are the same in other districts also.

In all, 228 such patients were identified during two cohort periods of start of treatment, namely from January, 1988 to August, 1988 and September, 1988 to February, 1989. Attempts were made by the Centre's staff to visit all these patients. However, 12% could not be traced as the addresses were incorrect and 5% had migrated. Of the remaining 189 patients, 11% had died and 2% had either completed the treatment or had their regimen changed. Reasons for getting 'lost' were elicited from the other 164 patients or their relatives and the information is given below:-

Reason*	% of patients 'lost'
Adverse reactions	12%
Symptom free	24%
Out of station	11%
Distance/Transport problems	12%
Too sick/old	2%
Rx. elsewhere	34%
Job loss/loss of wages	10%
Dissatisfaction with PHI	11%
Personal reasons	22%
Denies identity	2%
Lack of faith in Rx.	12%
Total patients interviewed	164

* The reasons are not mutually exclusive.

Bacteriology: In all, 108 patients were contacted and sputum specimens were collected from 65 patients. While 7 refused to give sputum, 36 patients were unable to produce sputum. All sputum samples were subjected to bacteriological investigations. Thirty-four (52%) were found to be still smear positive and thirty-three (51%) were culture positive (see table below).

Smear result	Culture result				
	Positive	Negative	Cont.	NTM	Total
Positive	28	2	4	0	34
Negative	5	20	4	2	31
Total	33	22	8	2	65

Two patients yielded a positive smear but were negative on culture. In one of these patients (smear 1+) the transit time between sputum collection and receipt at the Centre was 10 days. The other patient, with a 2+ smear,

was culture negative (transit time: 1 day) up to 12 weeks of incubation. A repeat specimen from the same patient yielded identical results.

All positive cultures were tested for sensitivity to streptomycin (S), isoniazid (H) and rifampicin (R). Of these, 14 (42%) were found to be excreting bacilli sensitive to all drugs. The remaining 19 were resistant to one or more drugs, as set out in the table below.

	Sensitive	Resistant					
	to SHR	S	H	R	SH	HR	SHR
No. of specimens	14	8	7	1	1	–	2*

*Mixed growth in both cases.

(started:1989; expected year of completion: 1990.)

Augmentation of case-holding component of the District Tuberculosis Programme by utilisation of multi-purpose workers

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

Aims and objectives: To assess the value of utilisation of the MPW in improving the case-holding component, for all patients initiated on treatment under the DTP in W.Godavari District during June to December 1989.

Training component: Two teams consisting of a Medical Officer and a Statistical Assistant visited 7 Peripheral Health Institutions (PHIs) of West Godavari District in Andhra Pradesh initially and briefed the Medical Officers, Laboratory Technician and Multi-Purpose Workers about their role in augmenting case-holding activity of DTP. MPWs were provided with specially designed forms, and were instructed to contact defaulters during their routine visits and find out the reason for default, motivate the patient to attend, and make appropriate entries in the form.

Prior to the start of the study, a KAP (Knowledge, Attitude and Practice) Questionnaire, regarding all aspects of tuberculosis was given to all the MPWs and supervisors of West Godavari District and the findings were encouraging.

Periodic monitoring visits are being made by the Centre's staff and necessary guidance given wherever a deficiency in defaulter retrieval action and form filling are found.

The study is in progress.

(started:1989; expected year of completion: 1990).

Knowledge, Attitude and Practice of medical officers and drug distributors involved in the programme at district level

The 'key' personnel at the peripheral level for running the tuberculosis programme are the medical officers, drug distributors and lab. technicians. Therefore, it is essential that they have full knowledge regarding the various aspects of the programme. The Centre has been involved in conducting training programmes for these personnel at the district level.

In order to find out the base level knowledge and the practice of these personnel, a questionnaire is being given to them before the training programme. This work has been undertaken in three districts during the year (see table below). The data from the questionnaires filled in by medical officers and drug distributors are being analysed; the data for lab. technicians is presented in page 35.

District	Medical officers	Drug distributors
Karnal	18	23
Ujjain	15	20
N.Arcot	47	45

(started:1988).

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 village Dai often lives in the same village and is expected to have a good rapport with the villagers. Hence, a pilot study was undertaken in Sriperumbudur talukh 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 63 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.

As a preliminary step, 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. Dais were given practical training in identifying chest symptomatics and in collecting sputum specimens from them, for transportation to the Centre. A total of 119 sputum specimens were collected (78 by the Centre's staff along with Dais and 41 independently by the Dais) and examined by smear and culture for *M.tuberculosis;* nine (7.6%) sputum specimens (4 from 78 and 5 from 41 respectively) were found to be positive for AFB*.

In order to assess the opinion of the community about Dais and the services rendered by them, a medical social worker is interviewing (using a questionnaire) the heads of the house-holds or responsible persons in each family in 24 randomly selected villages / colonies representing all the 12 clusters. The social worker is also making a preliminary assessment, using a separate questionnaire, about the awareness of tuberculosis in general.

After this is filled a separate one page hand-out in local language (Tamil) containing basic and important information on tuberculosis is given to them, to read or get read. The same family member is interviewed a second time (post assessment) within a period of two weeks using the same questionnaire to assess the acceptability and utility of this hand-out in spreading the message among the public. So far, 175 persons have been interviewed. The study is in progress.

(started: 1989; expected year of completion: 1990).

Involvement of voluntary agencies in improving the District Tuberculosis Programme

As one of the measures to improve case-finding and case-holding in the District Tuberculosis Programme (DTP), attempts are being made to study the effect of involvement of voluntary welfare agencies in the programme. For this purpose, two agencies working in Madras City have been identified. These agencies have community workers, who visit the houses in the areas and participate in community development programmes.

As a preliminary step, training programmes, by way of lecture- demonstrations, were organised for the community workers of both these agencies regarding (1) symptoms of tuberculosis, and the role of the workers in identifying symptomatics and referring them for investigations, and (2) basic information on treatment and the importance of case holding.

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* These patients were treated with ethambutol 600mg plus isoniazid 300 mg daily, supplied once a month to CHAs, who supplied these drugs weekly to the patients through the Dais.

A total of 60 workers have been trained so far.

(started:1989; expected year of completion:1994).

Condition of the microscopes in the districts

In DTP, case finding is based on sputum microscopy. Hence, it is important to ensure that the microscopes are in good condition. For this purpose, a proforma was prepared to know the condition of the microscopes in the 18 districts. This proforma was sent to all PHIs in 10 districts and the Medical Officers were requested to fill up the proforma and post it back in the self addressed envelope sent along with it. The table on page 33, gives the number of microscopes that needed various types of correction in the different districts. Puri had a large number of microscopes to be corrected.

In three districts, based on the information collected, possible repairs were made during team visits. The details of the repairs undertaken are given in the table below.

District	Total number repaired	Mechanical	Optical	Mechanical & optical
Puri	41	15	17	9
Raichur	7	0	3	4
W. Godavari	12	3	1	8

(started: 1989; expected year of completion:1991)

Comparison of Z-N smear reading in the districts (PHIs) and at the Centre

As case finding and assessing the progress of patients during treatment and follow-up in DTP is based on sputum smear examination by Z-N method, it was planned to check at the Centre samples of positive and negative sputum slides collected from the districts. The table on page 34 shows the number of slides investigated from the respective districts during the year 1989.

District	Information on No. of microscopes	Type of defect requiring correction (No.)											
		Total No. with defects	Coarse adjustment	Fine adjustment	Stage	Sub-stage	Eye piece	Low power	High power	Oil immersion	Condensor	Mirror	Nose piece
N.Arcot	8	8	4	1	2	3	0	0	0	2	1	0	0
Puri	64	46	14	20	15	22	20	0	0	34	25	0	17
Baroda	22	18	2	1	4	2	3	0	3	6	1	2	0
Thane	10	6	0	0	2	0	2	2	2	3	0	0	0
Karnal	25	6	0	0	2	3	1	1	2	3	0	1	0
Kanpur	7	5	4	2	5	4	2	3	3	4	2	3	0
Rajkot	11	2	0	0	0	0	0	0	0	2	0	0	0
Raichur	30	13	4	5	2	6	3	0	0	6	7	0	2
Vidisha	4	4	4	1	0	2	1	1	1	3	0	0	0
W.Godavari	25	16	3	3	2	4	5	0	0	7	4	2	0
Total	206	124	35	33	34	46	37	7	11	70	40	8	19

District		++	−−	+−	−+	Total
N. Arcot		8	3	0	1	12
Puri		26	35	0	6	67
Baroda -	DTC	10	14	0	0	24
	PHI	23	20	0	4	47
Thane -	DTC	4	5	0	0	9
	PHI	13	3	0	1	17
Karnal		30	55	3	5	93
Kanpur -	DTC	14	24	0	2	40
	PHI	6	0	0	0	6
Nagpur		5	22	1	3	31
Raichur		316	80	1	6	413
Vidisha		27	25	0	4	56
Aurangabad		2	22	0	0	24
Varanasi		45	28	0	1	74
Sabarkantha		0	24	0	4	28
W. Godavari -	DTC	16	42	1	5	64
	PHI	0	12	0	3	15

++ = District and the Centre reported 'positive'

−− = District and the Centre reported 'negative'

+− = District reported 'positive' and the Centre reported 'negative'

−+ = District reported 'negative' and the Centre reported 'positive'

It can be seen that a few reported as negative at the districts were reported as positive at the Centre's laboratory. But the differences are not statistically significant.

(started:1989;completed:1989)

Knowledge, attitude and practice of laboratory technicians

Questionnaires were prepared to evaluate the knowledge, attitude and practice (KAP) of Laboratory Technicians (LTs) working in the various districts monitored by the Centre. The questionnaires had 50 questions about tuberculosis in general, laboratory procedures, smear preparation, staining, microscopy, documentation and DTP procedures. The questions had multiple answers - correct, incorrect and unrelated answers - of which the LTs were asked to tick one *before* the orientation lectures.

Information available on KAP of 109 LTs in 4 districts (Dehra Dun, Karnal, West Godavari and Raichur) and 45 LTs (15 DTC and 30 PHI) who attended the ICMR/WHO workshop on SCC held in February 1989 at Madras, is presented in the table on page 35.

% questions correctly answered	General	Lab. procedures	Smear preparation	Staining procedures	Microscopy	Documentation	DTP procedures
Less than 50%	7	9	13	24	7	3	18
50-75%	4	32	8	13	27	18	15
76-99%	29	0	25	53	53	3	21
	92	73	82	66	70	42	67
100%	112	113	102	49	56	62	82
No information or not answered	2	0	6	15	11	68	18
Total technicians	154	154	154	154	154	154	154

It might be seen that the over-all knowledge of the technicians was satisfactory since a majority (66-92%) of them had answered more than 75% of the questions correctly. As regards documentation, information was not available for 68 technicians. If this figure is excluded, 65 of the remaining 86 LTs (76%) had given correct answers to more than 75% of the questions.

The impact of the orientation lectures is proposed to be assessed by evaluating the LTs during re-training programmes.

(started:1989; completed:1989)

Comparison of bacteriological findings of peripheral laboratories (N.Arcot and Pondicherry) and the Centre's laboratory

North Arcot: Analysis of the culture results of 1922 specimens processed over a 4-year period (1985-89) at North Arcot Field Laboratory and at the Centre's laboratory are presented below:

		Centre				
		Neg	Pos	Cont	NTM	Total
N.Arcot	Neg	136	400	18	8	562
	Pos	71	1104	30	3	1208
	Cont	25	107	3	2	137
	NTM	5	10	0	0	15
	Total	237	1621	51	13	1922

The proportion of negative cultures was 29% at North Arcot and 12% at the Centre, the difference attaining statistical significance. Sixty-three per cent of specimens were found to be culture positive at North Arcot compared with 84% at the centre. Analysis of the year – wise results (not tabulated) showed an increasing trend in culture positives at North Arcot – 43% in 1985-86, 59% in 1986-87, 72% in 1987-88 and 78% in 1988-89. The corresponding proportions for culture positives at the Centre were 78%, 82%, 82% and 83% respectively. The contamination rate was higher at North Arcot (7%) than at the Centre (3%).

Detailed analysis of culture results during 1988-89 are presented below:

		Culture results at the Centre				
		Neg.	<100 col..	≥100 cols	UMB/ Cont./NA	Total
Culture results at N.Arcot	Negative	36	13	17	4	70
	<100 col	9	51	32	4	96
	≥100 Col	8	57	161	4	230
	UMB/Cont	4	10	8	0	22
	Total	57	131	218	12	418

These results are based on two different specimens from the same patient, which also could contribute to these differences.

Pondicherry: Analysis of the culture results of 3300 specimens processed during the period 1985-89 at Pondicherry and at the Centre are presented below:

		Culture results at the Centre				
		Neg.	Pos.	Cont.	NTM	Total
Pondy results	Neg.	1549	437	59	30	2075
	Pos.	37	1052	22	5	1116
	Cont.	43	43	5	4	95
	NTM	2	9	1	2	14
	Total	1631	1541	87	41	3300

Thirty-four per cent of specimens were culture positive at Pondicherry as against 47% at the centre. The lower percentage of culture positives could have been due to the inclusion of sputum specimens collected from patients during treatment. The differences in the rates of positivity at the two laboratories could have been due to different methods used for processing - the Petroff's concentration method at the Centre and the cetrimide swab method at Pondicherry, which is known to be inferior to Petroff's method in smear-negative specimens. The contamination rates were similar at the two laboratories.

(started : 1985; completed:1989)

CLINICAL STUDIES

STUDIES COMPLETED

High coverage for long-term follow-up of patients with spinal tuberculosis

The Centre conducted a multicentric controlled clinical trial on spinal tuberculosis, in collaboration with the orthopaedic surgeons of six Government Hospitals of Madras (see 1985-86, 1987 and 1988 annual reports). The regimens investigated (in brief) were:

1. RAD/6HR: Radical excision of the diseased vertebrae and chemotherapy with isoniazid plus rifampicin daily for 6 months.
2. AMB/6HR: As in (1) but without surgery.
3. AMB/9HR: As in (2) but with chemotherapy for 9 months.

In all, 303 patients were admitted to this study, and were followed up for more than 8 years at 3-, 6- or 12- monthly intervals. All patients were reminded about their check-up a week prior to the due date. A very high coverage of over 94% has been achieved even among patients residing outside Madras city (out-station patients). The corresponding figures for city and sub-urban patients are over 99% and 98% respectively. The details are given in the following table.

	No.of occasions							
	City		Sub-urban		Out-station		Total	
	No.	%	No.	%	No.	%	No.	%
Total attend-ances due	2236	*100*	1003	*100*	1195	*100*	4434	*100*
Punctual attendance	1763	*78.9*	717	*71.5*	806	*67.4*	3286	*74.0*
Attendance within grace period*	457	*20.4*	263	*26.2*	321	*26.9*	1041	*23.5*
Late attendance	1	*0.1*	3	*0.3*	8	*0.7*	12	*0.3*
Questionnaire received	2	*0.1*	3	*0.3*	7	*0.6*	12	*0.3*
Missed attendance	13	*0.6*	17	*1.7*	53	*4.4*	83	*1.9*

*Within half the interval between the due date for that attendance and the next.

As evident from the data, patients attended without any action on 74% of occasions. For the remaining 26% of occasions, the various types of defaulter action taken are set out in the following table:

Defaulter action	City		Sub-urban		Out-station		Total	
	No.	Average per default	No.	Average per default	No.	Average per default	No.	Average per default
Visits made	669	1.4	227	0.8	187	0.5	1083	0.9
Letters written	54	0.1	257	0.9	618	1.6	929	0.8
Phone calls made	11	0.02	25	0.1	28	0.1	64	0.1
Total defaults	473		286		389		1148	

For patients who failed to attend within 3 months from the due date, and who have completed 60th month follow-up, questionnaires were sent to get information regarding their status of health with special reference to the spine. Twenty-five questionnaires were sent (city 3, sub-urban 6, out-station 16), and 12 were received back with information.

An average of 1.8 actions were taken per default to obtain the high coverage over an 8-year period of follow-up.

(started: 1975; completed: 1989).

STUDIES IN PROGRESS

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

The Centre has been investigating fully supervised intermittent chemotherapeutic regimens of 6 months' duration in the treatment of sputum positive pulmonary tuberculosis (see 1986-87 annual report).

The regimens are:

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 0.75g.
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, selected at random, received 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 analyses of efficacy.

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 other 1021 patients had a favourable response at the end of chemotherapy; 1004 were assessed for relapse. The bacteriological relapses requiring treatment during follow-up of 48 months are presented in the table on page 40.

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

Twenty-eight (6%) of 498 thrice-weekly patients had a relapse; the rates were 5-6% for the various continuation regimens. Thirty-six (7%) of 506 twice-weekly patients relapsed during the same period, the rates being 6-10% for the various continuation regimens. The relapse rate of 6% in the thrice-weekly series was not significantly different from that of 7% in the twice-weekly series.

Of the 64 relapses, 34 (53%) occurred in the first year of follow-up, 10 (16%) in the 2nd year, 12 (19%) in the 3rd year and 8 (12%) in the 4th year. In the great majority of cases, the bacilli were drug-sensitive at the time of relapse.

Initial Rx (2 months)	Conti-nuation Rx (4 months)	Total patients assessed	Relapse requiring treatment									
					Month of relapse after stopping Rx							
			Total No.	%	1-6	7-12	13-18	19-24	25-30	31-36	37-42	43-48
	SRHow	102	6	6	2	0	1	0	2	1	0	0
RSHZ	RHow	103	5	5	3	0	1	0	0	0	0	1
thrice	SRHtw	99	5	5	1	0	1	0	1	1	0	1
weekly	RHtw	99	6	6	2	0	1	1	1	0	1	0
	SHtw	95	6	6	2	0	1	0	1	0	1	1
	Any	498	28	6	10	0	5	1	5	2	2	3
	SRHow	109	7	6	4	0	0	2	0	1	0	0
RSHZ	RHow	103	7	7	7	0	0	0	0	0	0	0
twice	SRHtw	98	6	6	0	1	1	0	2	0	0	2
weekly	RHtw	94	6	6	5	0	0	0	0	1	0	0
	SHtw	102	10	10	7	0	1	0	0	1	1	0
	Any	506	36	7	23	1	2	2	2	3	1	2

There were 223 patients with bacilli initially resistant to 1 or more drugs; 62 had bacilli resistant to streptomycin alone, 64 to isoniazid alone and 97 to both drugs. Of the 62 patients with resistance to streptomycin alone, 1 had an unfavourable response and 8 relapsed. Of the 64 patients with resistance to isoniazid alone, 11 (17%) had an unfavourable response and 3 relapsed. Considering patients with resistance to streptomycin and isoniazid, 22 (30%) of 73 who had received rifampicin in the continuation phase and

18 (75%) of 24 who did not receive rifampicin had an unfavourable response. ($P < 0.001$); a bacteriological relapse occurred in 6 of 51 and 2 of 6 patients, respectively.

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

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 these regimens were 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. R. Ramamurthy succeeded by Dr. Thara Natarajan). 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. In all, 1044 patients (527 Madras, 517 Madurai) have been admitted to the study so far, and the intake is continuing. The patients will be followed up for a period of 5 years.

(started:1986; expected year of completion of intake:1990).

Collaborative clinical trial of tuberculous lymphadenitis in children followed up up to 42 months after stopping treatment

The follow-up of patients admitted to the short course chemotherapy study of tuberculous lymphadenitis in children is being continued (see 1988 annual report). This clinical 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 Govt. Stanley Hospital, Madras (annual report 1985-86). The subjects were children aged 1-12 years with lymph node tuberculosis confirmed by histopathology or culture. Patients admitted to the study were treated with a fully supervised intermittent regimen for 6 months, consisting of streptomycin, isoniazid, rifampicin and pyrazinamide twice a week for 2 months, followed by streptomycin and isoniazid twice a week for 4 months. At the end of chemotherapy, a repeat lymph node biopsy was done in those with significant residual lymphadenopathy (exceeding 10 mm). Patients were assessed at regular intervals at the centre and at the referral hospitals.

In all, 197 patients were admitted to the study. Of these 27 patients were excluded; the other 170 have completed treatment and 166 have been followed up up to 42 months after treatment.

Of the 170 patients who completed treatment, 50 had repeat lymph node biopsies at the end of treatment. In 2 of these patients, the lymphnode culture was positive for *M.tuberculosis*. These were considered as failures of chemotherapy and received an additional course of treatment. Six patients had lymphnode histology suggestive of tuberculosis, of whom one developed a cold abscess 2 months after treatment and was retreated. The other 5 have completed the 42 months of post treatment follow-up uneventfully.

In the follow-up phase, 4 more patients required additional treatment for tuberculosis - one each for abdominal tuberculosis in the 4th month of follow-up, tuberculous meningitis in the 36th month, pulmonary tuberculosis and lymphadenitis in the 38th month and spinal tuberculosis in the 42nd month.

In all, therefore, 7 (4%) of 166 patients required additional treatment, either for relapse of lymphnode tuberculosis, or development of tuberculosis at other sites; the remaining 96% of the patients had a favourable outcome up to 42 months of post-treatment follow-up. The patients are being followed up till 60 months.

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

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 (see 1988 annual report). During the year, the study was extended to adults, in collaboration with the Surgery Department (Dr. D. Anantharaj and Dr. M. N. Kamaluddin).

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 in 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, 44 child and 38 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; 86-87; 1988) 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 Institute of Thoracic Medicine, 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 1989, 865 patients have been registered and of these, 182 patients have been admitted to the study. Of these, information up to 1 year is available for 169 patients (84 2RHZ/4RH, 85 SEH/EH).

Characteristics on admission: The mean age was 30 years (range 13-72 years); there were 81 males and 88 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 169 patients, 122 (72%) had intestinal lesions, 69 (41%) had peritoneal tuberculosis, 20 (12%) had hepatic tuberculosis and 12 (7%) patients had mesenteric tuberculosis; 58 patients had combined lesions.

In all, 129 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 122 patients with intestinal tuberculosis, 104 (85%) had radiographic evidence by barium contrast studies, of whom 52% had either direct histopathological or bacteriological confirmation, or tuberculosis elsewhere in the body (indirect evidence). 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 69 cases of peritoneal tuberculosis, 58 had histopathological confirmation. Ascitic fluid was positive for *M.tuberculosis,* by smear or culture, in 6. One patient had exudative ascites with sputum-positive pulmonary tuberculosis and 3 patients had peritoneal tubercles with exudative ascites.

Considering the 20 patients with hepatic tuberculosis, all except 1 had histopathological confirmation, while all the 12 patients with mesenteric tuberculosis had the diagnosis confirmed by histopathology.

The table below presents the status at the end of treatment.

Status at the end of Rx	2RHZ /4RH		SEH/EH	
	No.	%	No.	%
Symptom free	69	*96*	59	*87*
Clinically improved but still symptomatic	2	*3*	4	*6*
Change of Rx for clinical deterioration	1	*1*	3	*4*
TB death	0	*0*	2	*3*
Total patients with assessable response	72	*100*	68	*100*
Response not assessable				
Non-tuberculous death	4		6	
Received less than 75% of Rx	3		7	
Interruption of 25% or more of Rx	3		0	
Rx modification	2		4	
Total patients	84		85	

Out of 72 patients in the rifampicin series and 68 in the non-rifampicin series, 69 (96%) and 59 (87%) respectively were symptom-free at the end of treatment. Two patients (3%) in the rifampicin and four patients (6%) in the non-rifampicin series had clinically improved 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: 1990)

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). During the year, the study was extended to the Railway Hospital, Perambur (Prof. Z.A. 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 : $3RHZ_7/6RH_2$

Regimen II : $3RHZ_3/6RH_2$

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, 108 patients from General Hospital and 24 cases from Railway Hospital have been admitted to the study. The following analysis is based on 55 patients who have completed treatment and been followed up for 3 months.

Characteristics on admission: The age range was 6 to 74 years. The maximum proportion of patients (60%) were between 12 and 25 years of age. The initial induration of Mantoux to 1TU of PPD (RT23 with Tween 80) was nil in 27% and 10 mm or more in 55%. BCG scar was present in

24%. Pulmonary tuberculosis was present in 8 (15%) of the cases; 37 patients had a single lesion and 18 had multiple lesions in CT scan.

Clinical presentation: Of the 55 patients, 12 presented only with papilloedema, 12 patients had papilloedema with neurological deficit, 11 patients had only neurological deficit and 20 patients had only symptoms.

Status at the end of treatment: A patient was classified as having a favourable response at the end of treatment if all the following criteria were satisfied. (1) No increase in intra-cranial tension, barring dysfunction. (2) No residual neurological deficit; and (3) CT scan normal or if abnormal, volume and hyperdensity reduced by atleast 50% and HU value without contrast 80 or more.

Findings: The results are given in the following 2 tables.

Clinical status at the end of Rx	Reg.I No.	Reg.II No.	Both No.	%
Normal	24	22	46	*84*
Residual deficit	3	3	6	*16*
Expired	1	2	3	
Total	28	27	55	*100*

It can be seen that 46 (84%) of the patients were normal clinically at the end of treatment; 3 patients expired, all due to reasons associated with surgery.

Excluding the three patients who died, the following table gives the CT scan status at the end of treatment.

CT scan status	Reg.I No.	Reg.II No.	Both No.	%
Disappeared	21	17	38	*73*
Decreased more than 50%	3	2	5	*10*
Decreased 50% or less	1	3	4	*8*
No change	1	1	2	*4*
Increased	1	0	1	*2*
Fresh lesion	0	2	2	*4*
Total	27	25	52	*101*

Thus, the response with short course chemotherapy was good, and the findings in the two regimens were fairly similar.

Adverse reactions: In all, 11 patients of 34 in regimen I and 1 patient of 32 in regimen II had hepatitis; 2 patients, one in each regimen developed purpura and had rifampicin terminated. One patient (regimen II) developed the flu syndrome.

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

(started:1986; expected year of completion of intake: 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 X-ray 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 sensitive to isoniazid and rifampicin

By random allocation to either 3EmbHRZ_2/9HR_2, or 3EmbHRZ_2/6HR_2, namely ethambutol 1200 mg plus isoniazid 600 mg (with pyridoxine 10 mg) plus rifampicin 450 mg plus pyrazinamide 2.0 g twice a week for the first 3 months, followed by isoniazid plus rifampicin in the same dosages for either the next 6 months (total 9 months) or the next 9 months (total 12 months). Every dose is given under supervision. So far, 47 patients have been admitted.

(b) Patients with bacilli resistant to isoniazid

6SEmbRZ_2/6EmbRZ_2 or 6KEmbRZ_2/6EmbRZ_2: Streptomycin 0.75 g or, if the bacilli are resistant to streptomycin, kanamycin 1.0 g, plus ethambutol 1200 mg plus rifampicin 450 mg plus pyrazinamide 2.0 g twice a week for the first 6 months, followed by ethambutol plus rifampicin plus pyrazinamide in the same dosages twice a week for the next 6 months (total 12 months). Every dose is given under supervision; 17 and 14 patients, respectively, have been admitted so far.

(c) Patients with bacilli resistant to isoniazid and rifampicin

3S_3EmbEthZ_7/9EmbEthZ_7 or 3K_3EmbEthZ_7/9EmbEthZ_7: 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 self-administration for the remaining days. So far, 6 and 15 patients, respectively, have been admitted.

The intake to all the regimens is continuing.

(started: 1987).

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 in sterile bottles on 3 consecutive days 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. Out of these, urine culture was positive for *M.tuberculosis* in 5 patients. Among these 5 patients, one was referred back during assessment to the study. The other 4 patients were referred to the Nephrology Department, Govt. General Hospital for further investigations. In one patient, who completed the investigations, except for a mild distortion of calyces on one side, all the other findings were normal. The other 3 patients have not completed the investigations.

(started:1987; intake completed:1989).

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 close family contacts of patients with sputum-positive pulmonary tuberculosis. It is a double-blind study, limited to 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 eligible child 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 Mantoux 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 5-year follow-up is in progress.

(started:1974; expected year of completion of 5 year follow-up: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 published (1988 annual report). The survivors at the end of treatment are being followed up to find out the relapse rates and the course of the residual lesions. They are seen once a month up to 24 months, once in 3 months thereafter up to 36 months and subsequently 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) chest radiographs at 3-monthly intervals for patients who have persistent abnormality at the end of treatment, till they become normal; and (c) cerebro-spinal fluid (CSF) examination for cell count, biochemical and bacteriological examinations 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.

The study is in progress.

(started:1982; 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 were at random, it is highly 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 1sec(FEV_1)
3. $\frac{FEV_1}{FVC} \times 100\%$
4. Maximum Voluntary Ventilation(MVV)

In addition, electrocardiograms are recorded in each patient every year. A total of 175 patients were tested initially and are being followed up yearly.

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

Characterization of lower respiratory tract inflammation in patients with smear-negative but X-ray positive pulmonary tuberculosis

The technique of bronchoalveolar lavage (BAL) was utilised to characterise the inflammatory and immune-effector cells in the lung parenchyma of patients with sputum smear negative for AFB, but with radiographic appearances suggestive of pulmonary tuberculosis. Patients whose sputa or lavage fluid had shown growth of *M.tuberculosis* in culture were classified as having active pulmonary tuberculosis. All patients had symptoms for less than 3 months and none had received any anti-TB treatment in the past. The lavages in each patient were carried out first from a radiologically normal subsegment and then from radiologically abnormal subsegments. The total and differential cells were determined, separately from each site. Lavages were done from the middle lobe and lingula in miliary tuberculosis and the specimens were pooled. Of 57 suspected patients in whom BAL was done, 28 patients had bacteriological confirmation by sputum culture, including 21 in whom the lavage fluid culture was also positive for *M.tuberculosis.* Four patients had miliary tuberculosis.

Twelve patients investigated showed predominance of macrophages (range: 82-96%; mean + sd: 90.4% + 5.1%) with a lymphocyte range of 2-16% (mean + sd: 7.5% + 4.5%). Another group of 7 patients showed predominance of lymphocytes (range 22-69%; mean + sd: 40.7% + 17.4%) with a macrophage count of 26-75% (mean + sd: 55.6% + 17.4%). In 4 patients,

an increased granulocyte count (both neutrophils and eosinophils) was observed (mean neutrophils 18.5% + 13.8%; mean eosinophils 38.5% + 21.5%). All 4 patients with miliary tuberculosis had predominance of lymphocytes (range 37-82%; mean + sd: 60% + 18.9%). Thus, three distinct cell profiles, in one group with an increase in alveolar macrophages, in the second with an increase in lymphocytes and in the third with an increase in granulocytes were observed.

It is proposed to study 50 patients bacteriologically confirmed by culture.

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

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

Since wide changes in pulmonary functions 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 was 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. Thirty-five subjects aged between 7 and 14 years were studied. Although the study population is not random, attempts were made to obtain a representative cross-section of normal subjects of Madras city. To achieve this, the subjects included relatives of patients attending the Centre 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. $\frac{FEV_1}{FVC}$ X 100 %
4. Total Lung Capacity (TLC)
5. Functional Residual Capacity (FRC)
6. Residual Volume (RV)
7. $\frac{RV}{TLC}$ %
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.

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

Follow up studies in Tropical Eosinophilia (TE)

Earlier bronchoalveolar 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 T.E. 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) and who had been treated for 3 weeks with Diethyl Carbamazine Citrate (6mg/kg body weight). The follow-up of these patients at 1, 3, 6, 12, 24, 36, 48 and 60 months, utilising the technique of bronchoalveolar lavage and pulmonary function is highly satisfactory.

So far, 157 patients have been admitted to the study and it is proposed to investigate a total of 200 patients.

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

Controlled clinical study of multi-drug therapy in multibacillary leprosy

As mentioned in the previous (1988) annual report, 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, 37 patients have been admitted to the study – 11 patients to the first regimen, 9 each to the second and third, and 8 to the fourth regimen.

It is proposed to admit 60 patients to each regimen.

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

LABORATORY STUDIES

STUDIES COMPLETED

In vitro activity of Rifapentine and Rifabutin against South Indian strains of M.tuberculosis sensitive and resistant to rifampicin.

With the objective of evolving better regimens utilising newer drugs, the *in vitro* activity of rifapentine (MDL 473) was evaluated on strains resistant as well as sensitive to rifampicin (see 1988 annual report). Because of total cross resistance between rifapentine and rifampicin, it was proposed to test another rifamycin derivative, Rifabutin (LM 427, ansamycin), which has been claimed to be active against some rifampicin-resistant strains.

A total of 103 strains comprising of 52 sensitive to rifampicin and 51 resistant to rifampicin were tested against rifampicin, rifapentine and rifabutin concurrently. The compounds were dissolved in dimethyl formamide and incorporated in Lowenstein-Jensen medium to give final concentrations of 1, 2, 4, 8, 16, 32, 64 and 128 mg/l. A standard suspension of the culture was inoculated on two drug-free LJ slopes and one slope of each of the above concentrations, incubated at 37°C for 4 weeks and the Minimal Inhibitory Concentrations (MIC) determined.

		Rifapentine MIC									
		≤1	2	4	8	16	32	64	128	>128	Total
Rifampicin MIC	≤1	1	1								2
	2	2	3	1							6
	4		1	1							2
	8			6	1						7
	16		1	1	9	2					13
	32		1	3	9	8					21
	64							1			1
	128								8	2	10
	>128								2	39	41
	Total	3	7	12	19	10		1	10	41	103

Rifapentine: All 51 strains resistant to rifampicin (MIC≥128) were also resistant to rifapentine, confirming the earlier findings (1988 annual report). Thus rifapentine did not show any activity on rifampicin resistant strains. The other 52 strains were sensitive to both, 9 had identical MICs, 2 had a higher MIC for rifapentine by 1 dilution (in the lower concentrations) while

41 had a lower MIC for rifapentine (26 by 1 dilution, 10 by 2 dilutions and 5 by 3 or 4 dilutions). Thus in 15 out of the 52 strains, rifapentine had a 4-16 fold higher activity than rifampicin. The (Geometric) mean MIC of the 52 strains was 13.3 with rifampicin and 6.0 with rifapentine, showing an average of 2.2 times higher activity with rifapentine. The difference between the means was statistically significant ($p < 0.001$).

Rifabutin: With rifabutin 11 of the 51 rifampicin resistant strains were found to be sensitive. Of the remaining 40 rifampicin resistant strains, 22 had identical MICs and in the remaining 18 the MIC was lower by 1 dilution with rifabutin.

		Rifabutin MIC									
		≤1	2	4	8	16	32	64	128	>128	Total
Rifampicin MIC	≤1	2									2
	2	6									6
	4	1	1								2
	8	7									7
	16	10	3								13
	32	13	6	2							21
	64						1				1
	128		1		4			2	3		10
	>128							4	18	19	41
	Total	39	11	2	4		1	6	21	19	103

Considering the activity of rifabutin on rifampicin sensitive strains, 2 strains had an identical MIC while the remaining 50 had a lower MIC for rifabutin (8 by 1 dilution, 1 by 2 dilutions, 12 by 3 dilutions, 16 by 4 dilutions and 13 by 5 dilutions). Thus in 42 of the 52 strains, rifabutin showed a 4-64 fold higher activity than rifampicin. The (Geometric) mean MIC of all 52 strains for rifabutin was 1.3 compared to 13.3 with rifampicin, showing an average of 10.2 fold higher activity with rifabutin. The difference between the means were highly significant ($p < 0.001$).

To conclude, although rifapentine had a lower mean MIC of 6.0 mg/l compared with 13.3 for rifampicin in rifampicin sensitive strains, it had no higher activity on rifampicin resistant strains. But, with rifabutin, not only was the mean MIC of sensitive strains much lower than that for rifapentine, but 22 per cent of rifampicin resistant strains were found to be sensitive.

(started: 1988; completed: 1989)

In vitro activity of capreomycin and ciprofloxacin on South Indian isolates of M.tuberculosis.

Resistance to rifampicin in strains of *M.tuberculosis* resistant to streptomycin and/or isoniazid is of poor prognostic significance. Treatment of such patients poses a problem since the reserve regimens currently available are not very effective in these patients and are also toxic. The aminoglycoside, capreomycin and a 4-fluoroquinolone, ciprofloxacin are two of the newer anti-TB drugs which have been claimed to be active against *M.tuberculosis* resistant to other drugs including rifampicin. Hence the *in vitro* activity of these drugs was tested against South Indian isolates of *M.tuberculosis* sensitive as well as resistant to streptomycin, isoniazid and/or rifampicin.

A total of 123 cultures was tested. In addition, the standard sensitive strain H37Rv was tested on different occasions. The drugs were incorporated in Lowenstein-Jensen medium in concentrations of 1, 2, 4, 8, 16, 32 and 64 mg/l and inoculated with a standard bacterial suspension. The slopes were read after 28 days' incubation and the Minimal Inhibitory Concentrations (MIC) determined.

The distribution of the strains according to MIC for capreomycin is presented in the table below:

Strains	No. tested	% strains with MIC of							
		1	2	4	8	16	32	64	>64
H37Rv	6					(33)*	(67)		
SHR-Sens.	53					11	64	19	6
SH/H-Res.	16					(12)	(63)	(25)	
SHR/HR-Res.	54					11	56	19	15
Total	123					11	60	20	9

*Figures in parantheses indicate percentages based on small totals.

The distribution of strains by MIC of capreomycin was fairly similar among the sensitive and resistant strains. However, 3 (6%) of 53 sensitive strains yielded an MIC of more than 64 as against 8 (15%) of 54 resistant to SHR/HR and none of 16 resistant to SH/H.

The distribution of MIC to ciprofloxacin is presented in the table on page 58. It was observed that the distributions were similar, there being no difference between sensitive and resistant populations. This could perhaps be due to lack of cross-resistance between quinolones and streptomycin, isoniazid or rifampicin.

Strains	No. tested	% strains with MIC of						
		≤1	2	4	8	16	32	64
H37Rv	6		(100)*					
SHR-Sens.	53	4	23	55	19			
SH/H-Res.	16		(31)	(56)	(12)			
SHR/HR-Res.	54	2	22	57	19			
Total	123	2	24	56	18			

*Figures in parantheses Indicate percentages based on small totals.

(started:1989; completed: 1989)

Proportion Sensitivity test (PST) for Tuberactinomycin (TUM)

Earlier (annual report 1987) the results of a study on *In vitro* susceptibility of *M.tuberculosis* isolated from South Indian patients to TUM, Kanamycin (Kan) and Streptomycin (SM) were presented. PST for TUM was done to obtain more information.

The test cultures were coded and the PST were set up with 8 and 16 mcg/ml concentrations. In each batch, standard reference strain H37Rv was set up as the control. The cultures were read and the proportions resistant were expressed as per the standard procedures followed at the Centre.

Proportions resistant to TUM at 16 mcg/ml, of H37Rv obtained on 6 occasions are given in the table below:

H37Rv Test Number	Proportion resistant (%) at 16 mcg/ml TUM
1	*3.1*
2	*1.3*
3	*5.6*
4	*13.3*
5	*4.5*
6	*31.3*

It can be seen from the table that, for the reference strain H37Rv, the proportions of resistance to TUM ranged from 1.3% to 31.3% on 6 occasions tested and on 5 occasions it was less than 15%. Two criteria of proportion resistante,, namely 25% and 50% at 16 mcg/ml were tentatively considered for the purpose of analysis.

A total of 38 strains were available for analysis by MIC and PST methods. An MIC of 100 or more mcg/ml was used as the criterion of resistance to TUM. The results of MIC criterion were compared with PST criteria in the following table.

MIC (mcg/ml)	TUM proportion resistant %				Total
	<25% (sen)*	≥25% (res)	<50% (sen)	≥50% (res)	
<100 (sen)	32	5	36	1	37
≥100 (res)	0	1	0	1	1
Total	32	6	36	2	38

* sen = Sensitive; res = Resistant

It can be seen that, of the 37 sensitive strains by MIC criterion, 5 by PST 25% criterion and only one by PST 50% criterion were classified as resistant respectively. The lone resistant culture by MIC criterion was classified as resistant by both the PST criteria. Therefore the PST 50% criterion seems to agree with the MIC 100 mcg/ml criterion in the classification of cultures for TUM susceptibility.

(started:1987; completed:1989)

Effect of administration of rifampicin on response to the ACTH stimulation test in patients with pulmonary tuberculosis.

Administration of ACTH is known to cause an increase in plasma levels of cortisol through stimulation of the adrenal cortex. A negative response to ACTH has been reported to be virtually pathognomonic of adrenocortical insufficiency. Investigations undertaken earlier at our Centre have shown that despite high circulating levels of cortisol, a substantial proportion of patients with pulmonary tuberculosis had a lack of adrenal reserve (or functional adrenocortical insufficiency) on the basis of response to stimulation of the adrenal cortex with synacthen, a synthetic fragment of ACTH (see 1987 annual report). Rifampicin is a known inducer of the hepatic microsomal enzyme system and has been shown to induce the metabolism of a number of

steroid drugs. An investigation was therefore undertaken to study the effect of different rhythms of rifampicin administration (daily and twice-weekly) on response to synacthen on admission and during the first 4 weeks of treatment in patients with pulmonary tuberculosis, in comparison with the findings in patients receiving a daily regimen that did not contain this drug.

Sputum smear-positive patients were randomly allocated to one of the following regimens :

1. R-7 : Rifampicin (450 mg) plus ethambutol (800 mg) plus isoniazid (300 mg) plus pyrazinamide (1.5 g) given daily.
2. R-2 : Rifampicin (450 mg) plus ethambutol (1.6 g) plus isoniazid (600 mg) plus pyrazinamide (2.0 g) given twice-weekly.
3. NR-7 : Ethambutol (800 mg) plus isoniazid (300 mg) plus pyrazinamide (1.5 g) given daily.

For the synacthen stimulation test (on admission and at 1, 2 and 4 weeks after start of chemotherapy), plasma cortisol levels were determined at 8 a.m. and at 1/2 hour and 1 hour after an intramuscular dose of synacthen 0.25 mg. Patients were classified on the basis of the increase in the plasma cortisol level at 1 hour over the respective basal value as positive (≥300 nmol/L), negative (<200 nmol/L) or doubtful (200-299 nmol/l) responders to synacthen. Those with a doubtful response were reclassified on the basis of the increase at 1/2 hour as positive (≥ 200 nmol/l) or negative responders (<200 nmol/l).

A total of 61 patients (54 male and 7 female) were admitted to the study; of these 17 were allocated to the R-7 series, 23 to the R-2 series and 21 to the NR-7 series; 4 patients (1 R-7, 1 R-2 and 2 NR-7) were excluded from the analysis due to reasons such as culture negativity on admission, inadequate (<80%) chemotherapy and failure to attend on one or more of the designated test-days. The analysis is therefore based on 57 patients (16 R-7, 22 R-2 and 19 NR-7). None of the patients was diabetic or pregnant or on any form of steroid treatment. The culture grading for *M. tuberculosis* was 3+ in 35 patients, 2+ in 19, 1+ in 2 and 1-19 colonies in 1 patient.

The mean basal plasma levels (8 a.m.) of cortisol in the 3 groups on admission and at 1, 2 and 4 weeks after start of treatment are presented in the top table on page 61.

In the R-7 series, the mean plasma cortisol level at 1 week was about 29% higher than on admission (P=0.06). Further treatment caused a decrease in the levels and the mean value at 4 weeks was about 14% less than that at 1 week (P=0.1), and similar to that on admission. In the NR-7 series, there was an increase (11%) in the mean level at 1 week (P=0.06)

Week	Mean ± standard deviation of basal plasma cortisol levels (nmol/l)		
	R-7	R-2	NR-7
0	471 ± 167	445 ± 127	466 ± 149
1	582 ± 136	452 ± 147	516 ± 122
2	550 ± 97	444 ± 178	481 ± 133
4	502 ± 126	450 ± 136	410 ± 138

and further treatment caused a decrease and the mean value at 4 weeks was about 21% less than that at 1 week ($P < 0.01$); the decrease (12%) between the mean values on admission and at 4 weeks was also significant ($P < 0.01$). There was practically no change in the basal plasma cortisol levels at the different weeks in the R-2 series.

Comparing the 3 series, the mean values on admission were similar ($P > 0.2$). At the first week, the mean value in the R-7 series was higher than those in the R-2 series ($P = 0.01$) and the NR-7 series ($P = 0.09$). At 2 weeks, only the difference between the R-7 and the R-2 series was significant ($P = 0.04$), and at 4 weeks, only the mean value in the NR-7 series was significantly lower than that in the R-7 series ($P = 0.05$).

The number of patients with a positive or negative response to synacthen on admission and at 4 weeks in the three series are given in the following table.

Response to synacthen on admission	Response to synacthen at 4 weeks								
	R-7			R-2			NR-7		
	Pos*	Neg	Total	Pos	Neg	Total	Pos	Neg	Total
Pos	5	3	8	11	5	16	8	0	8
Neg	2	6	8	4	2	6	7	4	11
Total	7	9	16	15	7	22	15	4	19

* Pos indicates positive response and Neg indicates negative response to synacthen.

On admission, 8 of 16 R-7, 16 of 22 R-2 and 8 of 19 NR-7 patients had a positive response to synacthen, the total number of patients with a positive response being 32 (56%) of the 57 patients admitted to the study. The proportion of positive responders in the R-2 series was higher than those in the other two series; the differences, however, were not significant ($P \geq 0.1$). In the R-7 series, 5 patients were positive and 6 negative responders both at 0 and 4 weeks; 3 patients who had a positive response initially

became negative responders and 2 others who were negative initially had a positive response at 4 weeks. In the R-2 series, the classification did not alter in 13 patients; of the remaining 9 patients, 5 who had a positive response initially became negative responders and 4 others who were negative initially had a positive response at 4 weeks. In the NR-7 series, the classification was the same at 0 and 4 weeks in 12 patients; 7 patients who were initially negative became positive responders, and none of the patients who were initially positive became negative responders at the end of 4 weeks. The proportions of patients with a positive response to synacthen at 4 weeks were 7 of 16 R-7, 15 of 22 R-2 and 15 of 19 NR-7 patients. While the differences between R-7 and R-2, and that between R-2 and NR-7 series were not significant, that between R-7 and NR-7 was statistically significant ($P = 0.04$). Further, the increase in the proportion of positive responders at 4 weeks over that at 0 week in the NR-7 series was significant ($P = 0.02$).

Sputum became negative for tubercle bacilli in 5 of 16 R-7 patients, 3 of 22 R-2 patients and in 5 of 19 NR-7 patients at 1 month after start of treatment. No association was observed between bacteriological status and response to synacthen, either on admission or at 4 weeks.

Plasma cortisol levels and adrenal function in tuberculous patients are governed by the stress due to the disease and possible damage of the adrenals due to tuberculous infection. Hence, treatment with effective regimens should result in a decrease in the circulating plasma concentrations and an increase in the proportion of patients with a positive response to synacthen. Both of these effects are seen in patients who did not receive rifampicin (NR-7); however, in patients who received this drug (R-7 and R-2), the plasma cortisol levels at the 4th week were similar to those on admission and no increase was observed in the number of positive responders. On the contrary, some patients who had a positive response initially became negative responders after 4 weeks, a phenomenon not observed in the NR-7 series. These findings suggest that rifampicin is exerting a deleterious effect on the adrenocortical function in tuberculous patients. The mechanism of action is not clear; the sustained high plasma cortisol levels in patients receiving rifampicin appear to argue against a mechanism involving a more rapid systemic clearance of cortisol induced by the drug. Whether formation of antibodies to rifampicin or other immunological reactions are responsible remains to be investigated.

(started : 1988; completed : 1989).

Diurnal variation of salivary cortisol levels in patients with pulmonary tuberculosis during treatment with anti-tuberculosis drugs

Investigations undertaken earlier at our Centre in healthy subjects and patients with pulmonary tuberculosis had shown that the diurnal variation of plasma cortisol levels was disturbed in tuberculous patients, and that the pattern of changes in the salivary levels was similar to that observed in

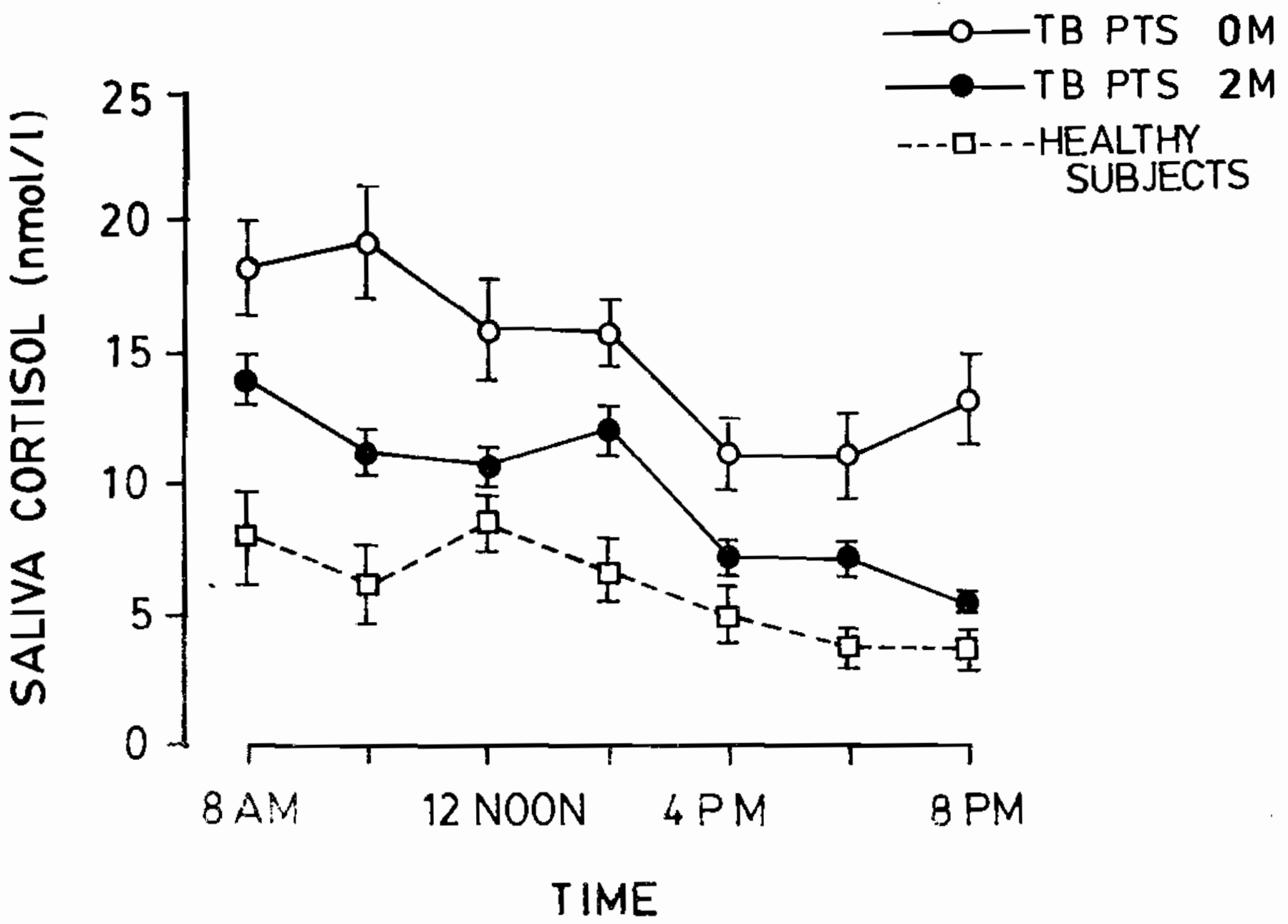
plasma (annual report, 1987). The salivary levels of cortisol in the patients were significantly higher than those in the healthy subjects; further, while there was a decline in cortisol levels in saliva (and in plasma) between 8 A.M. and 8 P.M. in the healthy subjects, there was a decrease upto 6 p.m. followed by a significant increase between 6 p.m. and 8 p.m. in the tuberculous patients. To examine if the abnormal pattern reverted to normal during treatment with anti-tuberculosis drugs, an investigation was undertaken to study the diurnal variation of salivary levels of cortisol on admission and at 2 months after start of treatment.

A total of 61 sputum smear-positive patients were admitted to the study and were randomly allocated to a daily rifampicin regimen (R-7), a twice-weekly rifampicin regimen (R-2) and a daily regimen without rifampicin (NR-7). The details of the drugs and the dosages are given on page 60. Of the 61 patients, 6 were excluded from analysis due to reasons such as culture negativity on admission, inadequate chemotherapy (<80%) or failure to attend on the 2nd occasion at 2 months. Of the 55 patients in the analysis, 16 were allocated to the R-7 regimen, 21 to the R-2 regimen and 18 to the NR-7 regimen. The diurnal variation of cortisol production, as assessed by the salivary levels of cortisol determined at 2-hourly intervals from 8 a.m. to 8 p.m., was studied on admission and at 2 months after the start of treatment. The salivary cortisol levels were determined employing a RIA procedure developed at our Centre using commercially available kits meant for plasma cortisol estimations.

The mean salivary cortisol levels on admission and at 2 months in the 3 groups are presented in the table on page 64. Further, the over-all means of the values in the 55 patients (amalgamating the findings in the 3 groups) at 0 and 2 months, together with those for healthy subjects from an earlier study, are presented in the figure on page 65. The vertical lines denote the standard error.

The findings with the 3 regimens were similar and were therefore amalgamated for further analysis. As observed in the previous study, in patients with pulmonary tuberculosis before the start of treatment, there is a decline in salivary levels of cortisol between 8 a.m. and 6 p.m. followed by an increase, the differences between the mean values at 8 a.m. and 6 p.m. ($P<0.001$) and 6 p.m. and 8 p.m. ($P=0.03$) being significant. After 2 months of treatment, there is an appreciable decrease in salivary cortisol ranging from 24% at 8 a.m. to 59% at 8 p.m., all the differences being significant ($P\leq0.02$). Further, there is a decline in salivary cortisol levels upto 8 p.m., the differences between the mean value at 8 a.m. and those at 6 p.m. and 8 p.m. being highly significant ($P<0.001$ for both). It may be noted that the rise observed after 6 p.m. at 0 month is not observed at 2 months; indeed, the mean value at 8 p.m. was significantly *lower* than that at 6

Group	Mth	Mean ± standard deviation of salivary cortisol levels (n mol/l)						
		8 a.m.	10 a.m.	12 Noon	2 p.m.	4 p.m.	6 p.m.	8 p.m.
R7	0	19.0 ± 9.5	22.1 ± 13.5	17.2 ± 11.1	18.1± 6.5	12.3 ± 11.8	13.1 ± 13.9	14.6 ± 10.7
	2	13.3 ± 4.9	12.4 ± 5.4	11.3 ± 4.5	11.4 ± 4.4	6.0 ± 2.2	7.6 ± 3.8	5.7 ± 3.1
R2	0	15.9 ± 9.2	15.1 ± 7.1	12.2 ± 6.7	13.6 ± 5.6	9.5 ± 6.1	10.1 ± 10.6	11.6 ± 10.0
	2	14.2 ± 6.1	10.2 ± 4.1	9.8 ± 2.8	10.4 ± 3.2	6.2 ± 3.4	5.5 ± 3:4	4.6 ± 2.0
NR7	0	20.2 ±17.4	21.3 ± 20.0	18.7 ± 18.4	16.1 ±11.9	11.8 ± 9.1	10.5 ± 7.4	13.9 ± 15.3
	2	14.1 ± 5.8	11.2 ± 8.5	10.8 ± 5.3	14.4 ± 7.9	9.1 ± 6.1	8.4 ± 5.1	6.2 ± 3.1
All 3 groups	0	18.2 ± 12.7	19.2 ± 14.6	15.8 ± 13.1	15.7 ± 8.6	11.1 ± 9.1	11.1 ± 10.9	13.2 ± 12.3
	2	13.9 ± 5.7	11.2 ± 6.3	10.6 ± 4.3	12.0 ± 5.7	7.1 ± 4.5	7.1 ± 4.3	5.4 ± 2.8


TB PTS 0M
TB PTS 2M
HEALTHY SUBJECTS
SALIVA CORTISOL (nmol/l)
25
20
15
10
5
0
8 AM
12 NOON
4 PM
8 PM
TIME

p.m. The findings presented in the figure show that the pattern of change in salivary cortisol is fairly similar to that in the healthy subjects.

These findings therefore demonstrate that the diurnal variation of cortisol release by the adrenal cortex is disturbed in patients with pulmonary tuberculosis but that the pattern returns to normal following 2 months (or probably less) of treatment with effective anti-tuberculosis regimens.

(started:1988; completed:1989).

Pharmacokinetics of isoniazid and rifampicin in patients with renal failure

Kidneys play a major role in the elimination of drugs and their metabolites from the system. In patients with renal failure, elimination of most drugs is retarded, and therapeutic doses as administered to normal individuals could lead to sustained high plasma levels that could be toxic. The dosages of drugs employed for the treatment of tuberculosis in patients with renal failure are largely empirical, being about half of those administered to those with normal renal function. Such an empirical reduction could still lead to toxic plasma levels of the drugs in patients with severe renal failure, while effective levels may not be attained in those with a mild degree of impairment. This difference could be further accentuated in case of drugs such as isoniazid, whose metabolizing enzymes display genetic polymorphism. Detailed investigations were therefore undertaken to correlate plasma levels and pharmacokinetic variables such as exposure (area under the time-concentration curve), peak concentration and plasma half-life of the drugs with different degrees of renal failure, to attempt to devise suitable drug dosages.

An investigation was therefore undertaken in collaboration with the Nephrology Department of the Government General Hospital, Madras (Prof. Muthusethupathi) to study the single dose pharmacokinetics of isoniazid 7.5 mg/kg and rifampicin 12 mg/kg in patients with different grades of renal failure, and on the basis of these findings, to estimate appropriate dosages of these drugs to be employed in tuberculous patients with renal failure.

Patients reporting to the General Hospital with renal failure which was stable for over a week were admitted to the study. The patients were classified as having mild, moderate or severe degree of renal failure based on creatinine clearance values of 21-50, 11-20 and 5-10 ml/min, respectively. In addition, a control group of healthy subjects with normal renal function was also investigated. The hepatic function, as assessed by the activities of AST and ALT in plasma were normal in all the subjects.

The isoniazid acetylator phenotype was tentatively determined on the basis of the salivary concentration of isoniazid at 5 hours after an oral dose of isonized 100 mg (criterion for a rapid acetylator ≤ 0.4 mcg/ml) and was confirmed on the basis of the plasma half-life of isoniazid (criterion for a rapid acetylator < 2.6 hours) determined during the pharmacokinetic investigation.

On the day of the test, isoniazid 7.5 mg/kg and rifampicin 12 mg/kg were administered on an empty stomach and blood was collected at 1, 2, 3, 6, 8 and 24 hours. Further, urine excreted over the period 0-24 hours was collected and the volume measured. Plasma from the blood specimens and aliquots of urine were stored at -20°C. Isoniazid and rifampicin concentrations in plasma were determined within 48 hours of collection, and urine concentrations of isoniazid, acetylisoniazid, rifampicin and desacetylrifampicin within 7 days, employing standard techniques, after coding the specimens. On the basis of these concentrations, the mean peak concentration, exposure, plasma half-life and the proportions of the drugs and some of their metabolites excreted in urine over a 24-hour period were calculated. In addition, several renal function parameters such as plasma concentrations of urea and creatinine and urine concentrations of creatinine were determined employing an automated blood chemistry analysis system, and creatinine clearance values were calculated.

A total of 16 healthy subjects (9 male, 7 female) and 35 patients with renal failure (19 male, 16 female) were admitted to the study. The renal failure was mild in 3, moderate in 15 and severe in 17 patients. Since the number of patients with mild renal failure is small, findings in these patients were amalgamated with those having moderate renal failure. The mean values for age and body-weight, plasma concentrations of urea and creatinine, and creatinine clearance in the 3 different groups separately for the slow and rapid acetylators, are presented in the following table.

Group	Acetylator phenotype	Mean values of the following				
		Age (years)	Body-wt. (kg)	Plasma urea (mg/dl)	Plasma creatinine (mg/dl)	Creatinine clearance (ml/min)
Healthy subjects (n = 16)	Slow (n = 10)	35.8	51.7	21.5	0.82	88.1
	Rapid (n = 6)	34.0	47.8	17.8	0.88	82.6
Mild or moderate (n = 18)	Slow (n = 12)	41.1	46.5	87.8	4.0	14.4
	Rapid (n = 6)	41.3	50.7	75.7	3.7	20.9
Severe (n = 17)	Slow (n = 9)	30.8	41.1	136.6	5.7	6.3
	Rapid (n = 8)	44.1	41.0	133.6	6.1	7.0

The mean dosage of isoniazid administered to slow and rapid acetylators among the healthy subjects, patients with mild or moderate renal failure and those with severe renal failure were 7.8 and 7.7, 8.0 and 7.6, and 8.2 and 7.9 mg/kg, respectively; the mean dosages of rifampicin in the three groups were 11.6, 11.8 and 12.1 mg/kg, respectively.

The distribution of patients by the time at which the highest plasma concentrations of isoniazid (amalgamating the findings in the slow and the rapid acetylators) and rifampicin were attained are presented in the table below.

Group	Drug	No. of subjects with highest concentrations observed at					Total
		1 hr	2 hr	3 hr	6 hr	8 hr	
Healthy subjects	Isoniazid	13	3	-	-	-	16
	Rifampicin	2	11	2	1	-	
Mild or moderate	Isoniazid	14	4	-	-	-	18
	Rifampicin	4	11	3	-	-	
Severe	Isoniazid	16	1	-	-	-	17
	Rifampicin	2	13	1	1	-	

These findings suggest that the rates of absorption of the 2 drugs appear to be similar in all the 3 groups investigated, and that there is a delay in the gastrointestinal absorption of rifampicin in comparison to that of isoniazid.

The mean serial plasma isoniazid concentrations in slow and rapid acetylators together with the mean peak concentration, the exposure and the plasma half-life are presented in the table on page 69.

In slow acetylators, the mean isoniazid concentrations were higher in the 2 groups of patients from the 3rd hour, the differences being substantial at 6, 8 and 24 hours. In rapid acetylators, the mean values in both groups of patients were slightly higher than in the healthy subjects at all time points. The mean concentration in patients with mild or moderate renal failure appeared to be similar to that in those with severe failure. In slow acetylators, the differences in the mean values for peak concentration, exposure and half-life between patients with mild or moderate failure and those with severe failure were not significant ($P > 0.2$). While the differences in the mean peak concentrations in both the groups of patients and in healthy subjects were not significant ($P > 0.2$), the exposure and half-life were higher ($P < 0.01$). In rapid acetylators there appeared to be a slight increase in the mean values

Hours after drug administration	Geometric mean of plasma isoniazid concentrations (mcg/ml)					
	Slow acetylators			Rapid acetylators		
	Healthy subjects	Mild or moderate renal failure	Severe renal failure	Healthy subjects	Mild or moderate renal failure	Severe renal failure
1	11.2	9.4	10.9	6.3	6.4	7.7
2	9.6	9.6	10.0	4.3	4.6	5.0
3	7.5	8.7	9.0	2.6	2.9	3.1
6	3.9	5.9	6.1	0.6	1.2	1.2
8	2.7	4.9	4.8	0.2	0.8	0.7
24	0.2	1.0	1.1	0.1	0.3	0.2
Mean peak concentration. (mcg/ml)	11.5	10.8	10.9	6.7	6.4	7.7
Exposure (mcg/ml. hours)	61	95	94	19	24	25
Half-life (hours)	3.4	6.3	5.8	1.6	2.1	2.1

for exposure and plasma half-life in the patients than in the controls; however, none of the differences between the three groups of subjects was statistically significant ($P > 0.05$).

The mean serial rifampicin concentrations together with the mean peak concentration, exposure and plasma half-life are presented in the table on page 70.

The plasma rifampicin concentrations at the different time-points and the mean values for peak concentration, exposure and plasma half-life were broadly similar in the 3 groups and none of the differences was significant ($P > 0.05$).

The correlations between some renal function parameters such as creatinine clearance, plasma creatinine or urea and the mean peak concentration, the exposure and the half-life of isoniazid were examined in the slow acetylators and it was observed (data not presented) that the correlations were poor in all the cases ($r \leq 0.28$).

Hours after drug admn.	Geometric mean of plasma rifampicin concentrations (mcg/ml)		
	Healthy subjects	Patients with renal failure	
		Mild or moderate	Severe
1	5.7	5.4	5.0
2	13.0	12.2	10.8
3	11.1	10.2	9.1
6	7.8	7.6	6.3
8	5.9	5.4	4.6
24	0.19	0.17	0.09
Mean peak concentration. (mcg/ml)	15.2	13.2	11.8
Exposure (mcg/ml. hours)	88	81	71
Half-life (hours)	5.0	5.1	4.9

The excretion of isoniazid and acetylisoniazid in slow and rapid acetylators and those of rifampicin and desacetylrifampicin in the three groups are presented in the table on page 71.

In slow acetylators, the proportion of the dose excreted as isoniazid or acetylisoniazid in urine collected over the period 0-24 hours was much lower in the patients than in the healthy subjects ($P < 0.001$). The excretion of both isoniazid ($P = 0.02$) and acetylisoniazid ($P < 0.01$) was lower in patients with severe renal failure than in those with mild or moderate failure.

In rapid acetylators, the excretion of the dose administered as isoniazid was significantly lower in patients with severe renal failure than in the healthy subjects ($P < 0.01$); the differences in the excretion between patients with mild or moderate failure and the healthy subjects or those with severe renal failure were not significant ($P = 0.2$). The differences between the 3 groups in the excretion as acetylisoniazid were significant ($P \leq 0.02$).

Drug/ metabolite	Acetylator phenotype	Mean of the proportion of dose (%) excreted in urine (0-24 hrs)		
		Healthy subjects	Patients with renal failure	
			Mild or moderate	Severe
Isoniazid	Slow	27.0	12.8	7.4
	Rapid	9.1	4.6	3.3
Acetyl-isoniazid	Slow	18.1	6.2	3.9
	Rapid	36.6	11.4	4.8
Rifampicin	Both groups	6.3	1.9	1.4
Desacetyl-rifampicin	Both groups	3.6	1.1	0.7

In contrast to the plasma levels of rifampicin where no differences were observed between the 3 groups, the excretion of the dose administered either as the unchanged drug or its primary metabolite, desacetylrifampicin, was significantly less in both groups of patients than in the healthy subjects ($P < 0.001$); the differences between the two groups of patients were however not satistically significant for either compound ($P \geq 0.09$)

These findings suggest that despite the inhibition in the renal excretion of Isoniazid and acetylisoniazid or of rifampicin and desacetylrifampicin, the plasma levels of isoniazid in rapid acetylators and of rifampicin in patients with renal failure are not affected to any appreciable extent. Kidneys play a relatively minor role in the elimination of isoniazid in rapid acetylators, acetylation being predominant, and of rifampicin and its metabolites, which are excreted mainly through the bile. These findings suggest that the doses of rifampicin and that of isoniazid in rapid acetylators in tuberculous patients with renal failure should be similar to those administered to tuberculous patients with normal renal function. In slow acetylators, there was an increase in the plasma levels in patients with renal failure and therapeutic doses as administered to patients with normal renal function could be potentially toxic. The correlations between some renal function parameters and the pharmacokinetic variables such as mean peak concentration, exposure and plasma half-life of isoniazid were poor and it is therefore not possible to adjust the dose suitably. However, a reduction in the dose is essential and it is preferable that these patients are treated with daily regimens containing the drug. Administration of half the normal dose should be adequate as even a 50% reduction in the exposure will not exert any therapeutic penalty, as evidenced by the success of daily regimens of isoniazid in rapid acetylators with pulmonary tuberculosis, who have substantially lower exposure and plasma half-life values than the slow acetylators.

(started: 1987; completed: 1989).

Renal function in patients with pulmonary tuberculosis during treatment with daily and intermittent regimens containing rifampicin

A few reports have suggested that administration of rifampicin, particularly intermittently and in high dosage, could be nephrotoxic. This has been attributed to hypersensitivity reactions caused by rifampicin antibodies. The nephrotoxic effect of rifampicin, if any, could be similar to that of antibiotics like gentamycin, viomycin and kanamycin which are known to cause acute tubular necrosis. Information on the renal function in patients receiving rifampicin and other anti-tuberculosis drugs is scarce; we, therefore, undertook an investigation to determine some renal function parameters in patients receiving daily and fully intermittent regimens of rifampicin and in healthy subjects.

Patients with pulmonary tuberculosis were randomly allocated to one of the following 3 regimens:

1. $2EHRZ_7/6EH_7$: Ethambutol, isoniazid, rifampicin and pyrazinamide daily for the first 2 months, followed by ethambutol and isoniazid daily for the next 6 months, the total duration of treatment being 8 months.
2. $2EHRZ_2/4EHR_2$: Ethambutol, isoniazid, rifampicin and pyrazinamide twice-a-week for 2 months followed by ethambutol, isoniazid and rifampicin twice-a-week for the next 4 months, the total duration being 6 months.
3. $2HRZ_2/4HR_2$: Same as regimen 2 above, but without ethambutol.

The dosages of the drugs during daily and twice-weekly treatment were 300 mg and 600 mg respectively for Isoniazid, 600 mg and 1200 mg for ethambutol, and 1.5 g and 2.0 g for pyrazinamide; the dosage of rifampicin was 450 mg for both.

Samples of blood and urine simultaneously were collected on admission, at 1 month and at the end of treatment (6/8 months) from 77 patients on the daily regimen, 71 on the twice-weekly regimen containing ethambutol and from 62 on the twice-weekly regimen without ethambutol. Serum and urine concentrations of sodium and creatinine and of serum urea on admission were determined using an autoanalyzer.

All the findings, before, during and at the end of treatment in patients on the 2 twice-weekly regimens were similar and were therefore amalgamated. The mean values of some renal function parameters in 77 patients on the daily regimen (R7) and 133 patients on the twice-weekly regimens (R2) at the 3 different time-points and those of 20 healthy subjects (determined on one occasion) are presented in the table on page 73.

Renal function test	Group	Mean ± standard deviation at the following months		
		0	1 m	6/8 m
Serum creatinine (mg/dl)	Healthy subjects	1.03 ± 0.14	-	-
	R7	0.79 ± 0.16	0.77 ± 0.17	0.72 ± 0.18
	R2	0.83 ± 0.18	0.80 ± 0.18	0.77 ± 0.19
Serum sodium (mmol/l)	Healthy subjects	142.4 ± 1.5	-	-
	R7	136.3 ± 4.3	137.7 ± 4.2	139.6 ± 2.8
	R2	136.6 ± 4.8	138.5 ± 3.7	140.0 ± 3.4
FENa (%)	Healthy subjects	1.29 ± 0.56	-	-
	R7	0.91 ± 0.59	0.95 ± 0.57	1.01± 0.64
	R2	0.98 ± 0.53	1.22 ± 0.66	1.11± 0.55

The mean serum urea values on admission (not tabulated) were 13.9 mg/dl in the R7 patients and 15.6 mg/dl in the R2 patients and 18.4 mg/dl in the healthy subjects. On admission, none of the differences between the R7 and R2 patients was significant; the amalgamated means (± standard deviation) were 0.82±0.17 mg/dl for serum creatinine, 136.5±4.63 mmol/l for serum sodium, 0.96±0.55 % for FENa and 15.0±4.6 mg/dl for serum urea. The mean values of all four parameters were significantly lower than in the healthy subjects ($P < 0.01$ for all).

During daily treatment (R7), there was an increase in the sodium values and the means at 1 month and at the end of chemotherapy were higher than that on admission ($P = 0.03$ and < 0.001), respectively. On the contrary, there was a slight decrease in serum creatinine concentrations and the mean value at the end of treatment was about 9% lower than that on admission ($P = 0.001$). There was a slight increase in the mean FENa values with treatment; neither of the differences was, however, significant ($P > 0.2$).

With twice-weekly chemotherapy too, there was an increase in the serum sodium concentrations and a decrease in serum creatinine concentrations, and the mean values at the end of treatment were significantly different from those on admission ($P < 0.001$ for both). In contrast to the observation in the R7 patients, the mean FENa value at 1 month was about 24% higher than on admission in the R2 patients ($P < 0.001$); further treatment caused a 9% decrease and the mean value at the end of treatment, though slightly

higher than on admission ($P = 0.04$), was significantly less than that at 1 month ($P = 0.01$).

The two significant findings of this investigation are: (a) the low serum concentrations of both sodium and creatinine in tuberculous patients on admission in comparison with those in healthy subjects, and (b) an increase in serum sodium concentrations and a decline in the serum creatinine concentrations with treatment. The normal range for serum sodium is 135 to 145 mmol/l; hyponatremia (< 135 mmol/l) was observed in 68 (31%) of 210 patients on admission and in none of the 20 healthy subjects. The proportions of patients with hyponatremia decreased with treatment with anti-tuberculosis drugs given daily or intermittently, and the proportions at 1 month and at the end of treatment were 17% and 2%, respectively. Hyponatremia in patients with pulmonary tuberculosis could be due to adrenocortical insufficiency or due to the syndrome of inappropriate AVP (SIADH) secretion.

To examine whether hyponatremia was due to the circulating levels of cortisol, plasma sodium concentrations (and those of creatinine) were determined before and 1 hour after stimulation of the adrenal cortex with a synthetic preparation of ACTH (synacthen) in 19 healthy subjects and 17 patients with sputum smear positive pulmonary tuberculosis. The concentrations of plasma cortisol were also determined by a RIA technique and the mean values are presented in the following table.

Plasma constituent	Mean ± standard deviation of the plasma values			
	Healthy subjects		TB patients	
	Before synacthen	After synacthen	Before synacthen	After synacthen
Sodium (mmol/l)	142.5 ± 1.6	142.2 ± 1.4	138.2 ± 3.4	138.1 ± 3.0
Creatinine (mg/dl)	1.03 ± 0.15	1.01 ± 0.15	0.92 ± 0.16	0.89 ± 0.17
Cortisol (nmol/l)	377.4 ± 109.0	924.6 ± 130.3	485.3 ± 160.0	768.1 ± 205.5

Before administration of synacthen, the plasma sodium and creatinine concentrations were significantly lower ($P \leq 0.04$) and the plasma cortisol concentrations appreciably higher ($P = 0.02$) in patients with pulmonary tuberculosis than in healthy subjects. Stimulation with synacthen caused an appreciable

increase in plasma cortisol levels in both groups. Based on the increase in plasma cortisol levels (over the basal value) at 1/2 hr (not tabulated) and 1 hr after administration of synacthen, none of the 19 healthy subjects and 6 of the 17 TB patients had a negative response to synacthen, suggesting adrenocortical insufficiency (or lack of adrenal reserve) in a high proportion of patients with pulmonary tuberculosis. There was practically no change in the plasma sodium and creatinine concentrations after synacthen in both healthy subjects and patients, suggesting that high levels of cortisol (or the lack of adrenal reserve) had no effect on the concentrations of these two constituents.

The syndrome of inapproporiate AVP (arginine vasopressin secretion) is characterized by hyponatremia caused by water retention attributable to persistent AVP release. In patients with nonmalignant pulmonary disease, the lung tissue acquires the capacity to synthesize and release AVP autonomously or reduces left atrial filling which stimulates central AVP release. AVP has been demonstrated in tuberculous lung tissue but not in uninvolved lung or in suspensions of tubercle bacilli. With effective anti-tuberculosis treatment, there is probably a decrease in AVP release leading to a decrease in water retention and this results in an increase in the plasma sodium concentrations. While SIADH (and water retention) can explain the hyponatremia in the patients on admission and the subsequent increase in plasma sodium concentrations, it is difficult to find an explanation for the decrease in plasma creatinine concentrations during treatment. Administration of rifampicin has been shown to significantly enhance the urinary excretion of both uric acid and pyrazinoic acid, and it has been suggested that this effect of the drug is possibly due to an inhibition of the tubular reabsorption of these two organic acids (see 1981 annual report). Whether rifampicin exerts a similar effect on the reabsorption of creatinine leading to an enhanced excretion of this compound in urine, thereby causing a decrease in plasma levels, remains to be investigated.

Nephrotoxicity during treatment with fully intermittent regimens of rifampicin has been extremely rare in our patients. Thus, only 1 of a total of 762 patients with pulmonary tuberculosis who were treated with fully intermitent regimens containing rifampicin had chronic nephrotic syndrome possibly attributable to the drug. This, and the biochemical evidence presented in this report, suggest that administration of rifampicin either daily or intermittently in a dosage of 12 mg/kg carries little or no risk of nephrotoxicity in South Indian patients with pulmonary tuberculosis.

(started: 1988; completed; 1989)

The FENa test in patients with chronic renal failure

Azotemia, i.e. increase of plasma concentrations of non-protein nitrogenous substances, principally urea and creatinine, is a common manifestation in patients with renal failure. Azotemia is frequently classified as pre-renal, renal

and post-renal. Renal function tests normally employed such as determination of serum urea or creatinine concentrations or of creatinine clearance do not differentiate between pre-renal azotemia and tubular necrosis (renal azotemia). It has been observed that handling of sodium is distinctly different in these conditions; the renal tubule avidly reabsorbs filtered sodium in pre-renal azotemia while it is restricted in acute tubular necrosis. A single test, the FENa (the excreted fraction of filtered sodium), takes advantage of the difference in the tubular handling of Na^+ and thus differentiates between the two conditions.

The FENa is the excreted fraction (%) of filtered sodium and is the ratio of renal clearance of sodium to the glomerular filtration rate, the latter represented by creatinine clearance for convenience. Thus, the formula is :

$$\text{FENa (\%)} = \frac{\text{U Na x P cr}}{\text{P Na x U cr}} \times 100$$

where U and P are concentrations in urine and plasma of sodium (Na) and creatinine (cr). The FENa can be determined on simultaneously collected samples of plasma (serum) and urine at any moment. FENa (%) values less than 1 are indicative of pre-renal azotemia and those greater than 3 suggest tubular necrosis (renal azotemia) in patients with acute renal failure. Little or no information is available on the utility of the FENa test in patients with chronic renal failure. This test was therefore evaluated in a group of patients with chronic renal failure and healthy controls admitted to an investigation to study the pharmacokinetics of isoniazid and rifampicin in collaboration with the Nephrology Department of the Government General Hospital, Madras. The serum and urine concentrations of creatinine and sodium and the serum concentrations of urea were determined using an automated blood chemistry analysis system (Beckman-Astra 8).

Patients were classified as having severe, moderate or mild renal failure on the basis of the values for creatinine clearance, namely 5-10 ml/min for severe cases, 11-20 ml/min for moderate cases and 21-50 ml/min for the mild cases. The numbers of patients in the severe, moderate and mild categories were 17 (8 male and 9 female), 15 (10 male and 5 female) and 3 (1 male and 2 female) respectively; the number of healthy subjects investigated was 16 (9 male and 7 female). The mean age (and range) was 37.1 years (16-72 years) in the severe cases, 41.6 years (15-65 years) in the moderate cases, 47.0 years (18-72 years) in the mild cases and 35.1 years (21-52 years) in the healthy subjects. The mean values of several renal function parameters in the different groups are presented in the table on page 77.

None of the patients had oliguria (daily urine output of less than 500 ml) while 3 patients (2 moderate, 1 severe) had urine output volumes between 500 and 1000 ml; the volume was greater than 2500 ml in 6 of 16 healthy

subjects and in 4 (1 mild, 2 moderate, 1 severe) of the 35 patients with renal failure ($P = 0.06$).

There was an appreciable increase in the concentrations of plasma urea and creatinine with increase in the degree of renal failure. The excretion of

Renal function	Mean ± standard deviation of the following			
	Healthy subjects	Patients with renal failure		
		Mild	Moderate	Severe
Plasma urea (mg/dl)	20.1±5.8	64.7±52.3	87.6±38.8	135.2±35.3
Plasma creatinine (mg/dl)	0.84±0.23	2.8±1.7	4.2±1.2	5.9±2.0
Plasma sodium (mmol/l)	140.4±3.6	137.7±2.9	136.0±6.1	136.0±5.4
Urine output (ml/day)	2198±598	2323±839	1914±786	1780±456
Excretion of sodium (mmol/day)	173.8±88.9	81.9±59.7	83.4±60.1	91.7±37.0
Excretion of creatinine (mg/day)	1021±398	1077±489	788±213	529±168
Creatinine clearance (ml/min)	86.0±27.8	31.9±13.0	13.5±2.1	6.6±2.1
FENa (%)	0.98±0.45	1.74±2.00	3.61±3.53	8.45±4.40
No. of subjects	16	3	15	17

sodium was about 50% lower in patients with renal failure than in the healthy subjects; however, the excretion appeared to be similar in the 3 groups of patients with renal failure. There was no difference in the excretion of creatinine between healthy subjects and patients with mild renal failure: the mean values were however substantially lower in patients with moderate or severe renal failure. There was a progressive increase in the FENa values with decrease in the rates of creatinine clearance.

Of the 35 patients with renal failure, 6 (2 mild, 3 moderate and 1 severe) had FENa values of less than 1.00, 5 (all moderate) had values between 1.01 and 3.00 and the remaining 24 (1 mild, 7 moderate and 16 severe) had values greater than 3.00. The mean values (± standard deviation) of the plasma concentrations of urea, creatinine and sodium and the excretion of sodium and creatinine in urine collected over a 24-hour period, according to the FENa values are presented in the table below.

FENa	No. of patients	Mean±standard deviation of the following					
		Plasma urea (mg/dl)	Plasma creatinine (mg/dl)	Plasma sodium (mmol/l)	Urine creatinine (mg/24hr)	Urine sodium (mmol/24hr)	Creatinine clearance (ml/min)
< 1.00	6	108.8 ± 56.6	3.76 ± 1.68	134.7 ± 5.1	894.4 ± 432.4	26.9 ± 10.6	20.9 ± 13.0
1.00-3.00	5	82.0 ± 15.2	3.79 ± 0.71	136.2 ± 2.8	741.2 ± 122.2	52.0 ± 15.7	14.0 ± 3.0
≥ 3.01	24	112.1 ± 42.7	5.26 ± 1.97	136.5 ± 5.8	623.5 ± 236.4	109.8 ± 44.1	9.0 ± 4.3

Relatively weak, though statistically significant associations ($P < 0.01$) were observed between FENa and excretion of sodium per day ($r = +0.63$), FENa and creatinine clearance ($r = -0.54$), FENa and excretion of creatinine per day ($r = -0.46$) and between plasma creatinine and creatinine clearance ($r = -0.51$). The associations between FENa and plasma constituents such as sodium, creatinine and urea were still weaker ($r < 0.34$), suggesting that urinary excretion of sodium and creatinine and probably the volume of urine excreted per day have a much greater influence on the FENa value than the plasma constituents.

The FENa depends upon several factors such as the salt intake, hydration status, tubular function and the glomerular filtration rate (GFR) of the individual. This study shows that FENa increases as the GFR falls and with a fall in the GFR, the kidney tries to eliminate proportionately more sodium in an attempt to maintain the body fluid volume. Hence, there is a disproportionate increase in the FENa with a decrease in the GFR. This is referred to as the "magnification phenomenon".

The low FENa values (< 1.0) observed in a few patients are probably due to severe salt restriction. In salt depleted states, the kidney tries to conserve more sodium, and this in turn leads to low FENa values.

(started: 1988; completed: 1989).

Iron-sequestration mechanisms of mycobacteria

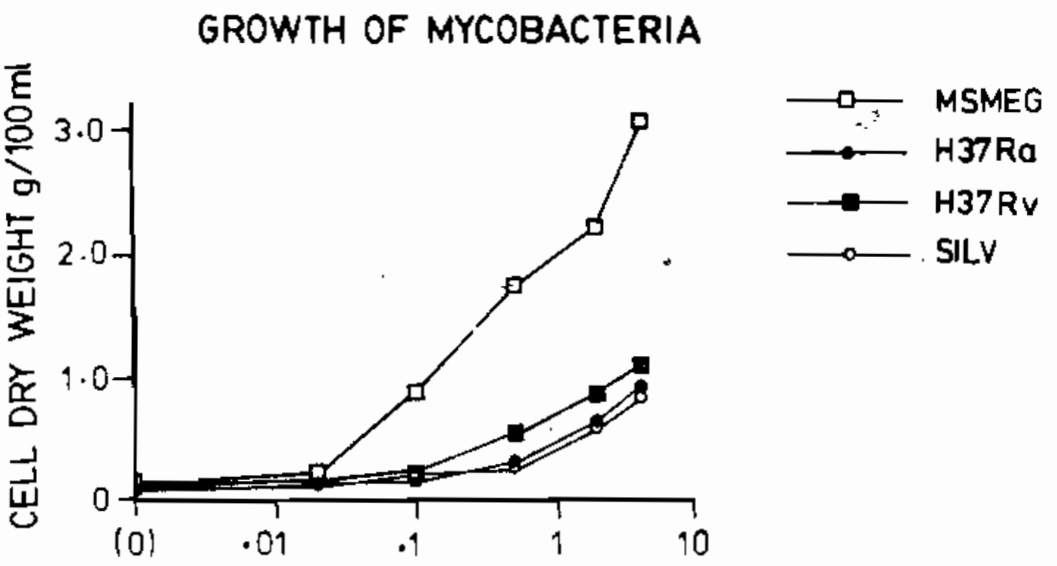
Iron is recognised to be a vital nutrient for the growth of microorganisms. The ability of microorganisms to survive and proliferate within the host tissues would depend, at least partly, on their ability to sequester free iron from their surroundings. It has been shown that the mobilization and uptake of iron is mediated by low molecular weight iron-chelating agents known as siderophores. Mycobacteria produce two types of siderophores, the exochelins and the mycobactins. The exochelins sequester iron from the surroundings and the mycobactins, which are constituents of the cell wall, are believed to help in the transport of free iron across the cell wall. Investigations were undertaken to study the influence of different concentrations of iron (in the free form or as hemoglobin) on the growth, and the production of exochelins and mycobactins *in vitro* by four different strains of mycobacteria. Further, the effect of addition of exochelins on the uptake of iron by mycobacteria as well as that of addition of iron-binding proteins such as transferrin and lactoferrin on the growth of mycobacteria and on the production of exochelins and mycobactins was also investigated.

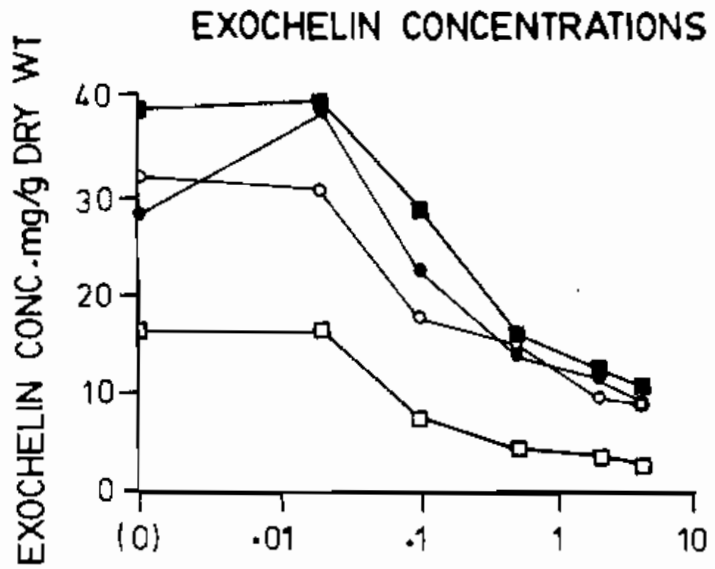
The four strains employed were a saprophyte *(M. smegmatis)*, a virulent strain *(M. tuberculosis H37Rv)*, an avirulent strain *(M. tuberculosis H37Ra)* and a South Indian Low Virulent strain of *M. tuberculosis (SILV)*. The four strains were grown on LJ media to their log phase and then subcultured into a synthetic medium made up of potassium dihydrogen phosphate, L-aspargine and glycerol (pH 6.8). This medium was made metal ion-free by autoclaving with alumina (chromatography grade) and then supplemented with Mg^{++}, Mn^{++}, and Zn^{++}.

Fe^{++} (in the form of $FeSo_4$) was added in concentrations of 0, 0.02, 0.1, 0.5, 2 and 4 mcg/ml (Ferrous iron is converted to ferric iron under aerobic conditions). The media were inoculated with the different strains and incubated at 37°C. The organisms were harvested on the 8th day for *M. smegmatis* and on the 35th day for the three *M. tuberculosis* strains.

I Effect of iron on the growth of mycobacteria and the production of siderophores: The four strains of mycobacteria were grown in the presence of concentrations of iron ranging from 0 to 4 mcg/ml. Cell dry-weights were determined using pre-weighed filters with drying to constant weight at 100°C. Exochelins were extracted from the cell-free culture filtrates with chloroform and mycobactins were extracted from the cells with ethanol and both the siderophores were estimated by gravimetric procedures. The means (of 6 estimates) are presented in the figure on page 80.

There was a significant increase in the growth of all 4 strains of mycobacteria with increasing concentrations of iron in the medium ($P < 0.001$),


GROWTH OF MYCOBACTERIA
CELL DRY WEIGHT g/100ml
3·0
2·0
1·0
0
(0)
·01
·1
1
10
MSMEG
H37Ra
H37Rv
SILV


EXOCHELIN CONCENTRATIONS
EXOCHELIN CONC.mg/g DRY WT
40
30
20
10
0
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·01
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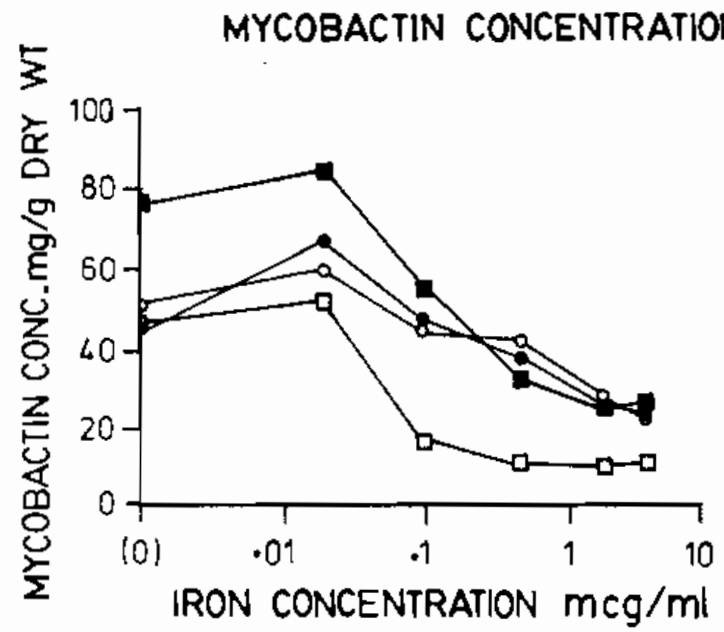
1
10


MYCOBACTIN CONCENTRATION
MYCOBACTIN CONC.mg/g DRY WT
100
80
60
40
20
0
(0)
·01
·1
1
10
IRON CONCENTRATION mcg/ml

the increase in the growth of the saprophytic *M. smegmatis* being substantially higher than that of the three *M. tuberculosis* strains. The growth of the virulent H37Rv strain was greater than that of either H37Ra or the SILV strain ($P<0.001$ for both), while it was similar in the avirulent and the low virulent strains.

A marked decrease was observed in the production of both exochelins and mycobactins with increasing concentrations of iron in the medium. The concentration of both these siderophores, particularly at the higher concentrations of iron, was appreciably lower with M. smegmatis than with any of the *M. tuberculosis* strains. As with the growth, the release of exochelins was higher with H37Rv than with H37Ra ($P=0.06$) or SILV ($P=0.02$); similarly, the production of mycobactins was also significantly higher with the virulent strain than with either the avirulent or the low virulent strains ($P<0.01$). The differences in either the release of exochelins or the production of mycobactins between the avirulent and the low-virulent strains were not significant ($P>0.2$).

The correlation co-efficients for the various associations observed are presented in the following table :

Association between	M. smegmatis	H37Ra	H37Rv	SILV
Iron concentrations and cell dry-weight	+ 0.91	+ 0.98	+ 0.93	+ 0.98
Iron concentrations and release of exochelins	− 0.62	− 0.67	− 0.75	− 0.69
Iron concentrations and production of mycobactins	− 0.52	− 0.81	− 0.70	− 0.83
Release of exochelins and production of mycobactins	+ 0.97	+ 0.94	+ 0.98	+ 0.95

All the correlation co-efficients were statistically significant ($P<0.01$; $H_0=0$). There was a strong linear association between the growth of the bacilli and concentrations of iron in the medium with the correlation co-efficients exceeding 0.90. The release of exochelins and the production of mycobactins were negatively correlated with iron concentrations but the association was not as strong as that observed with drycell-weight. The release of exochelins seemed to parallel the production of mycobactins.

These findings suggest that exochelins and mycobactins are essential under iron-deficient conditions (0 and 0.02 mcg/ml) for sequestration of iron. The decrease of the concentrations of these compounds with increasing concentrations of iron demonstrates that iron is taken up passively under iron-rich conditions. The virulent H37Rv appeared to be more capable of sequestering iron and thereby registering a higher growth due to its ability to synthesize greater amounts of both siderophores. Interestingly, the behaviour

of the low virulent strain appeared to be similar to that of the avirulent strain with respect to growth and the production of exochelins and mycobactins.

The South Indian low virulent strain of *M. tuberculosis* has been shown to be the causative organism of pulmonary tuberculosis in a large proportion of South Indian patients. No biochemical differences have so far been demonstrated between this strain and the virulent *M. tuberculosis* H37Rv strain. Iron has been shown to influence the virulence of a number of organisms such as *E. coli*. Findings reported here suggest that the ability of SILV to sequester iron is lower than that of the standard virulent strain, and this factor may possibly be responsible for the relatively low virulence of this strain.

Ii Log viable counts: In another experiment, the effect of increasing concentrations of iron on the multiplication, as assessed by the log viable counts, of the 4 strains of mycobacteria was examined. The organisms were initially grown (4 days for *M. smegmatis* and 2 weeks for the *M. tuberculosis* strains) in synthetic liquid media containing iron in concentrations ranging from 0 to 4 mcg/ml. They were then inoculated in 6 different concentrations (neat and in 5 dilutions ranging from 1 in 10 to 1 in 10,000) of the suspension of growth obtained above on to the L J slopes. The bacterial growth (log VC) was measured by counting the colonies following incubation at 37°C for 4 days for *M. smegmatis* and for 4 weeks for the three *M. tuberculosis* strains. The results (means of 6 estimates) are presented in the following table.

Strain	Mean log viable counts and standard deviations with initial growth in the presence of the following concentrations of iron (mcg/ml)					
	0	0.02	0.1	0.5	2.0	4.0
M. smegmatis	3.00 ± 0.27	3.84 ± 0.03	4.22 ± 0.20	5.27 ± 0.33	6.69 ± 0.37	7.90 ± 0.19
$H_{37}Ra$	2.96 ± 0.35	3.33 ± 0.21	4.36 ± 0.27	4.61 ± 0.16	5.41 ± 0.43	5.86 ± 0.19
$H_{37}Rv$	3.26 ± 0.14	3.64 ± 0.14	4.50 ± 0.19	4.92 ± 0.2[illegible]	5.72 ± 0.0[illegible]	6.89 ± 0.17
SILV	3.16 ± 0.22	3.40 ± 0.07	4.52 ± 0.22	5.10 ± 0.19	5.89 ± 0.06	5.93 ± 0.10

As seen with the dry cell-weight in the previous experiment, there was a significant increase ($P<0.001$) in the log VC of all the 4 strains following prior incubation with increasing concentrations of iron in the synthetic medium, the growth of the saprophytic *M. smegmatis* being substantially higher than with any of the *M. tuberculosis* strains. The increase in log VC was positively correlated with the increase in iron concentrations, the correlation co-efficients being 0.93 for *M. smegmatis*, 0.83 for H37Ra, 0.92 for H37Rv and 0.79 for the SILV strain ($P<0.01$ for all). The increase observed with the virulent H37Rv strain was significantly greater than with either the avirulent or the SILV strains ($P<0.001$). In contrast to the observation in the previous experiment, the growth of the SILV strain was significantly greater than that of the H37Ra strain ($P<0.001$). These findings demonstrate that the viability of the four strains is also enhanced by increasing concentrations of iron in the medium, and that it was greater with the pathogenic strains of *M. tuberculosis* (H37Rv and SILV) than with the avirulent H37Ra strain.

III Exochelins and uptake of iron by mycobacteria : Findings presented so far have shown that there is an increase in the growth of mycobacteria with increasing concentrations of iron in the medium and that the concentrations of exochelins and mycobactins, which are highest under iron-deficient conditions, register a marked decrease. It does not, however, follow that exochelins (and mycobactins) are involved in the uptake of iron by mycobacteria. An investigation was therefore undertaken to study the uptake of iron by the four strains of mycobacteria in the absence and in the presence of exochelins released by these strains. Two sets of flasks, each containing iron in a concentration of 15 mcg/ml were set up for each strain; one was used as a control and to the other was added exochelins (isolated from the same strain used for inoculation) at a concentration of 5 mcg/ml. The flasks were incubated at 37°C, and the residual iron concentrations in the media were determined, employing an atomic absorption spectrophotometer (flame system), at 2, 4, 6 and 8 days after inoculation with *M. smegmatis*, and at 1, 2, 3 and 4 weeks after inoculation with the three *M. tuberculosis* strains. The iron taken up by the bacilli was calculated as the difference between the initial concentration (15 mcg/ml) and the residual iron concentration. The mean and standard deviation of 6 replicates on each occasion are presented in the table on the page 84.

Addition of exochelins to media inoculated with *M. smegmatis* caused a significant increase in the iron uptake over the respective control values on all the 4 occasions ($P=0.03$ for 2nd day and 0.001 for the other 3 occasions). There was an appreciable increase in the uptake of iron in the presence of exochelins on all the four occasions with H37Ra and H37Rv ($P<0.01$ for all). With the SILV strain, however, the differences were not significant upto the 3rd week ($P\leq0.07$), and it was only at the 4th week that the increase over the respective control value attained statistical significance ($P<0.001$).

Strain	Addition to medium	Mean ± standard deviation of iron uptake (mcg/ml) at the following intervals after inoculation.			
		2nd day	4th day	6th day	8th day
M. smegmatis	None (control)	0.03±0.48	1.92±0.42	2.03±0.33	2.33±0.78
	Exochelin	1.48±0.51	4.32±0.33	5.75±0.27	6.17±0.16
M. tuberculosis strains		1st wk	2nd wk	3rd wk	4th wk
H37Ra	None	0.35±0.54	0.83±0.50	0.92±0.38	1.77±0.63
	Exochelin	1.33±0.62	2.27±0.55	5.23±0.36	5.78±0.24
H37Rv	None	0.42±0.51	0.82±0.56	1.70±0.74	2.50± 0.63
	Exochelin	2.43±0.25	3.71±0.42	5.48±0.29	6.02±0.29
SILV	None	0.55±0.43	0.95±0.43	1.30±0.52	2.02±0.31
	Exochelin	0.63±0.45	1.35±0.59	2.65±1.66	4.98±0.43

These findings suggest that exochelins are involved in the uptake of iron by mycobacteria. Investigations undertaken earlier have shown that the SILV strain produces exochelins and mycobactins, even though the concentrations of these two siderophores are lower than those produced by the virulent H37Rv strain. Whether the relatively low iron uptake even in the presence of added exochelins is related to the expression of receptor proteins (iron-regulated envelope proteins) on the mycobacterial envelope needs to be investigated.

IV Effect of hemoglobin on the growth and production of exochelins and mycobactins by mycobacteria

Hemoglobin is an iron-containing protein and is known to support the growth of several bacterial species. There is little or no information on the capacity of mycobacteria to survive and proliferate in the presence of hemoglobin as the source of iron. Investigations were therefore undertaken to study the growth *in vitro* of 4 strains of mycobacteria, and the production of exochelins and mycobactins by these strains in the presence of hemoglobin. The findings were compared with those obtained in the presence of equivalent concentrations of iron in the medium.

The concentration of hemoglobin to be added to the medium in lieu of iron was calculated on the basis of its molecular weight (64,500) and its capacity to bind 4 atoms of iron per mole. The synthetic medium was prepared as described earlier and iron was added in concentrations ranging from 0 to 4 mcg/ml to one set of flasks and hemoglobin was added in

Strain	Source of iron	Mean cell dry-weight (g/50 ml medium) in the presence of the following concentrations of iron (mcg/ml)					
		0	0.02	0.1	0.5	2.0	4.0
M. smegmatis	Free iron	0.102	0.116	0.411	0.795	1.150	1.890
	Hemoglobin		0.121	0.482	0.886	1.027	1.805
H37Ra	Free iron	0.033	0.051	0.082	0.158	0.309	0.452
	Hemoglobin		0.053	0.096	0.199	0.358	0.528
H37Rv	Free iron	0.049	0.058	0.110	0.261	0.411	0.526
	Hemoglobin		0.081	0.128	0.317	0.510	0.636
SILV	Free iron	0.030	0.052	0.097.	0.141	0.269	0.411
	Hemoglobin		0.063	0.115	0.170	0.342	0.440

concentrations of 5.8, 29, 145, 580 and 1160 mcg/ml (corresponding to the free iron concentrations from 0.02 to 4 mcg/ml set up above) to another set. The means (of 4 estimates) of cell dry weight and the concentrations of exochelins and mycobactins are presented in the tables above and below and page 86.

Strain	Source of iron	Mean exochelin concentrations(mg/g dry weight of cells) in the presence of the following concentrations of iron (mcg/ml)					
		0	0.02	0.1	0.5	2.0	4.0
M. smegmatis	Free iron	15.54	18.15	5.45	3.38	2.50	2.37
	Hemoglobin		20.01	9.27	7.24	3.23	2.75
H37Ra	Free iron	30.41	39.31	24.48	15.81	11.29	9.40
	Hemoglobin		43.65	28.58	17.58	14.00	10.30
H37Rv	Free iron	40.67	34.82	16.28	12.76	12.76	10.94
	Hemoglobin		53.03	35.05	21.27	15.68	13.10
SILV	Free iron	33.98	32.90	20.66	14.22	10.01	9.11
	Hemoglobin		44.21	26.14	20.52	13.11	10.80

As with free iron, there was a significant increase ($P<0.01$) in the growth of all strains of mycobacteria in the presence of increasing concentrations of hemoglobin as the source of iron. The mean cell dry-weight was higher with hemoglobin than with free iron at all concentration levels in the three *M. tuberculosis* strains ($P<0.01$) the differences ranging from 4 to 26% with H37Ra, 16 to 40% with H37Rv and 7 to 27% with the SILV. With *M. smegmatis,* the growth in the presence of hemoglobin was higher only at concentrations of 0.1 and 0.5 mcg/ml ($P<0.01$ for both), the over-all difference

in the increase in the cell dry-weight between 0.02 and 4 mcg/ml between free iron and hemoglobin was however not significant ($P > 0.2$).

Strain	Source of iron	Mean mycobactin concentrations (mg/g dry weight of cells) in the presence of the following concentrations of iron (mcg/ml)					
		0	0.02	0.1	0.5	2.0	4.0
M. smegmatis	Free iron	45.79	47.28	17.02	14.61	10.42	9.52
	Hemoglobin		65.91	25.94	18.08	15.17	11.49
H37Ra	Free iron	44.12	63.51	42.49	34.87	24.24	20.50
	Hemoglobin		75.79	49.52	38.97	29.32	25.06
H37Rv	Free iron	70.81	82.16	54.24	30.62	24.94	22.36
	Hemoglobin		89.82	62.34	37.80	28.97	26.26
SILV	Free iron	50.42	60.30	41.42	35.56	27.06	20.67
	Hemoglobin		64.65	49.89	41.03	30.68	23.89

The release of exochelins was also higher in the presence of hemoglobin than in that of free iron with all the four strains, and as with free iron, there was a decrease with increasing concentrations of hemoglobin iron in the medium. In the three *M. tuberculosis* strains, the differences between free iron and hemoglobin ranged from 10 to 24% with H37Ra, 20 to 52% with H37Rv and 19 to 44% with the SILV; the differences in the mean release of exochelins at the different concentrations were significant with the H37Rv and the SILV strains ($P = 0.05$), but not with the H37Ra strain. Corresponding to the significant differences observed in the dry cell weight at 0.1 and 0.5 mcg/ml concentrations, the release of exochelins at these two concentations was also significantly higher ($P < 0.01$) in the presence of hemoglobin with *M. smegmatis*.

The production of mycobactins was also significantly higher in the presence of hemoglobin than in that of free iron, the differences ranging from 12 to 22% with H37Ra, 8 to 23% with H37Rv, 7 to 20% with the SILV and 21 to 52% with *M. smegmatis*. There was a suggestion that hemoglobin induced the production of exochelins more than it did of mycobactins in the H37Rv and the SILV strains.

In the presence of hemoglobin, the growth and the release of exochelins was significantly higher with the virulent H37Rv strain than in either the avirulent or low virulent strains ($P \leq 0.02$). The production of mycobactins was also higher with the H37Rv strain than in the two strains; the difference was however significant ($P = 0.03$) only between H37Rv and the H37Ra strains.

These findings demonstrate that hemoglobin iron supports the growth of mycobacteria as well if not slightly better than free iron. There is an increase

in the production of siderophores, particularly that of the exochelins in the two pathogenic strains (H37Rv and the SILV), which is possibly responsible for the higher rate of growth observed in the presence of hemoglobin.

V Effect of iron-binding proteins : Most of free iron in the body is bound to iron-binding proteins such as transferrin (in serum), lactoferrin (in milk and other secretions and also macrophages) and ferritin (in liver and other tissues). To survive, the invading pathogens have to sequester iron from these proteins. An investigation was therefore undertaken to study the effect of two of these proteins namely, transferrin and lactoferrin on the growth and production of siderophores by mycobacteria in vitro.

The molecular weight of these two proteins is about 80,000 and one mole of each of these proteins binds with 2 atoms of iron. Three sets of flasks were set up for each strain; one flask contained iron in a concentration of 0.1 mcg/ml in the medium, the second, iron 0.1 mcg/ml plus transferrin 80 mcg/ml and the third, iron 0.1 mcg/ml plus lactoferrin 80 mcg/ml. The concentrations of proteins added is slightly more than adequate to bind the free iron. The dry cell-weight and the concentrations of exochelins and mycobactins produced (means of 4 estimates) are presented in the table below.

Strain	Addition to the medium	Cell dry-weight (g/50 ml medium)	Exochelin concentration (mg/g dry weight)	Mycobactin concentration (mg/g dry weight)
M. smegmatis	Iron only	0.419	7.74	17.91
	Iron + Transferrin	0.237	14.80	33.02
	Iron + Lactoferrin	0.265	14.16	28.35
H37Ra	Iron only	0.085	23.65	47.30
	Iron + Transferrin	0.036	55.67	55.67
	Iron + Lactoferrin	0.045	44.48	60.47
H37Rv	Iron only	0.120	27.14	54.32
	Iron + Transferrin	0.048	41.89	72.55
	Iron + Lactoferrin	0.057	35.58	71.16
SILV	Iron only	0.086	17.23	38.03
	Iron + Transferrin	0.032	31.67	63.36
	Iron + Lactoferrin	0.037	27.48	54.96

There was a decrease in dry-cell weight ranging from about 43% with *M. smegmatis* to about 63% with the SILV strain in the presence of transferrin ($P<0.001$ for all). In contrast, there was an appreciable increase in the concentrations of the exochelins released with the increase being maximum (about 135%) with the avirulent H37Ra strain and about 54% with the virulent H37Rv strain ($P<0.01$ for all strains). There was a significant increase in the concentrations of mycobactins also with all the 4 strains ($P<0.01$); the difference was, however, minimal with the H37Ra strain (about 18%).

A similar pattern of decrease in the dry-cell weight ($P<0.001$) and an increase in the synthesis of exochelins ($P\leq0.02$) or mycobactins ($P\leq0.06$) was also observed in the presence of lactoferrin. There was a suggestion that the inhibition in growth and the increase in the release of exochelins was slightly less with lactoferrin than with transferrin, particularly with the three *M. tuberculosis* strains, while the production of mycobactins was fairly similar.

Transferrin and lactoferrin have high association constants for ferric iron (10^{36}). Each is capable of reversibly binding two ferric ions and are usually only partly saturated with iron, transferrin being 25-30% saturated and lactoferrin about 55% saturated. In contrast to transferrin, lactoferrin binds iron tightly at low pH values (4.0 to 6.5), and this protein may have a role in the withholding of iron from invading microorganisms such as tubercle bacilli in an intra-cellular environment such as within the macrophages. The experiments reported here were carried out at a pH of 6.8 and as such it is possible that the exochelins might more readily sequester iron from lactoferrin than from transferrin, and this is reflected in the slightly higher growth (and lower synthesis of siderophores) observed in the presence of lactoferrin than in that of transferrin.

Despite an appreciable increase in the production of the siderophores, particularly the exochelins, the growth is only about 50% of that obtained in the medium containing iron only with all the four strains of mycobacteria. For the exochelins to be able to sequester iron from these iron-binding proteins, they must have association constants for iron greater than those of these proteins. Mycobacteria produce a number of exochelins; these have not been characterised and their association constants for iron are yet to be determined. Investigations to identify, characterise and to determine the association constants of these exochelins for iron are in progress.

(started: 1987 completed: 1989)

Development of an assay for studying the mycobactericidal potential of human monocyte derived macrophages

Earlier work at the Centre (see annual report, 1988) suggested that seven day old human peripheral blood monocyte derived macrophages (MDM) were

not able to cause inhibition of multiplication of intracellular *M.tuberculosis*, even after stimulation with autologous PPD induced T-cell factors. In order to find out whether these macrophages are capable of other functions like hydrogen peroxide (H_2O_2) production and killing of organisms other than mycobacteria, the staphylocidal assay was carried out. The macrophages: *S.aureus* ratio was similar to the previous experiment (1:2). The viability of intracellular *S.aureus* two and four hours after phagocytosis was determined by plating the macrophages lysate in nutrient agar at suitable dilutions and the readings were taken after 24 hrs.

It was found that MDM, whether stimulated with autologous T-lymphocyte supernatants or unstimulated, caused killing of the intracellular organisms as there was a decrease in the colony count at the end of 4 hrs.

The results of these experiments suggest that staphylocidal activity was well retained by seven day old MDM. This implies that macrophages are functional but are incapable of killing mycobacteria as reported in the previous year.

(started: 1986; completed: 1989)

Effect of lymphocyte factors on the production of hydrogen peroxide by human monocyte derived macrophage

When blood monocytes are maintained in culture, they come to resemble tissue macrophages by about the 4th day of culture. These macrophages are suitable for various macrophage function studies. Culture supernatants of lymphocytes stimulated with antigen or mitogen are known to contain macrophage activating factors.

The capacity of these supernatants to activate macrophage, as assessed by their H_2O_2 production, was studied. Mononuclear cells from ten bacteriologically proved pulmonary tuberculosis patients and ten blood bank donors were stimulated with 10mcg/ml of PPD for 48 hrs; supernatants were harvested and stored at -20°C.

MDM from three laboratory volunteers were maintained in culture and were stimulated with the lymphocyte supernatants (as mentioned above) from the 4th to the 6th day. H_2O_2 was estimated on the seventh day. The results of nmol of $H_2O_2/10^5$ MDM after stimulation with lymphocyte supernatants are shown as Mean ± S.D. in the top table on page 90..

It was observed that there was practically no difference between the tuberculous and the healthy control group in the capacity of stimulated MDM to produce H_2O_2. Besides, the individual lymphocyte supernatants did not show difference in their macrophage activating potential.

Volunteer	Tuberculous(10)	Control(10)
I	6.7 ± 2.2	6.1 ± 1.3
II	6.0 ± 1.2	4.6 ± 1.4
III	4.6 ± 1.1	3.8 ± 0.8

(started: 1989; completed: 1989)

Hydrogen peroxide release by OKIa1 (anti DR-monoclonal antibody) resistant alveolar macrophages in tuberculosis

Alveolar macrophages are the mononuclear phagocyte population lining the alveoli of the lung. These cells are mainly involved in killing and eliminating the pathogens. One of the killing mechanisms is the respiratory burst resulting in the release of toxic metabolites. The capacity of these cells to produce reactive oxygen intermediates upon stimulation (respiratory burst) is probably related to the cellular capacity for microbicidal function which represents one of the host defense functions against intracellular pathogens.

It is well known that HLA-Class II antigens (human leucocyte antigen - Class II antigens/immune associated antigens) such as HLA-DR, DP & DQ are mainly involved in immune function. Expression of Class II antigens is restricted to certain cells such as macrophages, monocytes, bone-marrow derived B-lymphocytes and activated T-lymphocytes. Attempts were made to find out the hydrogen peroxide releasing potential of HLA-DR positive and HLA-DR negative alveolar macrophages.

		H_2O_2 in nmols per 0.1×10^6 cells	
		Alveolar cells	Blood monocytes
Active TB.	1.	2.0	8.2
	2.	6.2	10.1
	3.	12.3	5.1
	4.	3.9	7.9
	5.	3.8	4.7
	6.	2.0	4.9
Inactive TB (treated)	7.	4.0	12.0
	8.	2.9	4.5
Other lung diseases	9.	10.8	27.1
	10.	2.0	10.3
Normal	11.	1.3	18.2

In the present study, phorbol-myristate acetate (PMA) triggered hydrogen peroxide (H_2O_2) producing potential of alveolar macrophages and the cor-

responding blood monocytes were compared. Further, the H_2O_2 producing potential of an alveolar macrophage population, which was resistant to OKIa1 (anti-DR monoclonal antibody in ascites form) monoclonal antibody was studied. The finding may be useful to understand the role of OKIa1 resistant alveolar macrophages in their microbicidal and immunological functions to the intracellular pathogens such as *M.tuberculosis.*

The alveolar macrophages of healthy subjects and patients with active tuberculosis, inactive tuberculosis, and non-tuberculous lung disease, produced less PMA induced hydrogen peroxide when compared to the corresponding blood monocytes as shown in the bottom table on page 90.

The alveolar macrophages that were susceptible to OKIa1 monoclonal antibody and complement ranged from 15 to 86%. The susceptibility is expressed as per cent cytotoxicity. The alveolar macrophage population which was resistant to OKIa1 monoclonal antibody and complement treatment produced more hydrogen peroxide than the control ascites (raised against SP 2/0 myeloma cells) and complement treated population and the cells which were not subjected to any treatment (see table below).

			H_2O_2 in nmols/ 0.1×10^6 Alveolar macrophages		
		% Cyto-toxicity	OKIa1 anti-body and complement treated	Control ascites and complement treated	Alveolar cells without treatment
Active TB	1.	86.1	7.0	2.7	2.4
	2.	44.3	5.3	3.9	3.8
Inactive TB (treated)	3.	42.1	9.0	3.5	2.0
	4.	52.5	15.0	5.0	3.5
	5.	37.5	13.6	8.0	4.0
	6.	45.4	7.9	6.7	4.4
	7.	15.0	10.6	6.3	3.9
Other lung diseases	8.	58.0	8.7	6.8	--
	9.	62.9	17.0	4.3	2.1
Normals	10.	60.0	2.2	1.3	1.3
	11.	47.0	8.7	4.3	2.8

On the other hand, peripheral blood monocytes which were resistant to OKIa1 monoclonal antibody produced similar levels of hydrogen peroxide (after stimulation with PMA) compared to monocytes treated with control ascites and complement (see table on page 92 for the levels in 4 subjects). More than 90% of the blood monocytes were resistant to OKIa1 monoclonal antibody and complement treatment.

Subject	Category	H_2O_2 in nmols/0.1 X 10^6 blood monocytes	
		Control ascites + Complement	*OKIa1 antibody + Complement
1.	Normal	18.5	19.8
2.	Active TB	8.2	7.0
3.	Non-TB	10.3	7.8
4.	Non-TB	7.0	7.5

* Per cent peripheral blood monocytes susceptible to OKIa1 monoclonal antibody and complement were 6-10%.

The present study reveals that alveolar macrophages are less efficient in their hydrogen peroxide producing potential than the blood monocytes. Further, alveolar macrophages that are resistant to OKIa1 (anti-HLA-DR monoclonal antibody) and complement may be a newly recruited monocyte-macrophage lineage not expressing a detectable level of Ia antigens on their surface. It will be useful to study the role of these cells in their microbicidal and immunological functions against intracellular pathogens such as *M.tuberculosis.*

(started: 1988; completed: 1989)

STUDIES IN PROGRESS

Cold staining method in comparison with the Z-N method for tubercle bacilli in sputum smears

A cold staining (CS) method described by Vasantha Kumari and others (Bull WHO - *64*, 741-743, 1986) for sputum smears was compared with the Ziehl-Neelsen (Z-N) method in a controlled comparison of 100 paired sputum smears and the findings reported in the 1988 annual report. A further 306 paired samples were examined in the same manner. It was decided to assess the intensity of colour of the bacilli achieved by the CS method, in order to see whether it affected the detection and grading of positives. The two methods were identical except for the heat applied to the stain in the Z-N method. Up to 10 minutes were taken to examine the smears and grade the positivity. For the purpose of analysis in this report, positives were graded into two categories: $<1+$ indicating less than 100 bacilli and $\geq 1+$ indicating 100 or more bacilli. The intensity of colour of the bacilli was recorded by giving a score of 2 for well-stained deep pink bacilli and a score of 1 for those stained pale pink.

The comparison of positivity obtained with the two methods is tabulated below:

CS method	Z-N method		All
	Negative	Positive	
Negative	128	27	155
Positive	18	133	151
All	146	160	306

There was 85% agreement between the two methods as regards the detection of positives. There were 18 specimens which were reported to be positive by the CS method but negative by the Z-N method, compared with 27 specimens positive by the Z-N method but negative by the CS method, the difference not being statistically significant (McNemar's test, $P>0.2$).

However, there was a highly significant difference between the two methods in the distribution of the scores given for intensity of colour of the bacilli (see top table on page 94)

	Colour Score	Z-N method 2	1	0*	Total
Cold method	2	112	0	7	119
	1	20	1	11	32
	0*	22	5	0	27
Total		154	6	18	178

*Smears negative by one method and positive by the other

Among 178 smears positive by either method, 154 had a score of 2 by the Z-N method as compared to 119 by the cold method ($p<0.001$). This shows that the cold staining method left many bacilli faintly stained.

Therefore analyses were done to examine how the intensity of colour affected the grading and detection of positives.

Cold method		Z-N method Positivity Grade			
Colour score	Positivity grade	Neg.	<1+	≥1+	All
Nil	Neg	0	22	5	27
1	<1+	11	4	8	23
	≥1+	0	2	7	9
		11	6	15	32
2	<1+	6	17	11	34
	≥1+	1	6	78	85
		7	23	89	119
	Total	18	51	109	178

It can be seen from the above table that only 9(28%) of the 32 smears with a colour score of 1 by cold method had a grade 1+ or more compared to 85 (71%) of the 119 smears with a colour score of 2 ($P<0.001$). Thus the grade of positivity was significantly lower among the smears which were poorly stained. In addition, significant association was also observed between the grades by the Z-N method and colour score by the cold method, the corresponding figures being 15(47%) of 32 and 89(75%) of 119 ($P<0.01$), respectively.

It may be concluded that the CS method was similar to the Z-N method in the detection of positive smears, though the Z-N method gave a higher proportion of positives graded as 1+.

(started:1989; completed:1989)

Fluorescein diacetate - Ethidium bromide staining method for viability of acid fast bacilli in sputum smears.

A staining method using Fluorescein diacetate (FDA) and ethidium bromide (EB) was described by Kvach and Veras as being useful in differentiating live and dead *M.leprae* in smears (International Journal of Leprosy 1982, *50*, 183-192). According to them the live bacteria stain green and dead ones red. Determination of viability of tubercle bacilli from smear examination without having to wait for culture is of particular value for patients on treatment, specially those on SCC, who go through a smear-positive culture-negative phase before conversion. It would help decisions regarding change or extension of treatment regimens.

In order to assess the usefulness and reliability of the method, three investigations were carried out. First, a number of non acid- fast bacteria were tested by the staining method in order to see how the presence of bacteria would interfere with the reading of the sputum smears.

Bacteria tested	No. of strains	Colour of stained bacteria	Hanging drop	Growth on sub-culture
Pneumococcus	1	Red		+
Staph. aureus	3	Red		+
Beta streptococci	2	Red		+
Ps. pyocyanea	4	Red	Motility +	+
Proteus species	2	Red	Motility +	+
E.coli	5	Red		+
Klebsiella	2	Red*		+
S.typhi	2	Red	Motility +	+
V.cholerae	1	Red	Motility +	+

* An occasional green bacillus was also seen.

It is seen from the table above that none of the non-acid fast bacteria tested took the green fluorescence of the FDA even though they were viable as confirmed by growth on subculture, and by motility in hanging drop preparation in the case of motile bacteria. All these bacteria took up the

red stain of ethidium bromide, showing that the live bacilli taking the green stain of FDA only and the dead ones staining red with EB is a phenomenon probably peculiar to the mycobacterial species. Mycobacterial culture suspension when stained with FDA/EB had green and red stained bacilli, the proportion varying according to the age of the culture.

Secondly, 182 samples of sputum were examined by the FDA/EB staining method. The processed deposits of sputum were used because sputum as such could not be stained directly (see 1988 annual report). Smears containing green fluorescent bacilli were called FDA positive.

FDA result	Routine smear and culture result				Total
	Positive by both	Negative by both	Smear pos. Cult. neg.	Smear neg. Cult. pos.	
Positive	100	1	6	0	107
Negative	20	47	5	3	75
Total	120	48	11	3	182

From the above table it can be seen that out of 182 samples, 131 were positive by routine smear, 123 by culture and 107 by the FDA method. Among them 100 were positive and 47 negative by all three methods. Twenty-three samples which were positive by culture, failed to show any FDA stained bacilli on smear, showing that the latter method was less sensitive than the culture method. These negatives occurred in similar proportions in all grades of culture positives (7 with 2+, 9 with 1+, and 7 with less than 20 colonies). Eleven samples were negative by the culture method but positive on routine smear, and it was this group that was suspected to have non-viable bacilli. By FDA method, 5 of them were negative and 6 were positive of which 5 smears had less than 5 bacilli in each. Unlike the routine direct smear, the smear for FDA is made from concentrated deposit of the sputum and the results are therefore not strictly comparable. Larger numbers of smear-positive culture-negative samples would yield more precise information on the specificity of the FDA/EB staining method.

The third part of the investigation was to relate the numbers of bacilli seen in the smear with actual viable count set up on L-J medium from the same deposit. Identical quantity of material (10 mcl) was used to make a smear and to inoculate a slope. The count on L-J medium was set up in 2 or 3 slopes whereas the smear count was based on a single smear. The smear count was invariably an under-estimate as it is not possible to ensure that the entire smear is examined. Even so, in 7 of 8 samples tested, the difference in the Log_{10} viable count per ml was less than one.

Thus the FDA/EB method of staining is useful in assessing viability of tubercle bacilli and the results are available immediately unlike culture methods

which take several weeks to yield results. However, a negative result does not indicate non-viability as the FDA/EB method is less sensitive than the culture method.

(started:1989; completed:1989)

Evaluation of bioluminescence assay for the estimation of viable tubercle bacilli

This study was undertaken to evaluate the bioluminescence assay of adenosine triphosphate(ATP) and to compare it with the standard method of viable counting. For this purpose, 32 cultures of *M.tuberculosis* stored at 4°C (30 for 6 months and 2 for 2 months) were used. Standard suspensions of these cultures were made and 1/10 dilutions were prepared according to the standard procedure. All these were coded before testing and the following estimations were done:

1. The total number of bacilli was estimated using Thoma counter.
2. Viable count was estimated by the standard method of viable counting on 7H11 plates incubated in CO_2 incubator.
3. Viable count was done by bioluminescence assay of ATP using Lumac Biocounter M2010A and reagents and procedures supplied by Lumac. In order to rule out contamination, these suspensions were first plated onto Muller-Hinton Agar and none of them were found to be contaminated. The results obtained employing both these methods are given in the table below.

Conventional method CFU/ml	ATP assay (RLU values)					
	$\geq 10^7$	10^6-	10^5-	10^4-	10^3-	Total
$\geq 10^6$	1	1				2
10^5 -		1	1			2
10^4 -			1	4		5
10^3 -		1	4	1	1	7
10^2 -		1	6	3		10
10^1 -		1				1
Negative		1		4		5
Total	1	4	14	12	1	32

All the cultures showed $\geq 10^7$ bacilli/ml as estimated by Thoma counter.

Viable counts estimated by ATP assay were found to be equal to or more than that estimated by conventional method. While 16 cultures showed $<10^3$ viable count by conventional method, all the cultures tested showed $\geq 10^3$ viable count by ATP assay.

In the conventional method, the cultures are incubated for 1 month, and are exposed to the problems of contamination and drying up. Low viable counts in the 16 cultures as estimated by conventional method could be due to these problems. These results suggested that bioluminescence assay can also be used in the estimation of viable tubercle bacilli; its main advantage is the availability of the result on the same day. It is proposed to employ this assay for estimating drug sensitivity of *M.tuberculosis* cultures.

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

Consistency of bacteriocin typing of M.tuberculosis cultures

Bacteriocin typing of *M.tuberculosis* cultures with a set of 9 indicator strains of rapidly growing non-tuberculous mycobacteria yielded 16 types. Further, to check the reproducibility and consistency of this typing, pretreatment cultures of some patients were screened. The cultures of only 5 out of 10 patients screened showed consistent results (annual report, 1988). So it was proposed to screen 2 pretreatment cultures from each of 50 patients, in order to assess the variability betwen cultures from the same patients in bacteriocin typing.

The number of viable organisms in a culture shows a decreasing trend during storage at 4°C. The inconsistency of bacteriocin typing observed in cultures of 5 patients may be attributed to this problem. So, the pretreatment cultures of patients, which had been stored at 4°C, are being subcultured on to 7H9 medium to bring them to the log phase. Later, they are being screened for the production of bacteriocin. The study is in progress.

(started:1988; expected year of completion: 1990)

Biological characterisation of M.avium-intracellulare-scrofulaceum isolates

There is a high prevalence of non-tuberculous mycobacteria (NTM) in the BCG trial area of South India. In 1981, NTM were isolated from 8.6% of 16907 sputum specimens collected from subjects belonging to the trial area. About one-third of the NTM isolated from sputum specimens in this area belonged to the *M.avium-intracellulare-scrofulaceum* (MAIS) complex. It has been shown by various workers that prior exposure to NTM has a modulatory effect on the immunity produced by BCG vaccination. For these reasons, it becomes important to obtain a biological profile of MAIS strains isolated in this area and to identify useful biological markers.

At present, MAIS isolates are differentiated from other NTM, and identified to species level based on a battery of tests. Falkinham et al. (Can. J. Microbiol. 1986, *32* : 10) have proposed a medium for the selective isolation and enumeration of MAIS, based upon the ability of these mycobacteria to utilise Tween 80 as the sole source of carbon, and grow them optimally at pH 5.5. Such a medium would simplify the identification protocol currently being followed. Further, on studying human and environmental isolates of MAIS, Falkinham et al. found that the clinical and environmental isolates differed significantly in the ability to grow on Middlebrook's 7H11 medium without enrichment, to grow at 43°C, and in the resistance to streptomycin, gentamycin, D-cycloserine, mercury or cadmium.

From the strains of NTM isolated from clinical specimens, about 100 have been selected. The growth characteristics of these strains and standard strains of *M.tuberculosis* H37Rv, H37Ra, *M.bovis* BCG, *M.avium intracellulare, M.scrofulaceum, M.kansasii, M.gordonae, M.phlei, M.fortuitum, M.microti, M.simiae, M.chelonei* and *M.vaccae* have been studied with respect to smooth or rough and transparent or opaque colony morphology, ability to grow on 7H11 medium without enrichment, at 43°C and on Falkinham's medium.

These strains will be identified using the standard procedures employed in our laboratory. Following this, susceptibility of these strains to drugs and heavy metal salts like streptomycin, kanamycin, gentamycin, D-cycloserine, rifampicin, ethambutol, pyrazinamide, HgCl and CdCl will be determined. Catalase will be extracted from these strains and subjected to heat susceptibility test. The strains will also be screened for the presence of plasmids.

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

Adenosine deaminase (ADA) levels in CSF of tuberculous meningitis (TBM)

ADA level in cerebrospinal fluid (CSF) was reported to be a useful diagnostic aid for TBM (Journal of Infectious Diseases *155,* 603, 1987). ADA (EC 3.5.4.4) is an enzyme that catalyses the deamination of adenosine into inosine and ammonia. This enzyme is secreted by T-lymphocytes and could be considered as a marker of T-lymphocyte turnover. Hence a study was initiated (see annual report 1988) to estimate the ADA levels in CSF of children with TBM.

The ADA levels in CSF of 23 culture positive TBM, 24 with culture negative but clinically diagnosed TBM and 9 non-tuberculous meningitis (non-TBM) patients are presented in the table on page 100.

The value of ADA (mean ± SD) in healthy subjects in CSF was reported as 0.4±0.6 U/litre. (Journal of Infectious Diseases, *155,* 1987). It can be seen from the table on page 100 that ADA levels varied from 0 to ≥10.0 U/l in both culture-positive and culture-negative TBM groups. In one of the non-Tb meningits

ADA (U/l)	TBM		Non-TBM
	Culture positive	Culture negative	
0.0	3	8	5
0.5-	3	11	3
2.0-	7	2	0
4.0-	4	0	0
6.0-	3	2	0
8.0-	2	0	1
≥10.0	1	1	0
Total	23	24	9
Mean ±SD	4.2±2.9	1.9±2.8	1.7±2.9
Range	0 - 11.5	0 - 12.9	0 - 9.9

cases the level was 9.9 U/litre. In published reports from abroad the mean ADA levels in CSF of TBM ranged from 8 to 14 U/litre. As the ADA levels of Indian TBM patients are not known, and in view of the low mean value of 4.2 U/litre, further investigations on the sensitivity of the assay procedures are required.

(started:1988; expected year of completion: 1990).

Adrenocortical function in tuberculous patients

A significant increase was observed in plasma cortisol levels at 1 week after the start of treatment with daily regimens that did or did not contain rifampicin in patients with pulmonary tuberculosis. This was followed by a decrease at 4 weeks, the decrease being more marked with a non-rifampicin regimen than with that containing rifampicin (see page 59). An enlargement of the infected tissue such as the lymph glands in patients with TB lymphadenitis and the lesions in the brain in patients with brain tuberculoma has been observed soon after the start of treatment. A similar phenomenon might occur in the adrenals too, as a consequence of which these glands could be hypertrophied resulting in an increased secretion of cortisol. The enlargement, if any, of the adrenals is being studied in about 15 patients with pulmonary tuberculosis before and at 1 week and 4 weeks after the start of treatment with daily regimens containing rifampicin, employing ultrasonography. The synacthen stimulation test is also being undertaken at the 3, time-points.

Further, the effect of rifampicin alone on the endogenous cortisol levels could not be established as the patients were under stress on account of

the disease resulting in an increase, and also on account of the probable infiltration of the adrenals by the tubercle bacilli which could cause a decrease in the cortisol output. It is proposed to obtain this information from about 15 healthy subjects before and after 1 week of rifampicin 600 mg administered daily. The ultrasonography of the adrenals will also be undertaken in these subjects at the two time-points.

(started: 1989; expected year of completion: 1991)

Further studies on the iron-sequestration mechanisms of mycobacteria

(a) **Characterization of the siderophores of mycobacteria:** Investigations undertaken earlier have shown that mycobacteria produce two types of siderophores, the extracellular exochelins and the intra-cellular mycobactins to sequester iron (see page 79). Investigations are in progress to separate and characterize these siderophores and also to determine their association constants for ferric iron.

Exochelins: Mycobacteria were grown under iron-deficient conditions (0.02 mcg/ml iron in the medium). After the prescribed periods of incubation, the contents were autoclaved, and the cells separated by filtration were used for extraction and characterization of the mycobactins. Exochelins in the culture filtrates were labelled with $^{55}FeCl_3$ and extracted with chloroform. The chloroform-soluble exochelins were separated on a silica gel G plate employing a solvent system of light petroleum ether : n-butanol : ethyl acetate (2:3:3) and then autoradiographed.

The chloroform-soluble exochelins from *M. smegmatis* separated into 6 bands containing radio-active iron, the Rf values being 0 (orlgin) and 0.10, 0.16, 0.39, 0.60 and 0.70. The separation patterns of exochelins from 3 strains of *M. tuberculosis* (H37Rv, the South Indian low virulent strain and H37Ra) were similar and a total of 7 bands were detected with Rf values of 0 (origin), 0.08, 0.26, 0.31, 0.38, 0.43 and 0.60. This shows that the *M. tuberculosis* strains produce exochelins that are different from the saprophytic *M. smegmatis.* Large-scale preparation and separation of exochelins is in progress. After separation on the TLC plates, these will be made iron-free, further purified, and the association constants for ferric iron determined.

Mycobactins: The mycobactins were extracted with ethanol from the autoclaved cells. The ethanol extract was evaporated to dryness, dissolved in methanol and the mycobactins in the methanolic solution were separated on a silica gel G plate developed with the same solvent system as for the exochelins. After air-drying, plates were viewed under ultra-violet light and the fluorescent compounds (mycobactins) detected.

Five bands (Rf values : 0.34, 0.46, 0.69, 0.73 and 0.84) were detected with *M. smegmatis* while only 4 bands (Rf values : 0.43, 0.46, 0.59 and

0.64) were observed with the three *M. tuberculosis* strains. Thus, the separation patterns of the mycobactins appear to be similar in the three *M. tuberculosis* strains, and *M. smegmatis* appears to be producing mycobactins different from the other 3 strains.

(b) Iron-regulated envelope proteins: These proteins are located on the cell-wall and the cell-membrane, and are believed to be expressed under iron-deficient conditions. These function as receptors and are presumed to have a role in the transport of iron into the cell. Experiments are in progress to separate, characterize and study the properties of these receptor proteins. Mycobacteria will be grown under iron-deficient (0.02 mcg/ml of iron in the medium) and iron-sufficient (4 mcg/ml iron) conditions and the cells harvested after the prescribed periods of incubation. The cells will be disrupted by ultrasonication and the cell-wall and cell-membrane fractions separated by ultracentrifugation. These will be solubilised in SDS and analysed by PAGE. The differences in the expression of these proteins, if any, between the 4 strains of mycobacteria and between conditions of iron-deficient and iron-sufficient growths will be studied.

(started: 1989; expected year of completion: 1991)

Studies on the mechanism of pyrazinamide action

It was believed that an acid environment (pH 5.6), as prevalent within the macrophages, was necessary for the activity of pyrazinamide against susceptible strains of mycobacteria. However, the hypothesis of a principally intracellular locus of action of pyrazinamide has been questioned recently. Mycobacterial strains susceptible to pyrazinamide have been shown to contain the enzyme, pyrazinamide deamidase, which converts the drug to pyrazinoic acid, while the enzyme is absent in drug-resistant strains. It has therefore been postulated that pyrazinoic acid is the active component. To gain a better understanding of the interactions between the hydrogen ion concentration (pH), presence of pyrazinamide deamidase and the effect of pyrazinamide and pyrazinoic acid, investigations were undertaken with two strains of mycobacteria namely, *M. microti* (a susceptible strain) and *M. bovis* BCG (a strain naturally resistant to pyrazinamide). The bactericidal action of pyrazinamide and pyrazinoic acid was tested in cell-free 7H9 liquid broth maintained at pH 5.6 and pH 7.0, and in a mouse macrophage culture system.

Preliminary investigations have shown that in the cell-free system, both pyrazinamide and pyrazinoic acid are active against *M. microti* at both pH 5.6 and 7.0, though the activity at pH 7.0 was less than that at pH 5.6. With *M. bovis* BCG, a slight bacteriostasis was observed with both pyrazinamide and pyrazinoic acid, at both pH 5.6 and 7.0. In the macrophage culture system, both pyrazinamide and pyrazinoic acid appeared to be equally effective against *M. microti.* With the BCG strain, however, there was no inhibition of growth either in the presence of pyrazinamide (as expected) or even in the

presence of high concentrations of pyrazinoic acid. This finding of the lack of activity of pyrazinoic acid against BCG and the earlier observation from the cell-free system of a slight bacteriostatic activity of pyrazinamide itself against BCG do not appear to support the hypothesis that pyrazinoic acid is the active component and that resistance to the drug is due to the absence of pyrazinamide deamidase. Further investigations with these two strains as well as with pyrazinamide-susceptible *M.tuberculosis* H37Rv and pyrazinamide-resistant *M. bovis* ravenal are in progress to confirm and expand these findings.

(started: 1989; expected year of completion: 1991)

Production of monoclonal antibodies against tuberculous antigens

In the last (1988) annual report, generation of twelve monoclonal antibodies had been reported. The generation of more monoclonal antibodies and characterization of some of the monoclonal antibodies raised earlier were two aspects of work carried out during the year.

During the year, twenty-three more fusions were carried out. Previous results had showed that a majority of monoclonal antibodies appear to react with immunodominant epitopes. Since these epitopes have not been found to be of interest for immunodiagnosis, efforts were directed towards getting antibodies different from those obtained so far. Also it was seen that a large proportion of hybrids that show positivity in the initial screening tend to lose activity during cloning. To avoid these problems, all the wells that showed positive hybrids were cultured in larger volume and cells were frozen in liquid nitrogen; 45 hybrids showing reactivity in preliminary ELISA screening have been frozen. The supernatants of these hybrids are being tested by immunoblotting. Of these the one showing interesting binding pattern will be revived and cloned further.

Out of the previously generated monoclonal antibodies three showed distinct binding patterns on immunoblots with H37Rv-CF antigen. Monoclonal antibody 31-3 bound to two lower molecular weight bands (11kD & 19kD), 5E9 recognized predominantly 29-31 kD antigens while monoclonal antibody 1H12 showed very broad reactivity between 30-200 kD range. Their cross-reactivity with mycobacteria other than tuberculosis and heterologous antigen, i.e., *E.coli* extract was studied in ELISA. While all three reacted strongly with immunizing antigen, *M.tuberculosis* H37Rv-CF, none reacted with *E.coli* extract. Antibody 31-3 also reacted with British strain SI and *M.terrae,* 5E9 reacted with *M.bovis* and BCG, and 1H12 reacted with *M.terrae, M.flavescence, M.scrofulaceum, M.bovis* and BCG. The optical densities (O.D.) obtained with various antigens in ELISA are shown in the table on page 104.

Antigen	Control SP 2/0 Super-natant	31-3	5E9	1H12
M.intracellulare serovar 8	0.248	0.274	0.283	0.263
M.Scrofulaceum	0.285	0.301	0.363	*0.471**
M.bovis	0.274	0.294	*0.533**	*1.549**
BCG	0.273	0.348	*0.413**	*0.635**
M.kansasii	0.222	0.404	0.233	0.319
M.flavescence	0.278	0.277	0.288	*0.422**
M.gordonae	0.184	0.189	0.239	0.261
M.terrae	0.187	*1.713**	0.207	*0.324**
M.fortuitum	0.215	0.178	0.220	0.213
M.chelonei	0.219	0.225	0.273	0.249
7219	0.246	0.242	0.280	0.269
S.I.	0.256	*1.275**	0.266	0.347
H37Rv-CF	0.223	*>3.00**	*1.969**	*2.514**
E.coli	0.280	0.264	0.309	0.344

* Reaction significantly higher than its counterpart with the respectlve SP 2/0 supernatant.

These three antibodies belong to IgG class and have been purified on Protein-A sepharose column. Target antigen of IH12 has also been affinity purified on antigen column. These antibodies and antigen IH12 will be evaluated for use in immunodiagnosis of tuberculosis.

(started: 1985; expected year of completion: 1991)

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

Affinity chromatography techniques to purify antigens relevant in diagnosis are being continued.

Affinity purified antibodies from a large pool of tuberculous serum were obtained and coupled to polyvinyl sulfone activated sepharose 4B as described in our earlier reports. The total culture filtrate antigen was passed through this column and absorbed. The unabsorbed antigens were washed with

phosphate buffered saline (PBS) and bound antigens were eluted with a battery of eluting agents such as:

1. 0.1M Glycine - Hcl pH 2.5
2. 4M urea
3. 3M potassium thiocyanate
4. 4M Guanidine hydrochloride

None of the eluting agents were able to desorb the antigens from the column in sufficient quantities. Changes in elution conditions such as length of incubation and temperature of elution also did not help.

The difficulty in elution of antigens from the antibody column could be attributed to high affinity constants between human IgG and antigen.

A portion of the sepharose 4B absorbent was boiled in sample buffer and analysed by SDS-PAGE. The coomassie blue stained gel revealed the presence of a number of bands, thus confirming the fact that the antigen-antibody complex could not be broken by any of the eluting agents and therefore most of the antigens were retained on the column itself.

Attempts are being pursued to affinity purify the culture filtrate antigens by passing them through normal human serum IgG absorbants. In this case, the commonly recognised antigens are bound to the absorbent, leaving more specific antigens in the supernatant. Difficulty of elution is circumvented by this procedure.

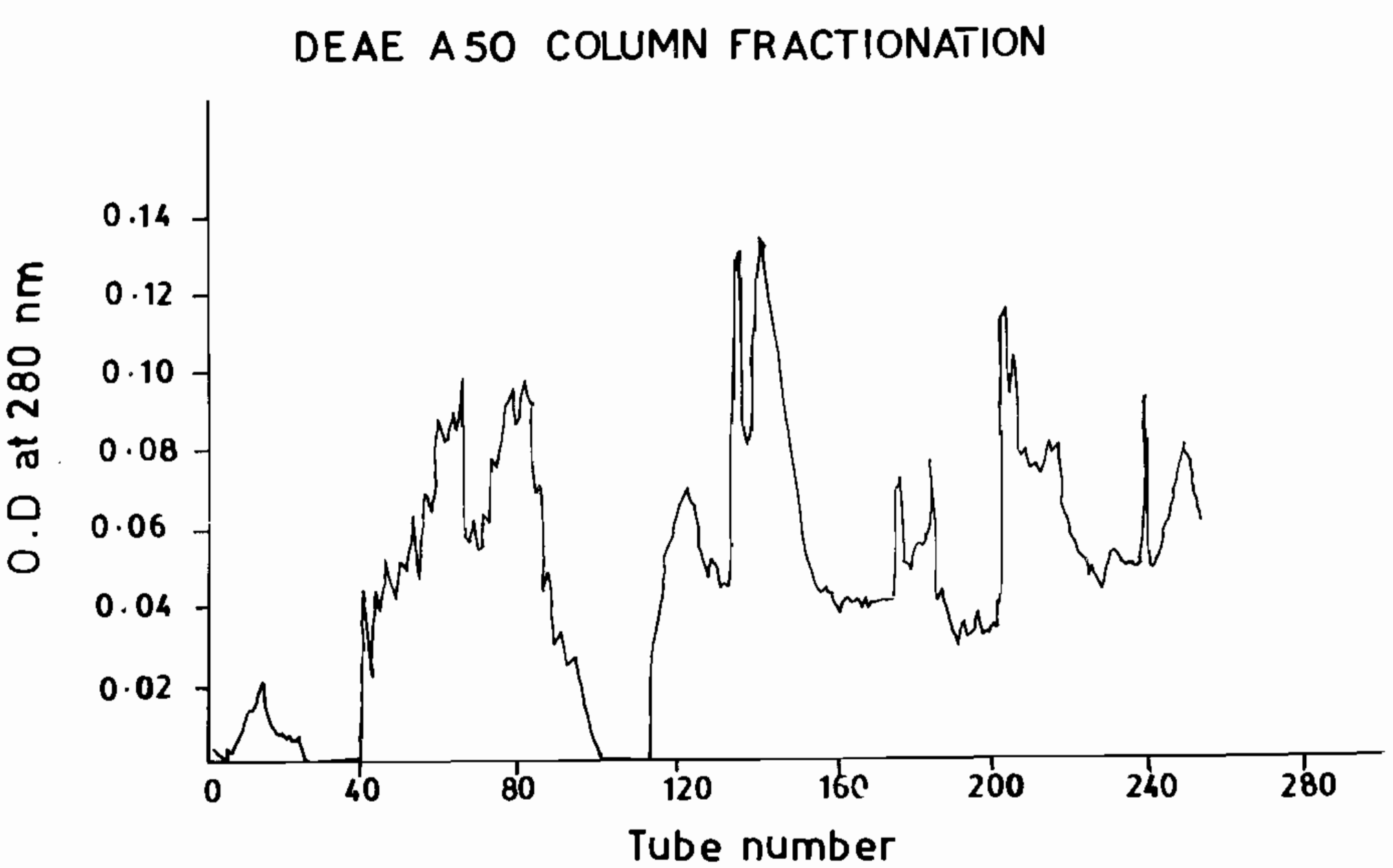
Experiments were undertaken to fractionate the culture filtrate into purified components using other physico-chemical techniques also. Ion exchange chromatography through DEAE-A50 Sephadex was carried out. Culture filtrate was separated into 15 fractions as shown in the figure in page 106.

The separation procedure will be repeated more number of times to collect sufficient concentration of each of the fractions and they will be evaluated in ELISA for their diagnostic potential.

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

Pulmonary defense mechanism against opportunistic/common bacterial infections in patients with pulmonary tuberculosis

Pulmonary defense mechanism against opportunistic/common bacterial infections in patients with pulmonary tuberculosis has been studied. Bactericidal activity of broncho-alveolar lavage fluids (soluble components) of healthy subjects and patients with active and inactive tuberculosis were studied using *Staphylococcus aureus* as an indicator system. An increased staphylocidal activity from 'O' hr to 4 hrs in the three - fold concentrated, cell-free supernatant of lavage fluids of normal subjects and inactive TB patients was observed.


DEAE A50 COLUMN FRACTIONATION
O.D at 280 nm
0·14
0·12
0·10
0·08
0·06
0·04
0·02
0
40
80
120
160
200
240
280
Tube number

But, decreased *S.aureus* killing was seen at 4 hrs of incubation in lavages only from patients with active tuberculosis. The study is in progress.

(started: 1989; expected year of completion: 1990)

Development of DNA probes for *M.tuberculosis*

The study on development of DNA probes for *M.tuberculosis* was started with the aim of detecting mycobacteria in clinical specimens where conventional detection is difficult. The efforts in the last year have been directed towards achieving this goal. The progress made is reported here.

DNA isolation and purification: *M.tuberculosis* H37Rv strain was grown in Sauton tween medium and 10ml of glycine was added to this culture, 18 hrs before harvest. Cells were treated with lysozyme followed by proteinase-K at 37°C. Subsequently the cells were treated with SDS at 37°C. The cell suspension was serially extracted with phenol, phenol-chloroform and chloroform-isoamyl alcohol. Finally, it was precipitated with ethanol.

Restriction endonuclease digestion and agarose gel electrophoresis: DNA was digested with restriction enzyme at concentrations five - fold higher than those specified by the commercial firm. Digestion was terminated by addition of stop buffer containing SDS. Bacteriophage T C1857 digested with Hind III was used as size markers. The digested DNA was size fractionated by electrophoresis in Tris-borate, EDTA buffer through 1% agarose gels. The gels were stained in an ethidium bromide solution and photographed under ultra violet light with polaroid type 667 film.

The figure in page 108 shows the restriction digestion of *M.tuberculosis* DNA with various enzymes.

Construction of recombinant plasmid genomic library: DNA (50 mcg) isolated from *M.tuberculosis* H37Rv was digested with restriction endonuclease BamH1. The resultant DNA fragments were ligated into the plasmid. Plasmid PBR-322, was digested with BamH1 and treated with calf intestinal phosphatase. Mycobacterial and vector DNA were mixed in varying ratio in ligation buffer and reaction mixtures were incubated overnight at 15°C and stored.

Escherichia coli strain HB101 competent cells were prepared by treatment with 0.1M Cacl2 and transformed with the recombinant plasmids by heat shock at 42°C. The colonies containing the recombinant plasmids were selected by plating on Luria-Bertini Agar (LBA) medium containing 100mcg of ampicillin/ml and frozen in 1ml aliquots in medium containing 15% glycerol. These stock cultures were stored at – 20°C.

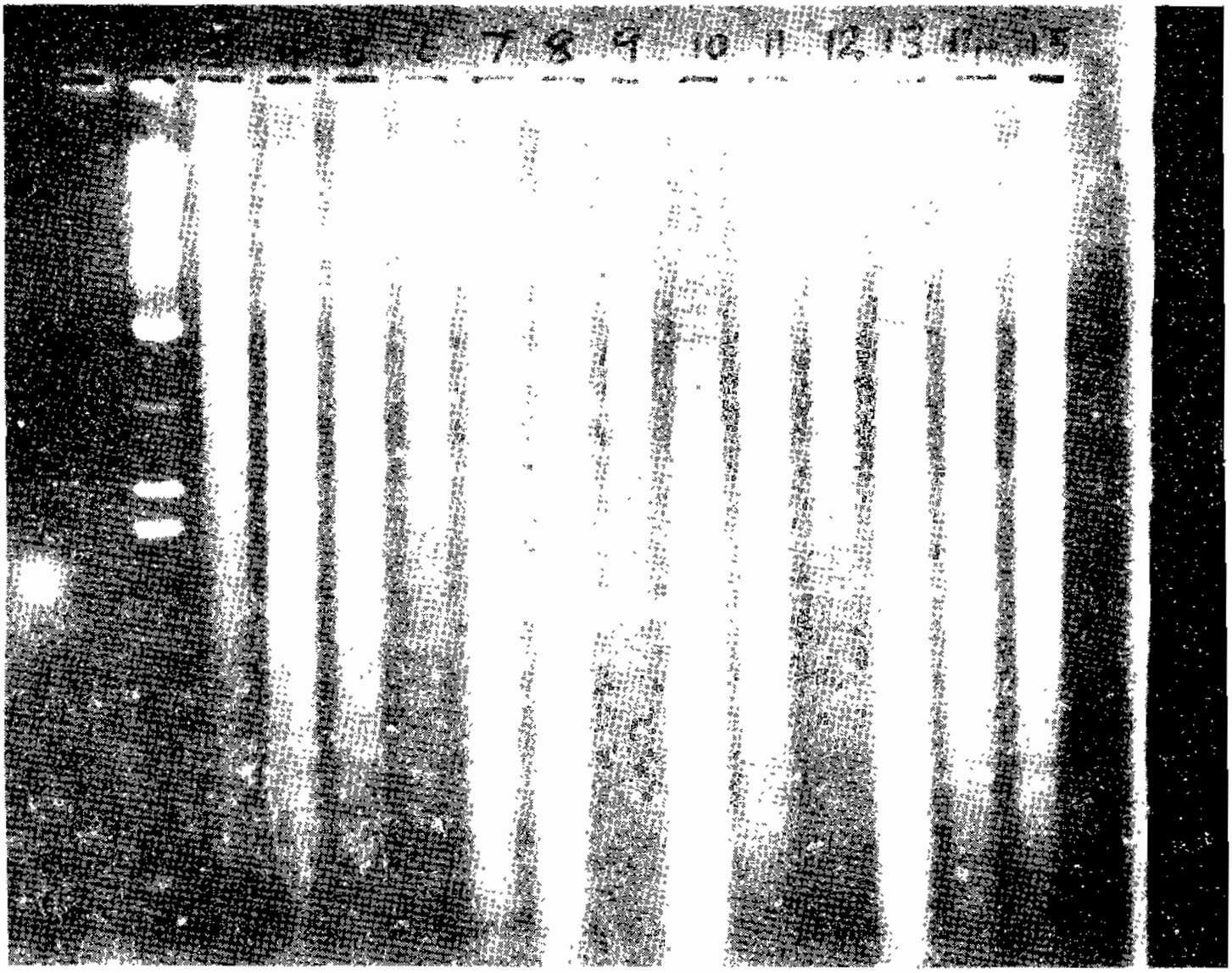
Screening the genomic library: The recombinant plasmids were analysed both by alkaline lysis method and hybridisation method.

In the hybridization method the host cells containing the recombinants were immobilized on the membrane by the Slot blot apparatus (Schleicher

M.tuberculosis DNA has been restriction digested with restriction enzymes.

Legend :

1 None	2 M.Wt.marker	3 Uncut DNA
4 $BamH_1$	5 Eco R_1	6 Hind III
7 PST I	8 Sal I	9 Sph I
10 Rsa I	11 Xho I	12 Xba I
13 Nar I	14 Sal Eco	15 BamH I Nar I



& Schuell). The filters were then treated with NaOH. Tris Hcl and Tris Hcl with Nacl to release plasmid DNA. The filters were baked in vacuo at 70°C to bind the DNA before hybridisation with Nick translated genomic DNA.

Nick translation: DNA was radio labelled with ^{32}P by Nick translation using DNAase I and DNA polymerase I. The labelled DNA was purified from unincorporated nucleotides by sephadex G50 chromatography. The Nick translated DNA was boiled for 10 minutes before it was added to the hybridization solution.

Hybridization and autoradiography: The nitrocellulose filters were incubated at least for 2 hrs at 65°C in hybridization solution contalning 6 x SSC (20 x SSC = 3mM Nacl 0.3M sodium citrate) 0.05% ficoll, 0.05 polyvinyl pyrolidine 0.1 mg of denatured herring sperm DNA /ml to minimize non specific binding and background. The denatured radio labelled probe was added to the bag and incubation was contrinued at 65°C for 18-24 hrs. Unbound probe was removed by washing the filters three times in 0.1% SSC with 0.1% SDS at 65°C for 30 minutes. The filters were allowed to dry and were then exposed to Kodak X-omat.

The figure in page 110 shows the autoradiogram of the standard *M.tuberculosis* and *E.coli* DNA (20, 2, 0.2, 0.02, 0.002 mcg and none) immobilised on NCP and probed with whole 32P labelled *M.tuberculosis DNA.* This was done to standardise the procedure of hybridization. The autoradiogram shows that the lowest detection limit of the *M.tuberculosis* probe is 20 ng and there was no recognition of *E.coli DNA.*

The autoradiogram of recombinant plasmids screened with *M.tuberculosis* DNA was obtained. The radioactive signals represent the recombinant plasmid clones that have the mycobacterial inserts as shown in the figure on page 111. The recombinant plasmids that show strong hybridisation signals will be further analysed. The work is in progress.

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

Evaluation of two-site antigen detection assay

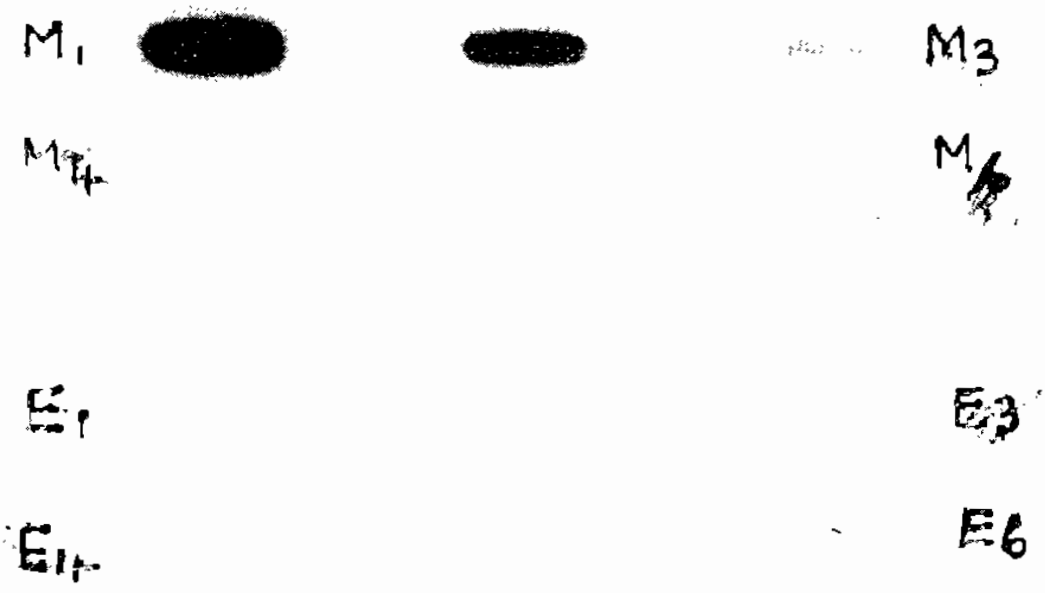
The development of enzyme linked immunosorbent assay (ELISA) has stimulated wide interest in serological tests for the diagnosis of tuberculosis either by measuring circulating antibodies or by detecting mycobacterial antigens in the clinical samples. Many reports have been published on antigen detection assays in CSF fluid, sputum and serum samples. In these studies, the authors have reported high sensitivity (ranging from 1 ng/ml to 20ng/ml) and specificity (ranging from 45% to 95%) for this assay. However, very little is reported about the reproducibility of these assays.

A study was undertaken to develop and standardize a two-site sandwich ELISA using polyclonal rabbit IgG antibodies raised against live H37Rv bacilli and evaluate its reproducibility. This report gives the findings.

Legend:

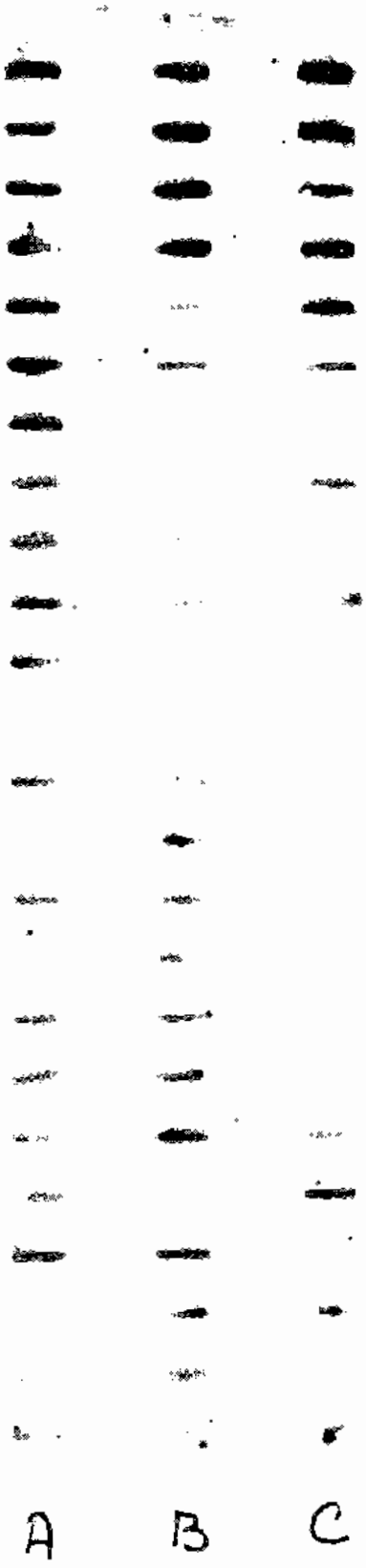
Autoradiogram of hybridisation of various concentrations of *E.coli* and *M.tuberculosis* DNA probed with ^{32}P labelled *M.tuberculosis* DNA.

M_1	:	M.tb DNA	20 mcg	E_1	:	E.coli DNA	20 mcg
M_2	:	,,	2 mcg	E_2	:	,,	2 mcg
M_3	:	,,	0.2 mcg	E_3	:	,,	0.2 mcg
M_4	:	,,	0.02 mcg	E_4	:	,,	0.02 mcg
M_5	:	,,	0.002 mcg	E_5	:	,,	0.002 mcg
M_6	:	,,	None	E_6	:	,,	None



Legend :

The transformed recombinant plasmids in *E.coli* HB_{101} containing various mycobacterial fragments were grown as single suspension and 100 mcl of the individual culture has been immobilised on the nitrocellulose membrane by the slot blot method. .The immobilised cells were alkali treated and hybridised with whole ^{32}P labelled *M.tuberculosis* probe. 3 clones from lane-A, 4 clones from lane-B and 5 clones from lane-C have strongly hybridised with *M.tuberculosis* probe.



Antigen: The antigen used here for standardization was prepared as follows. The live cells from 4-6 weeks old culture of *M.tuberculosis* (H37Rv strain) were harvested and washed thoroughly with PBS. Cells suspended in PBS with PMSF were sonicated for 10 minutes with maximum 80 vibrations per second inside the sterile hood under ice-salt mixture. The sonicate extract was centrifuged and supernatant was used as antigen.

Antisera: Antiserum to *M.tuberculosis* H37Rv was raised by immunizing rabbits with a live suspension of *M.tuberculosis* H37Rv cells. The antibody titre was checked after 3rd booster dose and high titre serum was collected and stored at -20°C.

Purification of IgG fraction: The rabbit immune serum was subjected to 80% ammonium sulphate precipitation to concentrate the proteins. It was later used to purify IgG fraction by affinity chromatography on Protein-A column.

A portion of this IgG was biotinilated and used as 2nd antibody for detecting the antigen.

Affinity purified antibodies: Affinity column of H37Rv sonicate antigen was prepared on sepharose 4B and above mentioned Protein-A purified IgG fraction was passed through it. The eluted antibodies were referred as affinity purified antibodies.

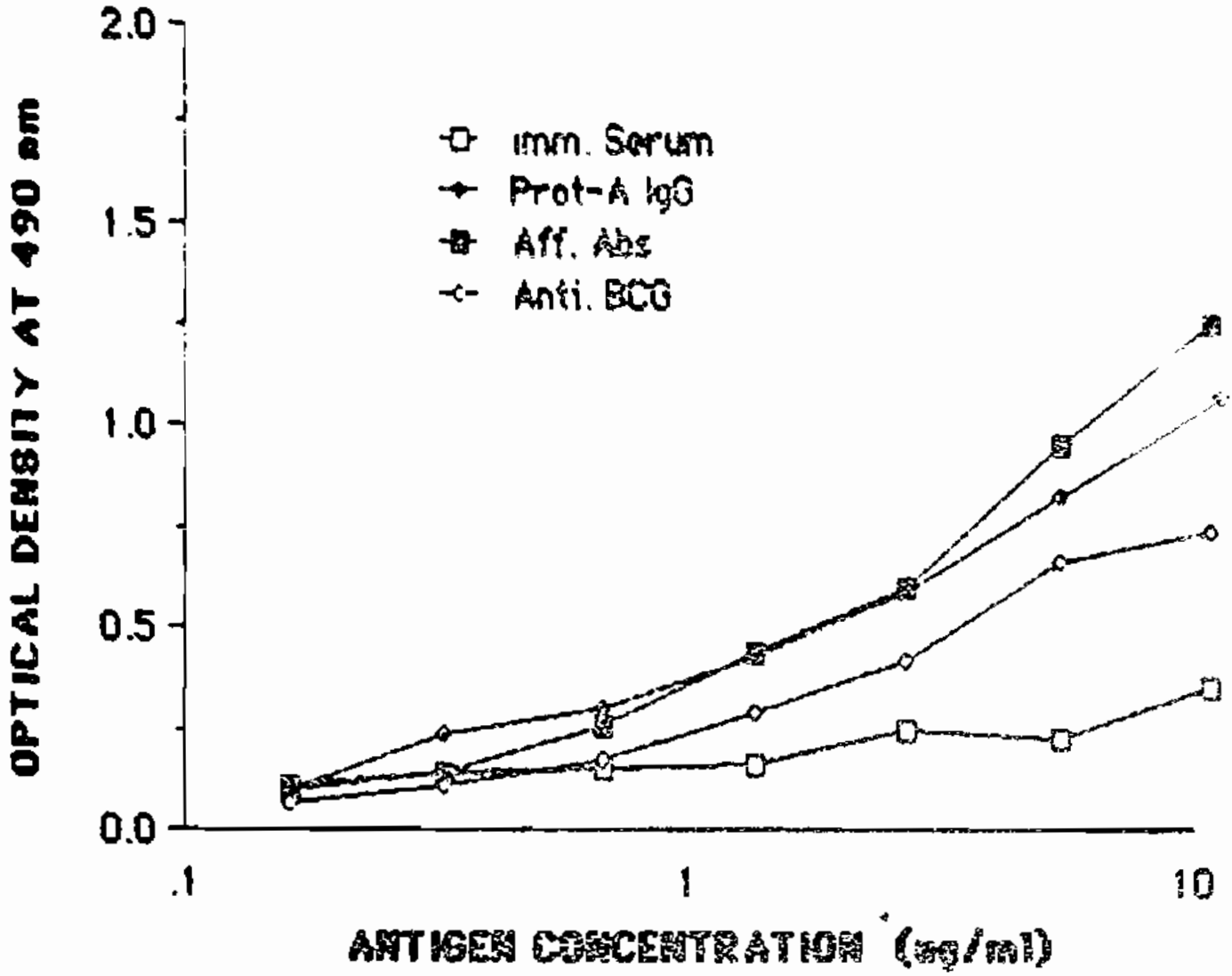
Commercial anti-BCG antibodies: Rabbit immunoglobulins to *M.bovis* (BCG) were obtained from Dakopatts (Copenhagen) and used for comparison. Preliminary experiments were done with H37Rv sonicate antigen alone.

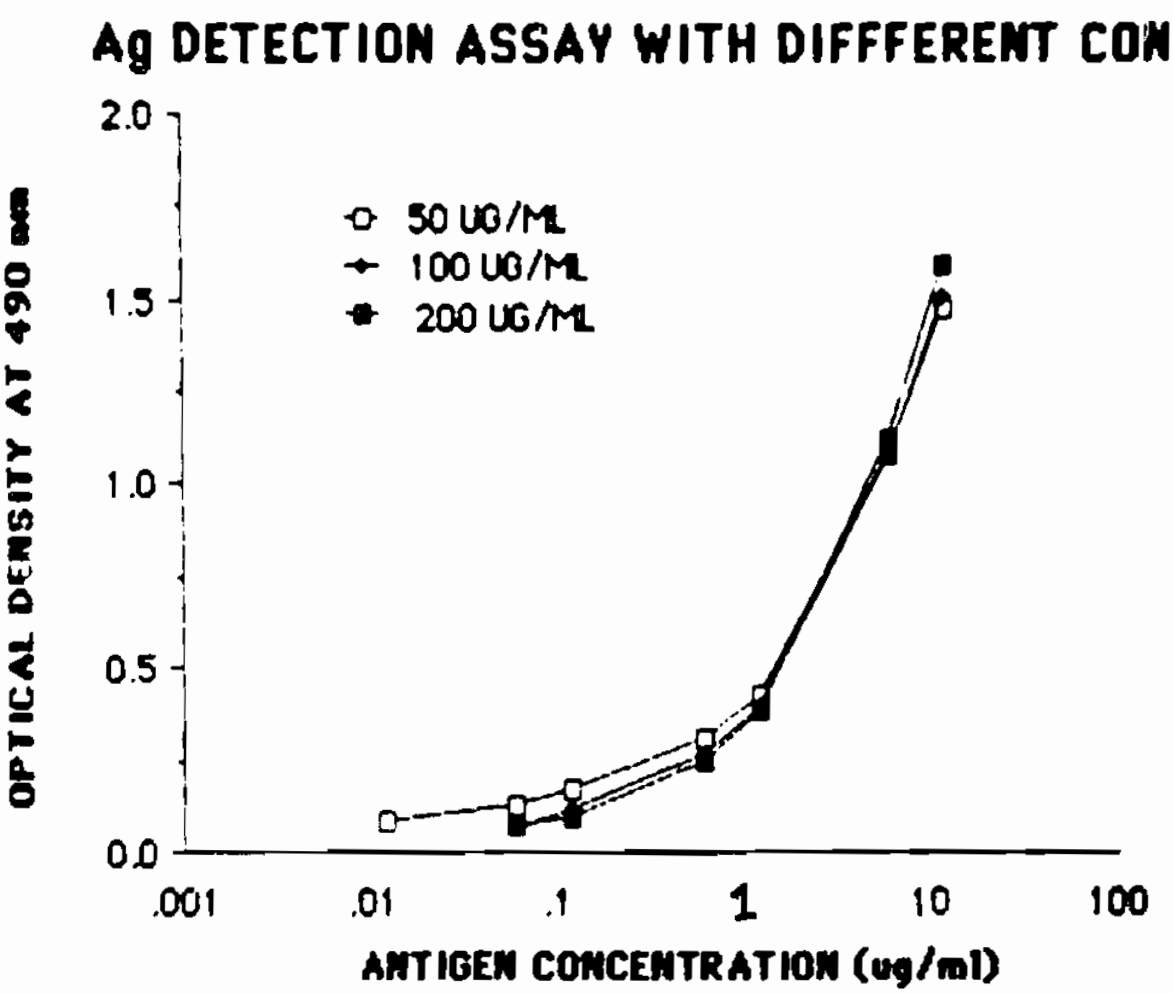
(a) **Selecting the trapping antibody:** Different antibodies as mentioned above (Immune serum, Protein-A purified IgG, affinity purified antibodies and commercial anti-BCG antibodies) were used at 100 mcg/ml concentration for trapping the antigen. The antigen concentration was tested from 1 ng/ml to 10 mcg/ml. The dose response curves were plotted as shown in the figure on page 113. Affinity purified antibodies showed higher response at higher antigen concentration. However, at low antigen concentration both affinity antibodies and Protein-A IgG showed similar response. Hence, Protein-A IgG was selected for trapping the antigen.

(b) **Selecting the optimum concentration of trapping IgG:** The dose response curve with various concentrations of IgG ranging from 50mcg/ml to 200mcg/ml was almost same (see figure on page 114) showing that 50mcg/ml of IgG was sufficient for trapping the antigen.

(c) **Detecting antibody concentration:** Of the two doses of biotinilated IgG (1:50 and 1:100) tested, 1:100 dilution was found to be better by studying the dose response curves (data not presented).

Ag DETECTION ASSAY - DIFF Ab SOURCES




Ag DETECTION ASSAY WITH DIFFFERENT CONC. OF IgG
OPTICAL DENSITY AT 490 nm
2.0
1.5
1.0
0.5
0.0
50 UG/ML
100 UG/ML
200 UG/ML
.001
.01
.1
1
10
100
ANTIGEN CONCENTRATION (ug/ml)

(d) **Cross reactivity with other mycobacterial antigens**

The cross reactivity of this assay was tested with 10 other mycobacterial antigens. The assay showed maximum response (O.D. 1.1 at 10mcg/ml of antigen concentration) with H37Rv antigen while with other species, the response ranged from 0.1 to 0.15 O.D. at the same antigen concentration.

(e) **Antigen detection assay in serum:** The sensitivity of the assay was further checked by mixing the antigen in serum sample low for mycobacterial antibodies (non-endemic control subject). The dose response curves were plotted for antigen alone and antigen mixed in serum as shown in the figure on page 116.. It showed consistently lower response when antigen was mixed in biological sample and the difference increased with increase in the antigen concentration.

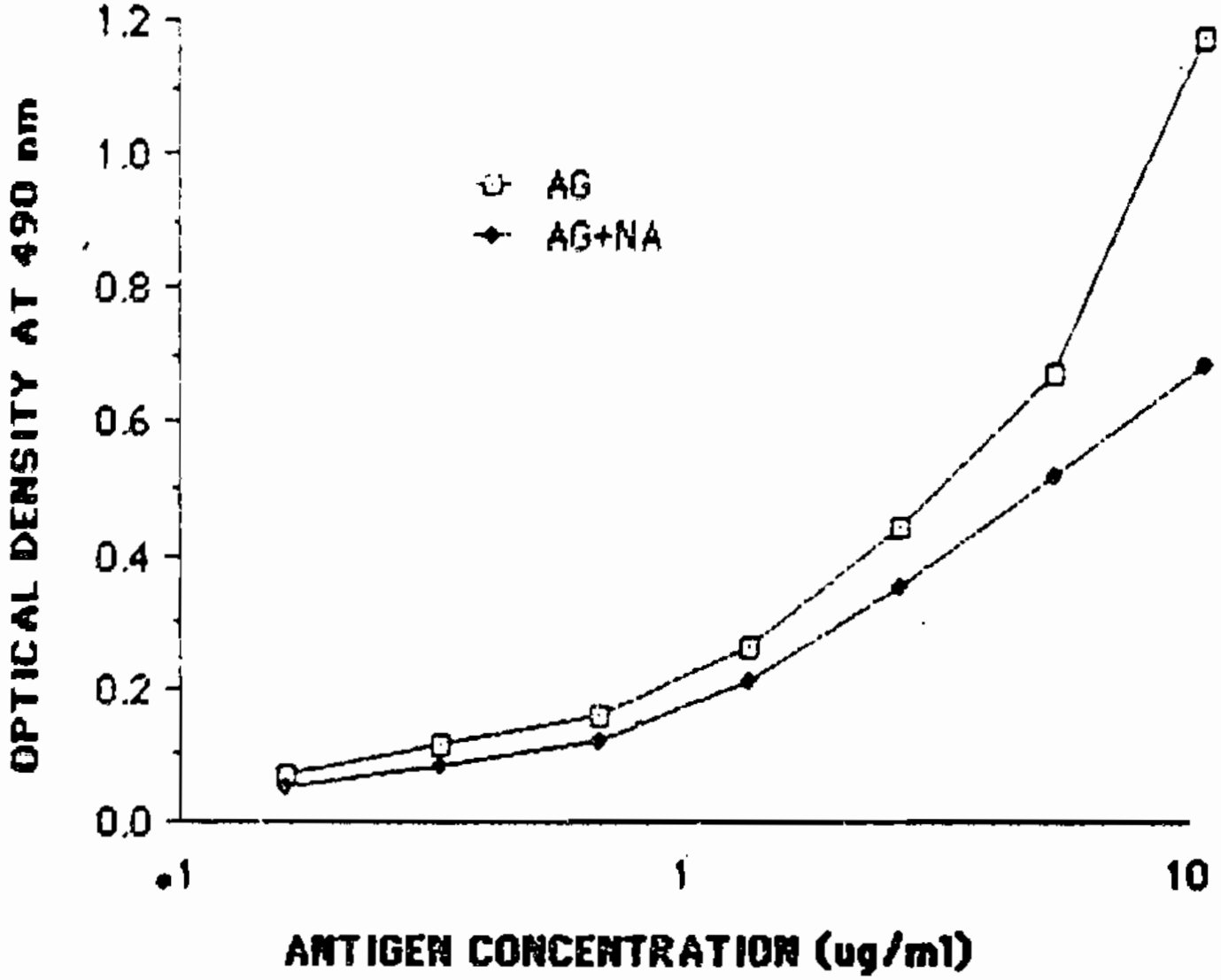
Reproducibility experiments: With the selected optimum conditions of trapping IgG (100mcg/ml) and detecting antibody (1:100), the reproducibility of the assay was studied. The two - site sandwich ELISA experiments with H37Rv sonicate antigen alone was repeated in 7 plates on different days under identical conditions. The antigen concentration used ranged from 0.15 to 10.0 mcg/ml.

The results of the reproducibility experiment are presented in the table below.

O.D.	X1	X2	X3	X4	X5	X6	X7	Mean	*S.D.	*CV%
0.55	0.24	0.37	0.47	0.03	0.24	0.12	0.91	0.34	0.29	*85.5*
0.65	0.66	0.84	0.99	0.30	0.57	0.44	1.42	0.75	0.37	*50.3*
0.75	1.20	1.42	1.62	0.71	1.00	0.88	2.01	1.26	0.45	*36.0*
0.90	2.19	2.46	2.76	1.51	1.77	1.70	3.00	2.20	0.56	*25.7*
1.00	2.96	3.26	3.62	2.16	2.37	2.35	3.74	2.92	0.64	*22.0*
1.20	4.72	5.07	5.57	3.66	3.72	3.83	5.37	4.56	0.81	*17.9*
1.40	6.73	7.13	7.80	5.41	5.26	5.54	7.18	6.44	1.00	*15.8*
1.50	7.83	8.25	9.00	6.37	6.10	6.47	8.16	7.45	1.10	*15.1*
1.65	9.57	10.03	10.91	7.90	7.43	7.96	9.69	9.07	1.30	*14.4*
1.75	10.80	11.28	12.25	8.98	8.37	9.00	10.77	10.21	1.40	*14.0*

* S.D. = Standard deviation; CV = Coefficient of variation

After making the transformation $X = Z^{0.66}$ where Z is the antigen concentration, it was found that the dose response curve was linear. For each plate, the regression lines were fitted by the method of least squares.


Ag DETECTION ASSAY - IN SERUM
OPTICAL DENSITY AT 490 nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
AG
AG+NA
.1
1
10
ANTIGEN CONCENTRATION (ug/ml)

Using the regression lines, the antigen concentrations were estimated for the hypothetical O.D. values ranging from 0.55 to 1.75. Column 1 shows the hypothetical O.D. values while columns 2 to 8 represent the estimated antigen concentration corresponding to the O.D. value. The columns 9 to 11 present the mean, S.D. and its coefficient of variation (CV), respectively.

It was found that the CV was high for low O.D. values and it consistently decreased with increase in the O.D. values. In order to keep the CV low, the assay should be so designed that it produces high O.D. values.

In this experiment, for a CV of 15%, the sensitivity of the assay will be 6mcg/ml.

(started: 1989; expected year of completion: 1991)

Analysis of immune complexes from tuberculous sera

As reported earlier (1988 annual report), attempts were made to purify the polyethylene glycol (PEG) precipitated immune complexes along the following lines.

1. Absorption to heat killed formalized staphylococcus aureus -A (Cowan I) strain - by their affinity to Protein-A.
2. Absorption to anti C3-d coated staphylococcus aureus-A cells by their bound C3.

The first of the two methods, i.e., absorption to protein-A gave consistently better resolution of antigenic bands. Using pooled tuberculous sera and pooled normal sera for precipitation, antigenic bands of molecular weight 82, 56, 35, 26, 23 and 18 kD. were seen to be present specifically in the tuberculous complexes.

Experiments are in progress to study the pattern in individual sera before, during and after treatment for tuberculosis.

(started: 1987; expected year of completion: 1990)

HLA studies in tuberculosis

The objective of this project is to use a combination of serological and DNA probes to analyse the genotype of a number of individuals to define whether there exists an association between any serological or DNA marker and the occurrence of tuberculosis. Patients who have been treated for any form of tuberculosis and whose parents or children have also been treated for tuberculosis of any form, are selected for the study. However, these families should have another family contact without manifestation of the disease. In addition to the 107 subjects from 22 families in 1988, 55 subjects from 13 more families were investigated. DNA from all these subjects have been prepared. Further work is in progress.

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

Histopathological classification of tuberculous lymphnodes

The present study, which was initiated in 1988, is aimed at delineating the host responses to *M.tuberculosis* at the microanatomical level in order to (a) understand the pathogenesis of tuberculosis better, and (b) define the diagnostic criteria for some of the unusual histological presentations of tuberculous lymphadenitis.

During the year, a further 263 lymphnode biopsies were examined and of these 103 (39.2%) were found to show evidence of tuberculosis. These could be classified into reactive (66%), hyperplastic (17.5%) hyporeactive (9.7%) and non-reactive (6.8%). Histometric procedures are currently being standardized to quantitate these histological changes.

In addition, 31 of these lymphnodes belonging to different categories were stained for the presence of mycobacterial antigens, immunoglobulins G and M and complement component C3d. Although a majority of these (27 of 31) grew *M.tuberculosis* in culture, it was not always possible to demonstrate AFB in the sections. However, mycobacterial antigens could be readily visualised in all these biopsies. It is hoped that when antibodies specific to *M.tuberculosis* are available, this technique could be used to confirm the diagnosis where it is doubtful on clinical and histological grounds alone.

Apart from mycobacterial antigens, the lymphnodes were stained for the presence of immunoglobulins G and M and complement C3d. A summary of the findings in the various groups is given in the table on page 119. The proportion of B lymphocytes (stained with anti immunoglobulin antibody) in the granuloma relative to the total lymphocytes is being worked out.

It is apparent from these findings that the immunopathogenetic pathways of the different groups are likely to be different. Currently, a method is being developed to quantitate the amount of antigen present in tne tissue. Also, it is planned to delineate the lymphocyte subsets in the various groups.

The introduction of histometric and quantitative histo-chemical methods would help to evolve a system of classification of tuberculous lymphnodes which will be of diagnostic and prognostic significance.

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

Type	No.of specimens examined	AFB culture positive	Morphology	Mycobacterial antigen	Immunoglobulins G & M	Complement C3d
Reactive	16	12	Organised granuloma, Epithelioid cells +, Lymphocytes, giant cells & plasma cells. Fine caseating necrosis	2+ in the caseous areas, epithelioid and giant cells	2+ in the areas of caseation, plasma cells & lymphocytes	2+ in the caseous areas, giant cells, epithelioid cells and polymorphs
Hyper-plastic	9	All 9	Organized granuloma, Epithelioid cells 3+, Giant cells & Lymphocytes ± to 1+	1 to 2+ in the Epithelioid and giant cells	Lymphocytes 2+	1+ in epithelioid cells
Hypo-reactive	3	All 3	Poorly organized granuloma, Macrophages 3+, Giant cells ±, Karyorrhexis +	2+ in the macrophages and necrotic areas	2+ in the necrotic areas	1+ in the macrophages
Non-reactive	3	All 3	Unorganized granuloma. Macrophages 3+, Polymorphs 2+, Karyorrhexis	2+ in the macrophages & necrotic areas	1+ in the necrotic areas	2+ in the necrotic areas, macrophages and polymorphs

EPIDEMIOLOGICAL STUDIES

Studies Completed

Tuberculosis prevalence survey in Raichur district

With the exception of the National Sample Survey of Tuberculosis conducted in 1955-58, no epidemiological data on tuberculosis are available at the national level, and particularly in districts where SCC is being monitored by the Centre. It was therefore proposed to conduct sample surveys in these districts.

As a first step, a tuberculosis prevalence survey was carried out in Raichur district of Karnataka State, with the specific objective of obtaining an estimate of the prevalence of bacteriologically positive (smear and/or culture) pulmonary tuberculosis in the district by examining all the 'chest symptomatics', aged 15 years and above, identified through the survey.

Procedures of the survey: Of the total population of 17.7 lakhs (1981 census), a random sample of villages and towns was drawn to give a sample size of about 65,000 persons. A complete census of the selected villages and towns was undertaken and all individuals registered. All those aged 15 years or above were questioned for symptoms suggestive of pulmonary tuberculosis. From the symptomatics, the nature and duration of the symptoms and the action taken, were elicited and recorded. From each person who complained of (a) cough of two weeks or more, (b) chest pain of one month or more, (c) fever of one month or more, or (d) haemoptysis (at any time), 2 sputum specimens (one spot and one overnight collection) were obtained. From the specimens, smears were made and examined for the presence of AFB by the Ziehl-Neelsen method at the camp site itself. The specimens were then sent to the Centre's laboratory for examination by smear, culture and sensitivity tests. In addition, 50% of the symptomatics who complained of cough of 7-13 days were also included for sputum examination, in order to find out the yield of cases from such symptomatics. Also, any person having a history of treatment for tuberculosis, irrespective of symptomatic status, was eligible for sputum examination. All smear or culture positive cases were intimated about the diagnosis and referred for treatment to the nearest health facility.

Study population and coverages obtained: The survey included 56 villages and 21 town blocks. Of 72,448 persons registered, 42,580 (58.8%) were aged 15 years and above. Of these, 40,657 (95%) individuals were examined for symptoms; 3,846 (9.5%) were eligible for sputum examination and sputum samples were collected from 3,685 (96%). High coverages were obtained in all age-sex groups (92% to 99%).

Distribution of symptomatics by age and sex: The table below gives the proportion of persons who were eligible for sputum examination by age, sex and different eligibility criteria. It can be seen that the overall proportion of symptomatics eligible for sputum examination increased with age and was higner among males than among females. It is also seen that the additional contribution of persons with cough of 7-13 days and those with a history of treatment for tuberculosis was very small (less than 3%). Considering the definition used for symptomatics under the District Tuberculosis Programme (DTP), the overall proportion eligible for sputum examination was 9.3% (11.8% for males and 7.0% for females).

Age-group (years)	Sex	Examined for symptoms	Eligible for sputum examination					
			DTP def.*		7-13 d** cough	History of Rx	Total	
			No.	%			No.	%
15-24	M	5695	225	4.0	3	4	232	4.1
	F	5855	129	2.2	5	1	135	2.3
	T	11550	354	3.1	8	5	367	3.2
25-34	M	4899	391	8.0	1	4	396	8.1
	F	5213	234	4.5	4	6	244	4.7
	T	10112	625	6.2	5	10	640	6.3
35-44	M	3827	467	12.2	6	5	478	12.5
	F	3561	275	7.7	4	3	282	7.9
	T	7388	742	10.0	10	8	760	10.3
45-54	M	2636	510	19.3	2	3	515	19.5
	F	2907	334	11.5	3	3	340	11.7
	T	5543	844	15.2	5	6	855	15.4
55+	M	2663	729	27.4	3	2	734	27.6
	F	3401	486	14.3	3	1	490	14.4
	T	6064	1215	20.0	6	3	1224	20.2
Total	M	19720	2322	11.8	15	18	2355	11.9
	F	20937	1458	7.0	19	14	1491	7.1
	T	40657	3780	9.3	34	32	3846	9.5

*Cough of ≥14 d / Chest pain, Fever of ≥1 m / Haemoptysis at any time

**Only 50% were eligible

Distribution of symptomatics by duration of symptoms: The table below gives the distribution of symptomatics by duration of symptoms. It can be seen that a vast majority of symptomatics had symptoms for atleast 1 month; 93% for cough, 97% for chest pain and 87% for fever. Similar figures for symptomatics having symptoms for more than 1 year were 51%, 54% and 25% respectively.

Duration of symptoms	Cough		Chest Pain		Fever	
	No.	%	No.	%	No.	%
1- 6 d	46	*1.4*	15	*0.6*	52	*5.4*
7-13 d	65	*1.9*	19	*0.7*	40	*4.1*
14-29 d	132	*3.8*	45	*1.8*	38	*3.9*
1- 3 m	888	*25.9*	642	*25.3*	376	*38.9*
4-12 m	544	*15.8*	459	*18.1*	215	*22.2*
>12 m	1759	*51.2*	1361	*53.6*	246	*25.4*
Total	3434	100.0	2541	100.0	967	100.0

Action taken by symptomatics: Of the 3685 symptomatics with sputum collection, information on action taken by them for symptoms was collected in 3679. It was seen that 47% of the symptomatics preferred to go to a private doctor, 23% to the nearest PHI or Taluk Hospital, and 1% took self medication or non allopathic medicine. As many as 28% did not take any action for symptoms.

Prevalence of disease: The table on page 123 gives the prevalence of tuberculosis by age and sex. The overall prevalence was 10.9 per 1000 among persons aged 15 years or more (95% confidence limits: 10.3−11.5). The prevalence increased with age and was about 3 times higher among males (16.3 per thousand) than among females (5.8 per thousand).

Under the District Tuberculosis Programme (DTP), the procedure is to examine a spot specimen for AFB using ZN technique. Considering only the sputum spot specimens collected and examined for AFB at Field Camp, the prevalence of sputum positive disease was 5.0 per 1000 (7.7 per thousand among males and 2.5 per thousand among females). Examination of an additional 'overnight' (ov) specimen for AFB, which is not feasible under DTP conditions, increased the prevalence rate to 6.3 per 1000. Repeating the smear examination of the spot and overnight specimens at the Central Laboratory using Fluorescence Microscopy yielded an additional prevalence of 0.8 and 0.5 cases per 1000 respectively. However, subjecting the sputum specimens for culture examination gave a substantial yield of 133 additional cases which worked out to a prevalence rate of 3.3 per 1000. Of these 133 cases, as many as 83 (62.4%) were culture positive on the spot specimen.

Distribution of sputum positive cases by symptom status: The top table on page 124 gives the distribution of sputum positive cases by symptom status.

It can be seen that of the 3685 symptomatics as many as 3193 (87%) had cough of 14 days or more and contributed 405 (92%) of the 440 sputum positive cases. There were 391 (11%) symptomatics without cough but with chest pain of one month or more and contributed 27 (6%) sputum positive cases. There were only 17 (0.5%) with fever alone and none of them was sputum positive. Others, comprising 84 (2%), yielded 8 (2%) sputum positive cases.

Age group (yrs)	Sex	Examined for		Number Sputum Positive						Rate per thousand
		symp-toms	spu-tum	ZN (spot)	ZN (ov)	FL (spot)	FL (ov)	Sm.-Cul +	All pos	
15-24	M	5678	215	10	1	3	-	4	18	3.2
	F	5844	124	7	2	-	-	4	13	2.2
	T	11522	339	17	3	3	-	8	31	2.7
25-34	M	4880	377	17	1	2	4	7	31	6.4
	F	5205	236	15	5	2	1	8	31	6.0
	T	10085	613	32	6	4	5	15	62	6.1
35-44	M	3812	463	45	6	6	2	23	82	21.5
	F	3557	278	14	6	-	3	3	26	7.3
	T	7369	741	59	12	6	5	26	108	14.7
45 +	M	5249	1199	79	19	14	9	67	188	35.8
	F	6271	793	16	11	5	2	17	51	8.1
	T	11520	1992	95	30	19	11	84	239	20.7
Total	M	19619	2254	151	27	25	15	101	319	16.3
	F	20877	1431	52	24	7	6	32	121	5.8
	T	40496	3685	203	51	32	21	133	440	
	Rate (per thousand)			*5.0*	*1.3*	*0.8*	*0.5*	*3.3*		*10.9*

Distribution of culture positive cases by drug sensitivity status: The table below gives the distribution of culture positive cases by drug sensitivity status to isoniazid, streptomycin and rifampicin. Of the 355 patients for whom drug sensitivity results were available, 234 (65.9%) were sensitive to all three drugs; 27 (7.6%) were resistant to both INH and rifampicin and another 78 (22.0%) to INH; 16 (4.5%) patients were resistant to streptomycin alone - Considering the sexes, 28.3% (75/265) of male and 33.3% (30/90) of female patients were resistant to INH and rifampicin or INH. In all, 17.7%, 29.5% and 7.6% of the patients were resistant to streptomycin, isoniazid and rifampicin respectively.

Symptom	Examined for sputum		Sputum p				
			S+C+	S-C+	S+C-	Total	
	No.	%				No.	%
Cough only of ≥ 14d (C)	1061	*28.8*	41	34	19	94	*21.4*
Chest pain only *of* ≥ 1m (P)	343	*9.3*	1	10	10	21	*4.8*
Fever only of ≥ 1m (F)	17	*0.5*	-	-	-	-	-
C + P	1388	*37.7*	91	56	37	184	*41.8*
C + F	*147*	*4.0*	17	5	1	23	*5.2*
P + F	*48*	*1.3*	1	4	1	6	*1.4*
C + P + F	*597*	*16.2*	71	21	12	104	*23.6*
Haemoptysis	*26*	*0.7*	1	1	-	2	*0.5*
Cough of 7-13d	*28*	*0.8*	-	-	*2*	2	*0.5*
History of Rx	*30*	*0.8*	1	2	1	4	*0.9*
Total	3685	*100.0*	224	133	83	440	*100.0*
C (all)	3193	*86.6*	220	116	69	405	*92.1*
P (without C)	*391*	*10.6*	2	14	11	27	*6.1*
F (without C,P)	*17*	*0.5*	-	-	-	-	-
Others	84	*2.3*	2	3	3	8	*1.8*

Age group (years)	Sex	No. culture positive	Sens. to S,H,R	Resistant to				
				S	H	S,H	H,R	S,H,R
15-24	M	12	10	0	1	1	-	
	F	10	6	2	1	1	-	-
	T	22	16	2	2	2	-	-
25-44	M	93	63	3	11	5	5	6
	F	44	26	3	2	4	4	5
	T	137	89	6	13	9	9	11
45 +	M	160	108	6	25	17	1	3
	F	36	21	2	7	3	1	2
	T	196	129	8	32	20	2	5
Total	M	265	181	9	37	23	6	9
	F	90	53	7	10	8	5	7
	T	355	234	16	47	31	11	16
	%		*(65.9)*	*(4.5)*	*(13.2)*	*(8.7)*	*(3.1)*	*(4.5)*
History of Rx.	No	244	186	11	26	13	5	3
	Yes	111	48	5	21	18	6	13

Resistance to INH was observed in 58 (52%) of the 111 cases with and 47 (19%) of the 244 cases without a history of treatment. Similarly, 38% of the 223 cases positive on both smear and culture were resistant to INH as compared to 15% of the 132 cases who were positive on culture only. While in the former group of patients, the resistance rate significantly differed in patients with (59%) and without (29%) a history of previous treatment, a majority (95%) of patients in the latter group did not give a history of previous treatment.

Sputum positivity and history of treatment: Nearly half (46.0%) of the 224 cases who were positive on both smear and culture gave a history of treatment for tuberculosis. Similar figures for the 83 smear positive, culture negative and 133 smear negative, culture positive cases were 14.5% and 6.0% respectively; 7.7% of the 3245 symptomatics who were sputum negative also gave a history of treatment.

Preferred place of treatment: The table below gives the distribution of persons with a history of anti-tuberculosis treatment according to place of treatment. It can be seen that, of the 334 patients from whom information could be obtained, as many as 194 (58%) went to private agencies for treatment. The remaining 140 (42%) went to government agencies of whom only 27 (8%) went to the nearest PHI.

Place of anti-TB treatment	Reason for preference						Total
	Better facility	No faith in others	Nearest place	Friend/ Doctor advice	Family doctor	N.A.	
Nearest PHI	3	1	20	2	-	1	27
Dist. TB centre	18	1	32	5	-	6	62
Taluk hosp.	9	1	11	1	1	3	26
Other govt. hosp.	8	4	3	7	2	1	25
Private doctor	72	26	6	49	7	12	172
Private hospital	16	2	2	1	-	1	22
Total	126	35	74	65	10	24	334

Reasons for preference: The table above also gives information on the reason for preferring the place of treatment. This information was obtained for 310 of the 334 patients. A majority (92%) of the patients who went to private agencies said that they received better facility (49%) or were advised by a friend, relative or doctor (28%) or had no faith in others

(15%). The reason given for going to a governmental agency was nearest place (51%) followed by better facility (29%).

(started: 1988 ;completed: 1989)

Survey for Tuberculosis in a Tribal Population in North Arcot District

The Jawadhu hills area in North Arcot district has a total population of about 96,000, mainly tribals of the Malayallee clan, distributed in 56 panchayats. Travelling in the area is difficult. The panchayats comprise several villages and hamlets. Many of them are approachable only by walking through kutcha roads or jungle trails. The population is served directly by three Primary Health Centres located in Jamunamarathur, Nammiyampattu and Pudhurnadu. Apart from these, the tribals also seek treatment in the nearest primary health centres on the plain, the ones at Alangayam, Odugathur and Kallapadi being the most popular.

A sample survey for tuberculosis was undertaken in this area. The objectives were to obtain prevalence rates for sputum positive and x-ray positive cases of pulmonary tuberculosis, as well as rates of tuberculosis infection in children below 10 years. It was also felt that doing a survey in a tribal area with hilly terrain, would lead to a better understanding of the difficulties likely to be faced in the implementation of the program.

The survey was conducted in 24 panchayats selected by stratified random sampling out of the total 56 panchayats. The following examinations were done:

1. House to house census and registration of all individuals.
2. Information on symptomatic status, of all individuals aged 15 years or more, and action sought for symptoms.
3. X-ray chest using MMR for all individuals aged 15 years or more.
4. One spot and one overnight specimen of sputum from all individuals with an x-ray abnormality, or with chest symptoms i.e. cough for ≥2 weeks, fever or chest pain for ≥1 month or haemoptysis.
5. Tuberculin testing with 1TU RT23 for all children aged 9 years or less.

The coverages obtained are shown in the table below.

Description	No. eligible	No. examined	Percentage
Tuberculin			
Testing	7420	7409	99.9
Test read	7409	6702	90
X-ray	16049	12746	79
Symptom	16049	15079	94
Sputum	3316	2805	85

It is seen that ≥ 90% coverages have been obtained for collecting information on symptomatic status and for tuberculin testing, and only 79% for x-ray and 85% for sputum. The relatively low coverage for x-ray and sputum is due to the difficult terrain and inaccessible areas that had to be covered on foot. This problem was most pronounced in 5 groups of villages, involving a population size of 2597. In the remaining, the coverages were around 90% for all examinations (see below).

Examination	No.of villages with coverages of			
	≤70%	71-80%	81-90%	>90%
Tuberculin	0	0	12	12
X-ray	4	1	7	12
Symptomatic	0	0	0	24

Five percent children had been vaccinated. The infection rate was estimated to be 6.3% in the unvaccinated.

Age (yrs)	Sex	*Without BCG scar*			*With BCG scar*		
		No. examined	No. reacting	%	No. examined	No. reacting	%
3M-4Y	M	1595	43	*2.7*	136	28	*20.6*
	F	1483	52	*3.5*	121	12	*9.9*
5-9	M	1717	170	*9.9*	43	12	*27.9*
	F	1554	135	*8.7*	53	17	*32.1*
Total	M	3312	213	*6.4*	179	40	*22.3*
	F	3037	187	*6.2*	174	29	*16.7*
G.Total		6349	400	*6.3*	353	69	*19.6*

In all, 151 bacteriologically positive cases had been detected. Their distribution according to the reason for collecting sputum is given in the table below. The bulk of sputum examined is because of symptomatic status.

Criteria for sputum exam.	No. eligible	No. examined (%)	No. positive
Symptom only	2598	2566 (98.8)	85
X-ray only	480	339 (70.6)	30
Both	222	200 (90.1)	36

However, only 36 out of 151 sputum positive cases had both symptoms and x-ray abnormality. In 85, the x-rays were normal; in 30 there were no symptoms. These would have been missed if only x-rays or only symptomatic status was used for screening.

Detailed analysis is in progress.

(started: 1989; completed: 1989)

Study of bacteriological quiescence and relapse in sputum positive pulmonary tuberculosis

This study was conducted in North Arcot district where the Centre has been monitoring Short Course Chemotherapy under programme conditions since 1983. The objective of the study was to estimate the proportion of smear positive patients who remain sputum negative after chemotherapy. All patients for whom treatment was initiated for smear positive pulmonary tuberculosis between 1.4.86 and 31.3.88 were included, irrespective of the regimen and the amount of treatment received. Records of all smear positive patients for whom anti-TB chemotherapy was initiated was obtained from the DTC and arranged talukwise. The PHI's in each taluk were then visited and these lists updated. The study population as identified (from the DTC and the PHIs) is shown below:

Number Registered		4233
Excluded		
Registered more than once	406	
Treated outside	77	876
Had treatment earlier	393	
For Analysis		
SCC	2305	
		3357
Non-SCC	1052	

Of a total of 4233 cards registered, cross indexing and house-visit identification showed that there were several cards for some patients, constituting double (or even triple) registrations. There were 406 such registrations within this period. Treatment had been started before 1.4.86 for 393 patients. Another 77 patients were from outside the district. Thus, 876 cards (21%) registered by the DTC as new cases during that period did not belong to the study population and were excluded. Of the remaining 3357 patients, 2305 had been started on SCC and 1052 on standard regimens.

All these patients were visited in their homes; two sputum specimens (1 spot and 1 overnight) were obtained from available patients, and sent to the laboratory at the Centre for smear and culture examination. At the time of home visit, information on symptomatic status as well as particulars of any other treatment taken were also collected.

Months after start of treatment	SCC No. Eligible	SCC No. Dead	SCC No. Contacted	Non-SCC No. Eligible	Non-SCC No. Dead	Non-SCC No. Contacted
<9	298	38	189	46	8	28
9-11	235	34	143	130	29	73
12-17	615	110	368	234	57	117
18-23	580	135	295	239	75	124
24+	577	126	318	403	115	175
Total	2305	443	1313	1052	284	517

The coverages obtained are shown in the above table. It was found that among 2305 patients for whom SCC was started, it was possible to contact 1313 (57%) and collect sputum from 1,291 patients (98%). Four hundred and forty-three (19%) had died, and 549 (24%) were not available in the address given on the cards. For standard regimens, of 1052 patients, 517 (49%) could be contacted and from 494 (96%) sputum could be collected, 284 (27%) had died and 251 (24%) were not found in the address given on the cards.

Bacteriological status in relation to drug collection: In all, 622 (48%) of 1291 patients in the SCC group and 251 (51%) of 494 patients in the Non-SCC group had completed more than 80% of chemotherapy (see table below). This, however, includes those who have had more than the prescribed doses of chemotherapy. Six hundred and fifty-two out of all 1785 patients (37%) had 50% or less of chemotherapy.

The proportion of cases remaining bacteriologically positive in relation to the amount of chemotherapy (the number of actual drug administrations/collections made divided by the total number of administrations/collections) is given below. It is seen that among 873 patients who had consumed >80% of drugs, 21.8% remain bacteriologically positive. It is also seen that among 652 patients who have collected <50% of treatment, 42.3% remained positive. This is not related to subsequent chemotherapy, as given in the top table on page 130.

	Short-Course Chemotherapy			Non-Short-Course Chemotherapy			All patients		
Drugs consumed/ collected	Total No.	Bact.Pos. No.	%	Total No.	Bact.Pos. No.	%	Total No.	Bact. Pos No.	%
<50%	456	194	*42.5*	196	82	*41.8*	652	276	*42.3*
50-80%	213	72	*33.8*	47	20	*42.6*	260	92	*35.4*
>80%	622	124	*19.9*	251	66	*26.3*	873	190	*21.8*
Total	1291	390	*30.2*	494	168	*34.0*	1785	558	*31.3*

Drugs consumed/ collected	With subsequent chemotherapy No.	% Positive	Without subsequent chemotherapy No.	% Positive
		SCC		
<50%	119	*42.0*	337	*42.7*
>50%	159	*35.8*	676	*20.6*
Total	278	*38.5*	1013	*27.9*
		Non-SCC		
<50%	32	*59.4*	164	*38.4*
>50%	62	*29.0*	236	*28.8*
Total	94	*39.4*	400	*32.8*

These data suggest that a substantial proportion of patients who have been irregular (either on SCC or standard drugs) remain bacteriologically positive even when subsequently treated with INH and thiacetazone.

Mortality: Overall mortality during the period of follow up was 27%. This was not related to age or sex. More than half the patients who had died had less than three months of chemotherapy. The exact cause of death could not be ascertained; it is also not known whether the patients were excreting bacilli at the time of death. However, most of the deaths were attributed to tuberculosis by the relatives.

Resistance to anti-tuberculosis drugs: Resistance to rifampicin either alone or in combination with INH and streptomycin was of the order of 12% among those remaining positive. The figures in those who had SCC and non-SCC were similar, i.e., those who have never had rifampicin also show 11% resistance to rifampicin. Resistance to INH was 66% and to streptomycin 26%; 5-7% of cultures showed resistance to more than one drug. Although this resistance

Initial treatment	No. Examined	Sens. to SHR	Resistance to H	S	R	SH	HR	SHR
SCC	389	136 (35)*	142 (37)	9 (2)	4 (1)	55 (14)	25 (6)	18 (5)
Non-SCC	168	39 (23)	59 (35)	4 (2)	- -	47 (28)	7 (4)	12 (7)
Total	557 **	275	201	13	4	102	32	30

* Figures in parenthesis shows percentages of those examined.

** One SCC patient for whom drug sensitivity results for INH is not available is excluded.

could have been brought about because of irregular chemotherapy, they are high enough to cause worry. A prospective longitudinal study (see below) is being undertaken to verify the rates for qulescence, relapse and resistance obtained in this study.

(started: 1988; completed: 1989)

STUDIES IN PROGRESS

Tuberculosis prevalence survey in North Arcot District

The sample survey for tuberculosis undertaken in Raichur District (see page 120) was extended during the year to North Arcot District in Tamil Nadu. The additional objectives of this survey were to obtain baseline figures for the prevalence of tuberculous infection among children aged below 10 years, and prevalence rates of x-ray positive tuberculosis.

The procedures were similar to those in the Raichur survey (Page 120). In one-third of the selected villages and towns, x-ray examination is done for those aged 15 years or above, irrespective of symptoms. Those whose x-rays are read as abnormal by at least one of 2 readers are eligible for sputum examination. All children aged 3m-9 years are tested with 1TU (RT23).

Of the estimated population of 50 lakhs, a sample of 83,585 was drawn. Of these 35,220 were eligible for x-ray examination. So far, 10,001 individuals have been registered from 5 villages. Of 6447 subjects aged 15 years or more, 5452 (85%) were interviewed. Of 3350 individuals eligible for x-ray examination, 2280 (68%) were x-rayed. In all, 1099 individuals were eligible for sputum examination and sputum was collected from 818 (74%). Of 2435 children eligible for tuberculin testing, 2048 were tested and in 1858 (76%), the reactions were read.

The work is in progress.

(started: 1989; expected year of completion: 1990)

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

The cross sectional study of the bacteriological status of smear positive pulmonary tuberculosis patients started on chemotherapy 6 months to two years earlier showed that about 31% of these patients remained positive. Another finding was that about half the patients among those who had as little as 50% of chemotherapy or less remained bacteriologically negative, even without subsequent chemotherapy (see page 130). In order to have a better understanding of the sputum conversion and relapse rates, and to identify the factors (other than chemotherapy) if any, influencing these, a prospective follow up of all patients for whom anti-tuberculosis chemotherapy is started has been undertaken.

Three busy health institutions (DTC, Arni and Gudiyatham) have been selected for this purpose. All sputum positive patients started on anti-tuberculosis

chemotherapy in these centres and living within a radius of twenty kilometres of the centre are registered for the study. These patients are visited in their homes within ten days of start of treatment and information on the following collected:- Symptoms and their duration, profession, history of previous chemotherapy and awareness about tuberculosis. Weight is also taken. One spot and one overnight specimens of sputum are also collected.

Subsequently these patients will be visited at 3, 6, 9, 12, 18 and 24 months and at each visit, sputum will be collected. Information on the number of defaults and the reasons for the same will also collected at 3, 6, 9 and 12 months. Information on change of treatment, and treatment outside the program is also being collected.

During the year, 137 cases have been registered, and 94 home visits were made. Seven patients could not be identified at the given address and three had moved away; 174 sputum specimens have been collected from the available 84 individuals. The study is in progress, and the intake will last about a year.

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

Assessment of Health Workers and development of case finding methodology in Childhood Tuberculosis

A randomised controlled trial to assess the protective efficacy of BCG against childhood tuberculosis was recommended by the Scientific Advisory Committee. This trial would involve the screening of a large number of children periodically in order that children likely to be suffering from tuberculosis may be picked out and screened further for tuberculosis. An earlier study showed that the field workers of the Centre can be trained to screen children and identify symptoms and signs attributable to tuberculosis (Annual Report 1988). However, the method of examination had not been standardised.

Since it is necessary to have standard methods of examination for any activity involving a large number of investigators, a study was initiated to:

(a) identify signs and symptoms with minimal observer variation,
(b) identify clinical features of children who would need investigations for tuberculosis, and
(c) develop a decision tree for referral of these children for investigations and treatment.

Standard definitions for each of the signs and symptoms have been evolved based on the experience of the previous study. All children aged below 12 years in a given population are examined by the health workers, and the presence or absence of symptoms such as prolonged cough or fever, or recurrent respiratory infections and signs like glandular swelling, or gibbus are being recorded on precoded forms. Children with any abnormality,

along with suitable controls, are being independently examined by a paediatrician and a medical officer. Investigations for tuberculosis are carried out in all children suspected to have the disease by either. This will help to identify signs and symptoms showing minimal observer variation. These can be used in the construction of a decision tree for referral of children for investigation. The validity of the decision tree is to be tested against the results of the investigations.

So far, 736 children have been examined by 4 trainee health workers. Of these, 271 (190 symptomatics and 81 controls) have been examined by the medical officers. The study is in progress.

(started: 1989; expected year of completion: 1990)

Retesting at 20 years for tuberculosis infection rates

Children aged 0-12 years from selected panchayats in Thiruvalangadu and Kadambathur panchayat unions have been tested at 0, 4, 10 and 15 years after the start of the BCG trial in order to see the trend of infection due to tuberculosis in the trial area. In keeping with this, retesting in the population aged 0- 12 years is being carried out in the same panchayats at 20 years. During the period under report, 27 of the 29 selected groups had been covered and 13,738 children aged 0-12 years have been registered. Of these, 12,268 (89%) had been tested with PPD-S and the size of reaction recorded.

The study is in progress.

(started: 1989, expected year of completion: 1990)

STATISTICAL STUDIES

Comparison of the logistic and Cox's regression models under fixed period outcomes

In prospective studies of tuberculosis the outcome is binary and is evaluated in all patients after a fixed time after entry. Response to a treatment is measured by the clinical, radiological, bacteriological, biochemical and immunological changes between the two evaluations. Since it is unethical to subject patients to frequent investigations, the actual response time is not observed accurately.

In this type of prospective study the aim is to estimate the risk of having the event both in the absence and presence of a factor such as a new drug or treatment. The usual method of analysis for data of the above type is to use the logistic regression model which gives estimates of the odds ratio and permits adjustments for relevant covariates. The other model used when the response time to an event is known is the Cox's proportional hazards model. One approach is to use standard computer programs (such as BMDP) for fitting the Cox's model and assume that the follow-up times are identical for all subjects. Such an approach differs from the logistic

approach both in terms of the distributional assumptions and also in terms of the functional relationship with the covariates.

Three measures of risk are discussed in relation to the above models. The first measure is the odds ratio generated by the logistic model and the other two measures are the relative risk and the hazards ratio generated by the Cox (proportional hazards) model. The aim of this paper is to compare the parameter estimates and associated test statistics of the logistic and Cox models when all patients are followed-up for the same period of time. This analysis evaluates the effect of using different models and of making different distributional assumptions for the outcome variable. The findings are illustrated with the data from clinical trials of tuberculosis conducted at the Centre.

For data analysis in prospective studies, the selection of a measure of association is not guided by epidemiologic principles, but by how the outcome is measured. When the outcome is binary (response vs non-response), whether the design is prospective or retrospective, the logistic model is often selected. When not only the occurrence of an event but also the time to an event is known, the proportional hazards model is often employed. It is important that the epidemiological estimates obtained are understood so that correct inferences can be drawn.

Regression models: The type of study under consideration is one in which all subjects are observed for a fixed time interval (0,T]. At the end of this period, the outcome indicating the disease status is known for all patients. In a study with two treatment groups, the probability that the event occurs can be represented by P0 and P1 for the standard treatment group and new treatment group respectively. The results from such a table can be displayed on a table as shown below.

	Outcome		
Group	No event	Event	Total
Standard	R0-M0	M0	R0
New treat.	R1-M1	M1	R1
Total	R - M	M	R

For the logistic model

$$P0 = \exp(\alpha_1)\ [1+\exp(\alpha_1)]^{-1}$$

and

$$P1 = \exp(\alpha_1+\beta_1)\ [1+\exp(\alpha_1+\beta_1)]^{-1}$$

where α_1 is the Intercept parameter and β_1 the regression co-efficient for the treatment variable.

A well known maximum likelihood estimate of the regression co-efficient is the logarithm of the odds ratio, i.e.

$\hat{\beta}1 = \text{Ln}[M1(R0-M0)/M0(R1-M1)]$

with variance estimated by

$\widehat{\text{Var}}(\hat{\beta}) = M0^{-1} + M1^{-1} + (R1-M1)^{-1} + (R0-M0)^{-1}$

For the proportional hazards model of Cox(1972), the hazard for an individual in the standard treatment group at time t is $\gamma(t)$ and for a treated individual is $\gamma(t)\exp(\hat{\beta}2)$ where $\hat{\beta}_2$ is to be estimated from the data. Prentice and Gloeckler (1978) has given the 'grouped data' version of the proportional hazards model. For standard treatment

$Po = 1-\alpha2$ and for the new treatment $P1 = 1 - \alpha2^{**}\exp(\beta2)$

where $\alpha2 = \exp[-\int_0^T \gamma(t)dt]$

The maximum likelihood estimate of $\beta2$ is obtained using

$\hat{\beta}2 = \text{Ln}[\text{Ln}(1-M1/R1)/\text{Ln}(1-M0/R0)]$

and the variance can be estimated by

$\widehat{\text{Var}}(\hat{\beta}2) = \exp(-2\hat{\mu})[M0/(R0(R0-M0)) + M1\ \exp(-2\hat{\beta}2)/(R1(R1-M1))]$

where $\hat{\mu} = \text{Ln}(-\text{Ln}\ \alpha2) = \text{Ln}[-\text{Ln}(1-M0/R0)]$

The parameter estimate, $\hat{\beta}2$ is the log of the hazard ratio.

An alternative estimate of β for discrete data obtained by maximizing the likelihood function is (Breslow 1974)

$\hat{\beta}3 = \text{Ln}(M1R0/M0R1)$

and the estimate of the asymptotic variance is

$\widehat{\text{Var}}(\hat{\beta}3) = (1/M1) + (1/M2)$

Thus the estimate of the log of the hazard ratio is exactly equal to the log of the relative risk.

The logistic and Prentice-Gloeckler models assume an underlying binomial distribution while Breslow's development of the Cox model assumes an underlying exponential distribution.

Comparison of parameters: The relation between the logistic and Cox model parameter estimates is displayed in the table on page 136.

P1	P0										
	5	10	15	20	30	40	50	60	70	80	90
10	3.8										
15	4.9	6.7									
20	6.0	8.1	9.7								
25	7.0	9.4	11.4	13.2							
35	9.3	12.1	14.4	16.6	20.9						
45	11.7	15.0	17.8	20.3	25.2	30.2					
55	14.6	18.4	21.6	24.5	30.3	35.7	41.8				
65	18.0	22.5	26.2	29.5	35.8	42.2	49.1	57.0			
75	22.6	27.9	32.2	36.0	43.3	50.7	58.5	67.4	78.2		
85	29.6	36.0	41.2	45.8	54.5	63.1	72.3	82.6	95.1	111.8	
95	44.8	53.6	60.6	66.8	78.2	89.4	101.2	114.3	130.1	150.8	183.9

The percent relative differences are the relative bias, since the maximum likelihood estimators are consistent. The agreement between the two estimates depends on P0 and to some extent P1/P0; when P0 is small (i.e. $<10\%$), the difference is $<10\%$ for a wide range of P1/P0. When the proportion with the event in the standard group is $\geq 15\%$, the logistic estimate is always greater than the proportional hazards estimate. Green and Symons (1983) showed that the logistic regression co-efficient will approximate that of the Cox model only when the follow-up time is short and the event is rare. With extended follow-up, the odds ratio is a poor estimator of the hazard ratio.

Similarly, it can be shown that the log relative risk is an inconsistent estimator of the log hazard ratio. For $P0 < P1$ the logarithm of the relative risk will be smaller than the logarithm of the hazard ratio, but the estimates converge as the event rate decreases.

The relationship between the odds ratio and the relative risk was examined by Cornfield (1951) and he noted that the odds ratio is a good approximation to the relative risk only when the event is rare. Here all the three measures of association are in good agreement for rare events. The logistic parameter is the largest in absolute magnitude, followed by the log hazard ratio and then the log relative risk.

Applications: Data from pulmonary and extrapulmonary tuberculosis studies are used to illustrate the comparisons among models.

The following table gives the bacteriological relapse during a 4-year period (Nazareth et al, 1971).

Treatment	No. of pts	Relapse	
		No.	%
Placebo	118	23	19
INH	127	12	9

The summary of the analysis of that data is shown in the table below.

Statistic	Logistic	Prentice-Gloeckler	Breslow
β	0.84	0.78	0.72
Var(β)	0.15	0.13	0.13
Risk	2.32	2.18	2.06
Wald	4.85	4.81	4.14

From the table we see that the different models give similar results when comparing 9% with 19%.

In the next application we consider data from a spinal tuberculosis study (ICMR/BMRC,1989). The table below gives the response status at the end of 36 months.

Treatment	No. of pts.	Status at 36 m	
		No.	%
R6	85	68	80
A6	83	72	87
A9	92	88	96

The summary of the analysis of the above data are given below.

From the above table we see that, the variance of the estimate $\hat{\beta}$ of Breslow is the lowest among the three. When comparing R6 Vs A6 all the three models give similar results in terms of risk and Wald's test. For the comparison of R6 with A9 the relative risk estimates are widely different for logistic and Prentice-Gloeckler. However the significance of the treatments is not affected; in contrast the Breslow's version gives a non-significant chi-square (Wald). Similar conclusions are obtained when comparing A6 with A9.

In summary, the proper measure of association in a prospective study is the relative risk. For a study with identical follow-up, the Breslow model will yield the estimate of relative risk for a 2x2 table. However the corresponding test statistic is conservative compared with the grouped data version of Cox's model. The odds ratio is only an approximation of the relative risk and useful only when the time period is short and the event is rare. Logistic

Statistic	Logistic	Prentice-Gloeckler	Breslow
		R6 Vs A6	
β	0.49	0.23	0.08
Var(β)	0.18	0.04	0.03
Risk	1.64	1.26	1.08
Wald	1.36	1.38	0.23
		R6 Vs A9	
β	1.70	0.67	0.18
Var(β)	0.33	0.04	0.03
Risk	5.50	1.95	1.20
Wald	8.68	10.47	1.22
		A6 Vs A9	
β	1.21	0.44	0.10
Var(β)	0.37	0.04	0.03
Risk	3.36	1.55	1.10
Wald	4.01	4.42	0.38

parameter has the largest absolute value and Breslow parameter has the least among the three. In conclusion, the Cox's model (Prentice-Gloeckler) seems to the more appropriate in the comparison of events with high probability.

(started : 1989, completed : 1989)

Estimation of error rates in medical screening procedures

Medical screening tests for drug use or exposure to AIDS antibodies have become more wide spread. Many concerns have been expressed about their routine use. The main concern arises because the prevalence of the disease in the general population is far less than that in a prescreened group, e.g., persons in a high risk group may give a high fraction of false positive classifications among the test results. All the screening procedures discussed in the standard texts assume that the accuracy rates - the sensitivity and specificity of the test and the prevalence rate of the disease in the population to be tested, are known. The traditional false negative rate or Neyman-Pearson type I error is defined as the proportion of "diseased" individuals who are negative on screening, or (1- sensitivity); the false positive rate, analogous to type II error, is defined as the proportion of "non-diseased" individuals who are positive on screening, or (1-specificity). These rates do not depend on prevalence.

The main purpose of this paper is to show the effect of the accuracy rates on the inference one can draw from a screening test. The ELISA test is used to screen blood for antibodies to the AIDS virus. An empirical study is considered in this paper to evaluate the predictive value of the test and

the components of the standard errors. It has been shown that the predictive value of the screening test does not depend on the sensitivity or specificity of the test alone, but far more on the prevalence of the disease in the population examined.

The Mathematical Frame Work : The purpose of a screening test is to determine whether a person belongs to class (D) of people who have a specific disease. The test result indicating that a person is a member of the class (D) will be denoted by S and a result indicating nonmembership by $\overline{S}$. The accuracy of a test is specified by two probabilities.

$\theta 1 = P[S/D] =$ the probability a person with disease is correctly diagnosed, called the sensitivity of the test, and

$\theta 2 = P[\overline{S/D}] =$ the probability a disease-free individual is correctly diagnosed, called the specificity of the test.

Here our focus is on the conditional probability P[D/S] that a person whom the test indicates as having the disease actually has it. Letting $\pi = P(D)$ denote the prevalence of the disease in the population tested, using Bayes' theorem, we have

$$\begin{aligned} P[D/S] &= P[D \cap S]\ P[S] \\ &= \pi\theta 1\ /(\pi\theta 1 + (1-\pi)\ (1-\theta_2)) \\ &= \pi\theta 1\ /(\pi(\theta 1 + \theta 2 - 1) + (1-\theta_2)) \end{aligned}$$

This probability is called the predictive value of a positive test (PVP).

In many mass screening programmes the prevalence π may vary among sub-populations so that it must be estimated separately in each one. This is accomplished by using the fact that the probability that a person tested will be diagnosed as ill is

$$\begin{aligned} p = P[S] &= \pi P[S/D] + (1-\pi)\ P[S/\overline{D}] \\ &= \pi\theta 1 + (1-\pi)\ (1-\theta_2) \end{aligned}$$

When a sample of n persons are tested, one estimates p by the proportion $\hat{p}$ of those who are classified in S. Solving for π in the above equation yields the estimate of the prevalence given by

$$\hat{\pi} = (\hat{p} + \theta_2 - 1)\ /\ (\theta_1 + \theta_2 - 1)$$

From this the estimate $\hat{C}$ for P[D/S] is given by

$$\hat{C} = 1/\hat{p}$$

The estimate of the prevalence may not be between 0 and 1 if θ_1 and θ_2 are low. To avoid such problems, a truncated version $\pi 1$ of π as $\pi 1 = \min(\max(\hat{\pi}, 0))$ is considered.

When θ_1 and θ_2 are not known but are estimated based on samples of size n1 and n2, where the screening test is used on persons whose disease status is known, we replace θ_1 and θ_2 by their estimated values. The asymptotic variance of $\hat{C}$ is given by $\{\theta_1(1-\theta_2)/(p(\theta_1+\theta_2-1)\}^2 \{p(1-p)/np^2\}$ $+\{\pi(1-\theta_2)/p(\theta_1+\theta_2-1)\}^2 \{\theta_1(1-\theta_1)/n_1\} + \{\theta_1(1-\pi)/p(\theta_1+\theta_2-1)\}^2 \{\theta_2(1-\theta_2)/n_2\}$

When $\theta 1$ and $\theta 2$ are known, the last two terms vanish. These terms reflect the variability in our estimate of the true positive rate due to uncertainty about the true value of the specificity and sensitivity of the screening test. From the formula we realise that when sensitivity and specificity are high, but the prevalence π is low, both the first and third terms increase, so that the use of screening tests on groups that have a low prevalence rate will often yield a low predictive value of positivity that has a large standard error.

Application to ELISA test for AIDS: The ELISA test for AIDS is used to screen donated blood for the AIDS antibody. An evaluation of the test yielded sensitivity = .977 and specificity = .926 (Weiss et al. 1988). The table below presents the estimated PVP, i,e., $E(\hat{C})$ and its approximating variances as a function of the prevalence rates and number tested. The proportion of variance of C that is due to uncertainty of θ is also given. From the table we see that the PVP is 90% or more only if the prevalence of the disease is 40% or more. When the disease prevalence is very low (e.g. AIDS) say 1% or less, the predictive value is as low as 12% and the variance due to estimation increases as the prevalence decreases.

Approximate variances of the estimated true positive rate $\hat{C}$ when $\theta 1 = 0.977$, $\theta 2 = 0.926$ as a function of prevalence, sample sizes n1, n2 and size of the population to be screened are tabulated on page 141.

Prevalence π	$E(\hat{C})$	$Var(\hat{C}) \times 10^{-2}$ ($\theta 1$, $\theta 2$ known)	$Var(\hat{C}) \times 10^{-2}$ ($\theta 1$, $\theta 2$ estimated)	%Var.due to estimation
	n = 500	n1 = 100	n2 = 300	
0.50	0.93	0.65	1.69	85.8
0.40	0.90	0.94	2.44	85.1
0.30	0.85	1.43	3.62	84.3
0.20	0.77	2.41	6.74	82.0
0.10	0.59	4.91	10.22	76.9
0.08	0.53	5.92	11.87	75.1
0.05	0.41	8.17	15.39	71.8
0.03	0.29	10.56	18.91	68.8
0.02	0.21	12.21	21.26	67.0
0.01	0.12	14.33	24.20	64.9

The estimated PVP values for different sample sizes screened and accuracy rates are given below.

Prevalence π	$E(\hat{C})$	$Var(\hat{C})\times10^{-2}$ ($\theta1$, $\theta2$ known)	$Var(\hat{C})\times10^{-2}$ ($\theta1$, $\theta2$ estimated)	%Var.due to estimation
	n = 5000	n1 = 100 n2 = 300	$\theta1$ = .95	$\theta2$ = .95
0.50	0.95	0.15	1.34	98.9
0.40	0.93	0.22	1.96	98.6
0.30	0.89	0.34	2.93	98.5
0.20	0.83	0.59	4.66	98.3
0.10	0.68	1.32	8.64	97.7
0.08	0.62	1.64	10.15	97.4
0.05	0.50	2.42	13.50	96.8
0.03	0.37	3.36	17.07	96.1
0.02	0.28	4.06	19.57	95.7
0.01	0.16	5.05	22.85	95.1

Prevalence π	$E(\hat{C})$	$Var(\hat{C})\times10^{-2}$ ($\theta1$, $\theta2$ known)	$Var(\hat{C})\times10^{-2}$ ($\theta1$, $\theta2$ estimated)	%Var.due to estimation
	n = 5000	n1 = 100 n2 = 300	$\theta1$ = .98	$\theta2$ = .98
0.50	0.98	0.06	0.83	99.4
0.40	0.97	0.09	1.23	99.4
0.30	0.95	0.14	1.88	99.4
0.20	0.92	0.26	3.12	99.3
0.10	0.84	0.69	6.44	98.9
008	0.81	0.91	7.89	98.7
0.05	0.72	1.57	11.63	98.2
0.03	0.60	2.61	16.61	97.5
0.02	0.50	3.65	20.95	97.0
0.01	0.33	5.59	28.16	96.1

To study the effect of n, n1, n2, $\theta1$, and $\theta2$, an empirical study is done varying the ranges of the above parameters. The sample size for screening is varied from 500 to 50,000 in increments of 500; n1, n2 are varied from 50 to 500 the increments being 50, and $\theta1$, $\theta2$ are varied from .85 to .99 and π is varied from .5 to .01. Some of the results are presented in the table below. From the table we see that the effects of sampling variability in the estimates of sensitivity and specificity on the variances of $\hat{C}$ are not affected by the size of the population screened.

We present the PVP(%) corresponding to the prevalence of 1% ($\pi = 0.01$) of the disease in the table on page 142.

		Sensitivity			
		0.85	0.90	0.95	0.99
Specifi-city	0.85	5	6	6	6
	0.90	8	8	9	9
	0.95	15	15	16	17
	0.99	46	48	49	50

From the table we note that when $\pi = 0.01$ the estimated PVP value is less than 50% indicating a rate of false positives exceeding 50%. Also when the specificity is 95% or less, even a test with 99% sensitivity will yield very poor results. On the other hand when the specificity is 99% the estimated PVP is considerably increased irrespective of the sensitivity.

Conclusions: The results show that when the prevalence is very high (ie., ≥40%) the expected value of $\hat{C}$ is very high and its standard error is small even accounting for uncertainty of the specificity and sensitivity. When π is small, not only $\hat{C}$ is less than 0.5 indicating a rate of false positives exceeding 50%, its standard error is also quite high. The effect of sampling variability in the estimates of sensitivity and specificity on the standard error of $\hat{C}$ is not affected by the size of the population screened.

The results also indicate that the sample sizes used to determine the specificity of a screening test may need to be increased. The optimal allocation of subjects used to determine the accuracy rates of the screening test related to the prevalence rate in the population, is given by

$$n1/n2 \simeq (\pi/(1-\pi)) \sqrt{((1-\theta_1)(1-\theta_2)/\theta_1\theta_2)}$$

If one desired to estimate the prevalence or predictive negative rate most efficiently, the optimal allocation is given by

$$n1/n2 \simeq (\pi/(1-\pi)) \sqrt{(\theta_1(1-\theta_1)/\theta_2(1-\theta_2))}$$

Similarly for estimating the false negative rate the optimal allocation is

$$n1/n2 \simeq (\pi/(1-\pi)) \sqrt{(\theta_1\theta_2 \ / \ (1-\theta_1)(1-\theta_2))}$$

Successive multiple screening of test positives can reduce the number of false positives, but it does not reduce false negatives. In populations where the overall prevalences are high, even less perfect tests will yield adequate predictive values.

(started : 1989; completed : 1989)

ELECTRONIC DATA PROCESSING

An important development during the year has been the formation of an Electronic Data Processing (EDP) department in July 1989, which will plan and organise, in a phased manner, overall computerization (data entry and verfication, data procesing and statistical analyses) of all the major research activities of the Centre.

Main-frame computer: The main memory for the VAX 750 computer system has been enhanced to 10 Mb by the addition of an 8 Mb primary memory control board. A new RA82 hard disk of 622 Mb capacity and a BMDP statistical software system have also been installed.

The VAX computer system continues to be used for analysing the voluminous data generated by the Chengalpattu BCG trial. About 20 lakh records from 140 tuberculin negative groups were processed to check for completeness as well as consistency of data for each individual, both within and between rounds, and corrections were incorporated wherever necessary. All culture positive cases from the 140 panchayats were listed and the results verified against original entries in the registers. About 13 lakh records were converted from EBCDIC format to ASCII representation. Key to the "vaccine" codes were also transferred to the intake analysis records of about 1.3 lakh individuals for decoding purposes.

For the data generated from three sample surveys carried out in rural, semi-urban and metropolitan areas among chest symptomatics, data processing was done and tables were obtained. Computerization of District Tuberculosis Programme (DTP) has been taken up.

Personal Computer: Several personal computers, available in the different departments, are being increasingly utilised for storing data from studies or experiments with limited numbers, tabulation and detailed analyses, and graphical representations. For preparation of the Annual Report, the entire text was stored on floppy diskettes using Word Processing software. The diskettes were handed over to the printers for obtaining laser prints and offset printing. This has enormously reduced the amount of time and effort spent in checking and correcting proofs at different stages.

Since a large number of staff members were interested in learning about computers and utilising them for their work, a one-month training course was organised, with introductory lectures by experienced staff members. A wide range of topics were covered, namely, (1) general introduction to personal computers, (2) key board operations, (3) data entry, data verification (Datastar), (4) basic concepts in programming (BASIC), (5) spread-sheets (Lotus 1-2-3), (6) data base management (dBASE III), (7) word processing (Word Perfect), and (8) statistical analysis (STATPAK, EPISTAT).

In addition, limited hands-on-training was given to small groups. An evaluation of the training course was undertaken by using multiple-choice question and feed back forms. The training course evoked enthusiastic response and has increased the awareness of the importance, role and limitation of computers among staff members and helped them to utilise computers for their scientific studies.

APPENDICES

TRAINING PROGRAMMES

WHO fellows

Mr. Hari Govind Shrestha and Mr. Tara Prasad Shrestha, Nepal for two weeks from 3-10-89.

Trainees

The following underwent training in different departments as follows:

Bacteriology

Dr.S.P.S. Nain, Asst. Scientist, from the College of Veterinary Sciences, Haryana from 28-4-89 to 4-5-89.

Twelve M.Sc. (Microbiology) final year students from Gulbarga University, Gulbarga, from 6-12-89 to 8-12-89.

Immunology

Miss.T. Bhooma, PG student from Madras University for 4 weeks in April and May 1989.

Mr.K. Anand Kumar, Research Fellow from Vector Control Research Centre, Pondicherry, for 10 days from 1-9-89.

Cardio-Pulmonary Medicine

M.Phil students from Dr.A.L.M. Post Graduate Institute of Basic Medical Sciences, Taramani, Madras on 17-2-89.

Dr.Sanjay Gupta and Dr.Surajajit Kar from Calcutta Hospital and Medical Research Institute, Calcutta, from 31-3-89 to 1-4-89.

Dr.N.Sankaran from Tirunelveli Medical College, Tirunelveli, from 17-7-89 to 22-7-89.

Dr.A.S. Natarajan from Govt. Thiruvotteswarar Hospital of Thoracic Medicine, Madras, from 7-8-89 to 12-8-89.

Dr.Dinesh Prabhu from Trichur Medical College, Trichur, from 30-11-89 to 8-12-89.

General

Dr.K. Rajendran, PG student, Thanjavur Medical College, Thanjavur, from 6-1-89 to 13-1-89.

Dr.S. Mahadevan, Asst. Professor, JIPMER, Pondicherry, for 1 week in February 1989.

Dr.S. Pinitsoontorn and Dr.S. Srisaenpang, Dept. of Community Medicine, Khon Kaen University, Thailand, from 28-2-89 to 10-3-89.

Nine DPH students from Madras Medical College, Madras, from 16-3-89 to 23-3-89.

Others

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

Medical students

Raja Muthiah Medical College, Annamalai University, Annamalai Nagar - 1 batch.

Post-graduate students

Three M.D.(TB) students and eight PG students (DTRD 2nd year) from Institute of Thoracic Medicine, Madras.

Nine PG students undergoing diploma in Public Health course at Institute of Community Medicine, Madras Medical College, Madras.

Dr.Shyam Sundar and Dr.Rupa Mokkapattal from Andhra Pradesh Chest Hospital, Osmania Medical College, Hyderabad, Andhra Pradesh.

Dr.Vikram Reddy, Dr.V.Gopalakrishnaiah and Dr.B.V.L.N.Raju from Kakatiya Medical College, Warangal, Andhra Pradesh.

Two M.Sc. (Microbiology) and two M.D. (Microbiology) students from Christian Medical College, Vellore.

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

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 - 2 batches.

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

Multi-purpose Health Workers from Seva Samajam Girls' Training Institute, Madras - 1 batch.

M.A. (Sociology) students from Madras School of Social Work, Madras - 2 batches.

Medical Social Work students from Loyola College of Social Sciences, Trivandaram, Kerala - 1 batch

ICMR-WHO SEMINAR AND WORKSHOP

A 2-day Seminar was held on the 24th and 25th of February 1989, for Professors of Medical Colleges, and Joint Directors (TB) and State TB Officers from the 18 districts where the Short Course Chemotherapy programme is being monitored by the Centre. Simultaneously, a Workshop was held for Laboratory Technicians and Statistical Assistants, with demonstrations in addition to lectures. The subjects covered and the particlpants ara given below.

Seminar for Medical Officers

Subject	Speaker
Diagnosis	Prof.K.Jagannath, Director, Institute of Thoracic Medicine, Madras.
Bacteriology of tuberculosis	Dr.C.N.Paramasivan, Deputy Director, Tuberculosis Research Centre, Madras.
Rationale of chemotherapy of TB	Dr.S.P.Tripathy, Addl. Director-General, Indian Council of Medical Research, New Delhi.
SCC - an overview	Dr.R.Prabhakar, Director, Tuberculosis Research Centre, Madras.
Treatment of failures	Dr.Rani Balasubramanian, Senior Research Officer, Tuberculosis Research Centre, Madras.
TB spine	Dr.R.Parthasarathy, Emeritus Medical Scientist, Tuberculosis Research Centre, Madras.
TB lymphadenitis	Dr.M.S.Jawahar, Senior Research Officer, Tuberculosis Research Centre, Madras.
TB meningitis	Dr.Padma Ramachandran, Asst. Director, Tuberculosis Research Centre, Madras.
Adverse reactions in clinical practice	Dr.K.C.Mohanty, Dept. of Tuberculosis and Respiratory Diseases, Grant Medical College and Sir J.J.Group of Hospitals, Bombay.
Bio-chemical/ pharmacological aspects of adverse reactions to anti-TB drugs	Dr.G.Raghupati Sarma, Deputy Director, Tuberculosis Research Centre, Madras.

Subject	Speaker
Social aspects of TB control	Dr.T.S.Natarajan, Social Scientist, Tribal Research Centre, Tamil University, Uthagamandalam.
Evaluation of the programme	Dr.S.Radhakrishna, Director, Institute for Research in Medical Statistics (Madras chapter), Madras.
NTP - an overview	Dr.K.Chaudhri, Director, National Tuberculosis Institute, Bangalore.
DTP - rationale and concepts	Dr.T.N.Nachinarkiniyan, Joint Director (TB), Govt. of Tamil Nadu, Madras.
Programme implementation	Dr.N.M.Sudarsanam, Asst. Director,Tuberculosis Research Centre, Madras.
Documentation	Mr.P.R.Somasundaram, Deputy Director, Tuberculosis Research Centre, Madras.
Management of 'suspects'	Dr.P.Jagota, National Tuberculosis Institute, Bangalore.

Common Programme for Laboratory Technicians and Statistical Assistants

Problems of tuberculosis in India -SCC In DTP	Dr. N. M. Sudarsanam
Case finding and case holding in the programme	Dr.Rajeswari Ramachandran
Documentation	Mr.P.R.Somasundaram

Programme for Laboratory Technicians

Sputum microscopy in DTP	Mr.P.Venkataraman
General principles of microscopy	Dr.N.Selvakumar
Maintenance of microscopes	Mr.C.Alexander

Demonstrations

on:	by:
Staining/examinations	Dr.N.Selvakumar, Dr.Vanaja Kumar, Mr.P.Venkataraman, Mr.C.Alexander, Mr.B.N.Gopalan and Mrs.Sara Mathew
Maintenance of microscopes	Mr.C.Alexander
Documentation	Mr.A. S. L. Narayana and Mr.S.Sivasubramanian
Techniques of smear taking, fixing and staining	Dr.N.Selvakumar, Mr. P. Venkataraman and Mr.C.Alexander
Smear reading	Dr. Vanaja Kumar, Mr. B. N. Gopalan and Mrs.Sara Mathew

Programme for Statistical Assistants

Subject	Speaker
Various registers and cards	Mr.S.Sivasubramanian
Monthly, quarterly and annual returns	Mr.A.S.L.Narayana
Use of computers for scrutinising, analysis and presentation	Mr.P.V.Krishnamurthy
Cohort analysis principles	Mr.G.S.Acharyulu
Monitoring of returns	Mr.B.Janardhanam

Demonstrations

on:	by:
Reporting procedures	Mr.V.Chandrasekaran
Analysis of sample treatment cards	Mr. B. Janardhanam and Mr.V.Chandrasekaran
Scrutiny of returns	Mr. A. S. L. Narayana and Mr.S.Sivasubramanian
Computerisation of data	Mr.P.V.Krishnamurthy

INDO-US WORKSHOP ON MAJOR ADVANCES IN TUBERCULOSIS RESEARCH

A workshop for scientists from India and the United States of America was held at the Centre from the 4th to the 7th December 1989, with the main objective of tackling the common problems encountered in the diagnosis and management of tuberculosis, utilising newer technologies and strategies. The participants represented various disciplines including chemotherapy, epidemiology, behavioral sciences, pulmonary medicine, pharmacology, biochemistry, microbiology, immunology and molecular biology. There were 78 participants, 14 from the United States of America and 64 from India.

The aim of the multi-disciplinary approach to the Workshop was to identify areas of major thrust in the respective disciplines and have detailed discussions, which could lead to development of protocols for bilateral studies.

The deliberations of the workshop were useful for initiating collaborative studies, and also for transferring the newly-available technologies to the laboratories in India with major interests in tuberculosis research.

STAFF DEVELOPMENT PROGRAMME

1. Dr. Sulochana Das underwent a 2-week training course on "B-cell biology" sponsored by the Department of Biotechnology, New Delhi, at Madurai Kamaraj University, Madurai, during January-February, 1989.
2. Mr.M. Nagarajan was awarded a 2-month W.H.O. Fellowship to undergo training in "Use of modern electronic computers for processing of morbidity, mortality and health related data", at the Centre for Health Statistics, Lansing, Michigan, U.S.A., from July, 1989.
3. Mr.K. Thyagarajan was awarded a 10-week W.H.O. Fellowship to undergo training in "Use of modern electronic computers for processing of morbidity, mortality and health related data" at the State Centre for Health Statistics, Raleigh, North Carolina, U.S.A., from July, 1989.
4. Dr.Prema Gurumurthy underwent a 3-day course on the "Principles of Practice of Liquid Chromatography (HPLC)" conducted by Waters Instruments Pvt. Ltd., at Bangalore, during August, 1989.

PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

Name of the conference, venue and date	Title of paper	Name of staff member
28th National Conference of National College of Chest Physicians, New Delhi, 10-12 January, 1989	Membrane diffusing capacity(Dm) and pulmonary capillary blood volume(Vc) in untreated tropical eosinophilia	Dr.V.K.Vijayan
South Zonal Conference of the Indian Association of Medical Microbiology, Annamalai University, Chidhambaram, 29 January, 1989	Bacteriological investigations for Short Course Chemotherapy under District Tuberculosis Programme conditions	Dr.C.N.Paramasivan
-do-	Cetylpyridinium chloride (CPC) as a preservative for the storage of sputum samples at ambient temperature	Dr.N.Selvakumar
-do-	Isolation of the catalase gene from *Mycobacterium tuberculosis* by constructing libraries in lambda phage vectors	Mr.Daniel Herbert
Southern Railway TB Association, Railway Hospital, Perambur, Madras, 16 August, 1989	The present status of treatment of tuberculosis	Dr.M.S.Jawahar
XIV Annual Conference of the Indian Immunology Society, Sewagram, Maharashtra, 12-14 October, 1989	Immuno-histology of tuberculous lymphadenitis	Dr.V.D.Ramanathan

Name of the conference, venue and date	Title of paper	Name of staff member
XIV World Congress of Neurology, New Delhi, 22-27 October, 1989	Short Course Chemotherapy in the treatment of brain tuberculoma - a controlled clinical trial	Dr.Rajeswari Ramachandran
XVI Biennial Conference of the Indian Association of Leprologists, Trichur, 10-12 November, 1989	Boosting effect of Rees' skin test on reactions to a repeat skin test with the same antigen (poster presentation)	Mr.R.S.Vallishayee
- do -	An immuno-histo-chemical study of neuritis in leprosy	Dr.V.D.Ramanathan
First Joint Conference on Tuberculosis and Chest Diseases (Continuing Medical Education Programme), Madras, 11 December, 1989	Role of short course chemotherapy in sputum negative pulmonary tuberculosis	Dr.R.Prabhakar
- do -	Newer anti-mycobacterial drugs and their role in the treatment of patients with pulmonary tuberculosis	Dr.C.N.Paramasivan
- do -	Basic principles of statistics in medicine	Mr.P.Venkatesan
First Joint Conference on Tuberculosis and Chest Diseases, Madras, 11-14 December, 1989	Long-term follow-up (4 1/2 - 8 years) of children treated for tuberculous meningitis in South India	Dr.Padma Ramachandran
- do -	Single-breath diffusing capacity in healthy young adult (15-40 years) South Indians	Dr.V.K.Vijayan
- do -	Sample survey of awareness of symptoms and utilisation of health facilities by chest symptomatics	Mrs.K.Thilakavathy

Name of the conference, venue and date	Title of paper	Name of staff member
First Joint Conference on Tuberculosis and Chest Diseases, Madras, 11-14 December, 1989	Controlled clinical trial in the treatment of tuberculosis of spine without paraplegia	Dr.A.M.Reetha
-do-	Determination of the isoniazid acetylator phenotype of adults and children based on salivary concentrations of the drug	Mr.S.Kallasam
-do-	Adrenocortical function in patients with pulmonary tuberculosis	Mrs. Chandra Immanuel
-do-	Drug sensitivity pattern of *M.tuberculosis* isolates from South Indian patients to rifapentine, rifabutin, 4- fluoroquinolones and capreomycin (poster presentation)	Mr.P.Venkataraman
First Joint Conference on Tuberculosis and Chest Diseases, Madras: Symposium on the use of therapeutic aerosols in the management of lung diseases, 14 December, 1989	Merits and demerits of various devices used for aerosol therapy	Dr. V.K.Vijayan
IX National Congress on Respiratory Diseases (Continuing Medical Education Programme), Hyderabad, 17 December, 1989	Exercise testing and its limitations	Dr.V.K.Vijayan
-do-	Problems in the re-treatment of multi-drug resistant TB	Dr.M.S.Jawahar

Name of the conference, venue and date	Title of paper	Name of staff member
IX National Congress on Respiratory Diseases, Hyderabad, 17-20 December, 1989.	Changes in lung function and their response to treatment in Tropical Eosinophilia	Dr.V.K.Vijayan
IX National Congress on Respiratory Diseases, Hyderabad: Symposium on "Respiratory Infections and Newer Chemotherapeutic Agents", 20 December, 1989	Basis for approach to anti-tuberculosis chemotherapy	Dr.M.S.Jawahar

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
International Clinical Epidemiology Network VII, Goa, 23-28 January, 1989	Dr.Manjula Datta	Signs of severity in acute lower respiratory infections in children
Short term training course on application of monoclonal antibodies in early diagnosis of malignant diseases, Cancer Institute, Madras, 6 February, 1989	Dr.Ramesh S.Paranjape	Immunological technique with special reference to immunoblotting
Short term training programme-cum-workshop, Cancer Institute, Madras, 15-18 March, 1989.	Dr.Manjula Datta Mr.P.V.Krishnamurthy	
Workshop at Centre for Biotechnology, Anna University, Madras, March, 1989	Dr.Rajiswamy	Tuberculosis - an overview
-do-	Dr.Sujatha Narayanan	Development and handling of molecular probes for the diagnosis of tropical diseases
Workshop on Epidemiology for Clinicians, Madras Medical College, Madras, 2-3 May, 1989	Dr.Manjula Datta Mr.P.V.Krishnamurthy	
Informal consultation on clinical and field trials of ivermectin for lymphatic filariasis, TDR/WHO, Geneva, 24-26 May, 1989	Dr.V.Kumaraswami	Ivermectin trials in India

Name of the event, venue and date	Name of staff member	Title of paper
Joint meeting of the International Child-Health Society and the Royal Society of Hygiene and Tropical Medicine, London, 26 October, 1989	Dr.Manjula Datta	Epidemiology of acute lower respiratory infections in children in South India
Two workshops on 'Research Methodology' for post-graduate students at Madras Medical College, 4-7 October and 7-9 November, 1989	Dr.Manjula Datta Mr.P.V.Krishnamurthy	
Indo-EEC Symposium on Leprosy and Other Mycobacterial Diseases, Cancer Research Institute (Tata Memorial Centre), Bombay, November 6-9, 1989	Dr. Ramesh S.Paranjape	Immunodiagnosis in tuberculosis
-do-	Dr.Rajiswamy	Macrophage functions in human tuberculosis
Workshop on 'Research Methodology' for Professors of Medicine (by MGR Medical University) at the Institute of Child Health and Hospital for Children, Madras, 18-20 December, 1989	Dr.Manjula Datta Mr.P.V.Krishnamurthy	
Workshop on DNA diagnosis and Filariasis, WHO and New England Bio Labs., Jakarta, 18-20 December, 1989	Dr.V.Kumaraswami	New horizons in the treatment of filariasis

LIST OF PUBLICATIONS

Papers published

1. Kannapiran, M., Chandra Immanuel, Krishnamurthy, P.V. and Raghupati Sarma, G. C-reactive protein levels in patients with pulmonary tuberculosis. *Lung India,* 1989, *7,* 34-36.
2. Raghupati Sarma, G. Isoniazid acetylator phenotype. *Indian Pediatrics,* 1989, *26,* 197-198.
3. Sanjeevi, C.B. and Narayanan, P.R. Antifilarial and anti- PPD IgM antibodies in cord blood. *Indian Journal of Pediatrics,* 1989. *56, 207-211.*
4. Kuppu Rao, K.V. and Vijayan, V.K. Maximal expiratory flow volume loop in South Indian sportsmen. *Indian Journal of Physiology and Pharmacology,* 1989, *32,* 93-99.
5. Vijayan, V.K. and Kuppu Rao, K.V. Membrane diffusing capacity (Dm) and pulmonary capillary blood volume (Vc) in untreated tropical eosinophilia (Abstract). *Indian Journal of Chest Diseases and Allied Sciences,* 1989, *31,* 51-52.
6. Indian Council of Medical Research/British Medical Research Council Working Party Study. A controlled trial of short-course regimens of chemotherapy in patients receiving ambulatory treatment or undergoing radical surgery for tuberculosis of the spine. *Indian Journal of Tuberculosis,* 1989, *36(Supp.),* 1-21.
7. Rajeswari Ramachandran, Rani Balasubramanian, Sivasubramanian, S., Venkatesan, P., Soundrapandian, S., Sharmugasundaram, T.K. and Prabhakar, R. Short course chemotherapy in the treatment of Pott's paraplegia. *Indian Journal of Tuberculosis,* 1989, *36,* 113-115.
8. Rani Balasubramanian, Rajeswari Ramachandran, Pauline Joseph, Nagarajan, M., Thiruvengadam, K.V., Tripathy, S.P. and Prabhakar, R. Interim results of a controlled clinical study of abdominal tuberculosis. *Indian Journal of Tuberculosis,* 1989, *36,* 117-121.
9. Paramasivan, C.N. Bacteriological investigations for short-course chemotherapy under district tuberculosis programme conditions (Summary). *Indian Journal of Tuberculosis,*1989, *36,* 140.
10. Prabhakar, R. (Moderator), Khasgiwala, S.C., Nachinarkinlan, T.M., Ranga Rao, I., Santha Devi, T., Saxena, G.P., Seetha, M.A., Somasundaram, P.R. and Sudarsanam, N.M. Symposium on "Assessment of short-course chemotherapy under programme conditions" (Summary). *Indian Journal of Tuberculosis,* 1989, *36,* 143.
11. Venkatesan, P. and Somasundaram, P.R. A probabilistic approach for modelling the joint action of drugs. *Biomedicine,* 1989, *9,* 13-18.

12. Vijayan, V.K. Practical application series: AIDS and pulmonologists. *Lung India,* 1989, *7,* 85-88.
13. Kallasam, S., Fathima Rahman, Narayana, A.S.L. and Raghupati Sarma, G. Determination of the acetylator phenotype employing concentrations of isoniazid in saliva. *Indian Journal of Tuberculosis,* 1989, *36,* 151-155.
14. Selvaraj, P., Rajiswamy, Vijayan, V.K., Prabhakar, R. and Narayanan, P.R. Hydrogen peroxide release by OKI A1 (anti DR-Monoclonal antibody) resistant alveolar macrophages in tuberculosis. *Indian Journal of Chest Diseases and Allied Sciences,* 1989, *31,* 141-149.
15. Somasundaram, P.R. Collection of complete and accurate data relating to controlled clinical trials in tuberculosis. In *Biostatistics: Issues in Medical Research,* Proceedings of the Fourth Annual Conference of the Indian Society for Medical Statistics. Ed: Kaliaperumal, V.G., Subbakrishna, D.K. and Sundararaj, N. Department of Biostatistics, NIMHANS, Bangalore, 1989, 162-166.
16. Vijayan, V.K., Pandey, V.P., Sankaran, K., Mehrotra, Y., Darbari, B.S. and Misra, N.P. Bronchoalveolar lavage study in victims of toxic gas leak at Bhopal. *Indian Journal of Medical Research,*1989, *90,* 407-414.
17. Nutman, T.B., Vijayan, V.K., Pinkston, P., Kumaraswami, V., Steel, C., Crystal, R.G. and Ottesen, E.A. Tropical Pulmonary Eosinophilia. Analysis of antifilarial antibody localised to the lung. *Journal of Infectious Diseases,* 1989, *160,* 1042-1050.
18. Chandra Immanuel, Jayasankar, K., Narayana, A.S.L., Santha, T., Sundaram, V. and Raghupati Sarma, G. Induction of rifampicin metabolism during treatment of tuberculous patients with daily and fully intermittent regimens containing the drug. *Indian Journal of Chest Diseases and Allied Sciences,* 1989, *31,* 251-257.
19. Santha, T., Nazareth, O., Krishnamurthy, M.S., Rani Balasubramanian, Vijayan, V.K., Janardhanam, B., Venkataraman, P., Tripathy, S.P. and Prabhakar, R. Treatment of pulmonary tuberculosis patients treated with short course chemotherapy regimens in South India - 5- year follow-up. *Tubercle,* 1989, *70,* 229-234.
20. Padma Ramachandran, Duraipandian, M., Reetha, A.M., Mahalakshmi, S.M. and Prabhakar, R. Long-term status of children treated for tuberculous meningitis in South India. *Tubercle,* 1989, *70,* 235-239.
21. Sayeed, Z.A., Sudarsanam, K. and Rajeswari Ramachandran. Tuberculoma of brain- study of prospective clinical, EEG,CT Scan data of fifty two patients (Abstract): *Neurology India,* 1989, *37,* 512E02.
22. Rajeswari Ramachandran and Kalyanaraman, S. Short Course Chemotherapy in the treatment of brain tuberculoma - a controlled clinical trial (Abstract): *Neurology India,* 1989, *37,* 512E03.
23. Kalyanaraman, S., Rajeswari Ramachandran, Ganapathy, K., Kanaka, T.S., Reginald, J., Velmurugendran, C.U., Kumaresan,G. and Sayeed, Z.A. CT guided

medical treatment of tuberculoma. *Proceedings of 9th International Congress of the Neurological Society* (Abstract), 1989, 86.

24. Ganapathy, K., Rajeswari Ramachandran and Kalyanaraman, S. Tuberculoma of the brain - Recent concepts. *Progress in Clinical Neurosciences, 1989, 3,* 103-113.
25. Kalyanaraman,S., Rajeswari Ramachandran, Velmurugendran, C.U. and Kanaka, T.S. CT guided medical treatment of tuberculoma in children (Abstract). *Child Nervous System,* 1989, *5,* 261.
26. Freedman,D.O., Nutman,T.B., Jamal, S., Kumaraswami, V. and Ottesen, E.A. Selective up-regulation of endothelial cell class I MHC expression by cytokines from patients with lymphatic filariasis. *Journal of Immunology,* 1989, *142,* 653-658.
27. Lal, R.B., Kumaraswami, V., Krishnan, N., Nutman, T.B. and Ottesen, E.A. Lymphocyte subpopulations in Bancroftian filariasis: activated (DR+) $CD8^+$ T cells in patients with chronic lymphatic obstruction. *Clinical & Experimental Immunology,* 1989, *77,* 77-82.
28. Moudgil, K.D., Gupta, S.K., Narayanan, P.R., Srivastava, L.M., Mishra, R.S. and Talwar, G.P. Antibody response to phenolic glycolipid I and *Mycobacterium w* antigens and its relation to bacterial load in *M.leprae* infected mice and leprosy patients. *Clinical and Experimental Immunology, 1989, 78, 214-218.*
29. Lowrie, D.B., Shuk Han Cheng, Raji Swamy, Narayanan, P.R., Sarma, R.V.S.N., Walker K.B., Aber, V.R. and Mitchison, D.A. BCG vaccination in South India does not enhance antimycobacterial activity in peripheral blood monocytes - direct contrast with results in Britian. *Journal of Medical Microbiology (Abstract), 1989, 28, XI.*

30 Sanjeevi, C.B. and Seshiah, V. Insulin and Insulin Antibody. *The Journal of General Medicine, 1989, 1, 47-49.*

Papers accepted for publication

1. Rajeswari Ramachandran, 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. Raghupati Sarma, G., Kailasam, S., Manjula Datta, Loganathan, G.K., Fathima Rahman and Narayana, A.S.L. Classification of children as slow or rapid acetylators of isoniazid based on concentrations of isoniazid in saliva following oral administration of body-weight and surface-area related dosages of the drug. *Indian Pediatrics.*
3. Thomas, A., Balakrishnan, A., Nagarajan, M., Prabhakar, R., Tripathy, S.P., Christian, M. and Somasundaram, P.R. Controlled clinical trial of two multi-drug regimens with and without rifampicin in bacteriologically positive cases of leprosy. *International Journal of Leprosy.*

4. 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.*
5. 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.*
6. 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.*
7. 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.*
8. Gupte, M.D., Anantharaman, D.S., Nagaraju, B., Kannan, S. and Vallishayee, R.S. Experiences with *Mycobacterium leprae* soluble antigens in a population endemic for leprosy. *Leprosy Review.*
9. Prema Gurumurthy, Fathima Rahman, Narayana, A.S.L. and Raghupati Sarma, G. Salivary levels of isoniazid and rifampicin in tuberculous patients. *Tubercle.*
10. Lal, R.B., Kumaraswami, V., Cathy Steel, and Nutman, T.B. Phosphocholine-containing antigens of Brugia malayi nonspecifically suppress lymphocyte functions. *American Journal of Tropical Medicine and Hygiene.*
11 Venkatesan, P., Viswanathan, K. and Prabhakar, R. A model for the analysis of repeated specimens in clinical trials. *Proceedings of the 6th Annual Conference of the Indian Society for Medical Statistics, Hyderabad, October, 1988.*
12. Venkatesan, P., Viswanathan, K. and Prabhakar, R. A non-parametric prediction interval for censored survival data. *Proceedings of the 6th Annual Conference of the Indian Society for Medical Statistics, Hyderabad, October, 1988.*

JOURNAL CLUB

Journal club meetings were held each week, at which published scientific articles were reviewed by staff members of various departments in turn. A synopsis of the papers to be presented and the reference details were circulated a few days prior to the meeting, in order to facilitate better participation of the audience in the discussion that followed the presentation. In all, 38 such meetings were conducted, and 37 staff members presented one or more articles of interest.

Research discussions were held once or twice every month, for presenting current activities of different departments. During the year, the staff members of Pulmonary physiology, Epidemiology and Chemotherapy divisions presented the studies that were in progress.

LECTURES BY VISITING SCIENTISTS

Subject	Speaker
History of the Tuberculosis Chemotherapy Centre, Madras	Prof. Wallace Fox, formerly of Medical Research Council, London, U.K.
Microbiological mechanisms in anti-tuberculosis chemotherapy	Dr.J.B. Selkon, Public Health Laboratory Services, Oxford, U.K.

DISTINGUISHED VISITORS

1. Dr.UKan Tun, Training Officer, Ministry of Health, Burma and Dr.Tin U, Director General of Health Services, Burma.
2. Dr.Raimondo Francesco, Consultant, for AIDS, Italian Govt. Italy.
3. Dr.Roberto Esposito, Associate Professor of Infectious Diseases, University of Milan, Italy.
4. Prof.A.S.Paintal, Director-General, ICMR, New Delhi.
5. Dr.Barry Walker, Royal Post graduate Medical School, London.
6. Dr.R.K.Shenoy, Professor of Medicine, T.D.Medical College, Alleppey.
7. Dr.S.K.Jain, Professor of Cardio-respiratory Physiology, V.P.Chest Institute, Delhi.
8. Dr.D.B.Lowrie, British Medical Research Council, London.
9. Dr.E.A.Ottesen, Chief, Section of Clinical Parasitology, National Institutes of Health, Bethesda, Maryland, U.S.A.
10. Dr.C.P.Ramachandran, Secretary, Steering Committee for Filariasis, TDR, W.H.O., Geneva.
11. Dr.V.K.Arora, Professor of Chest Diseases, JIPMER, Pondicherry.
12. Prof. A.Namasivayam, Professor of Physiology, Dr.A.L.M.PGIBMS, Taramani, Madras.
13. Dr.P.M.Udani, Retd. Professor of Paediatrics, Bombay.
14. Dr.P.A.Deshmukh, Retd. Consultant Physician, Jamshedpur.
15. Dr.Brian White Guay, Senior Director, Merck Sharp and Dohme Laboratories, TDR Group, U.S.A.
16. Prof. Donald W.Smith, Professor of Medical Microbiology, University of Wisconsin Medical School, Madison, U. S. A.

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 -	Planning Board, 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.
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, Indian Council of Medical Research, New Delhi.
- do -	Central Crisis Group for Chemical Disasters, Ministry of Environment and Forest, Government of India, New Delhi.

Dr. V.K. Vijayan	Expert Committee on Toxic Gas induced Pulmonary diseases, Bhopal Gas Tragedy Relief and Rehabilitation Department and Madhya Pradesh Council of Science & Technology.
- do -	Assistant Editor, *Lung India*, Madras.
- do -	Joint Organising Secretary (representing the National College of Chest Physicians of India), First Joint Conference on Tuberculosis and Chest Diseases, Madras.
Dr. V. Kumaraswami	Associate Editor, *Lung India*, Madras.
Dr.Manjula Datta, Mr.P.R.Somasundaram, Mr.R.S.Vallishayee, 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.

PRIZES AND AWARDS RECEIVED BY STAFF MEMBERS

Dr.V.K.Vijayan was nominated to deliver the " Dr. O.A. Sarma Endowment Lecture" on "An Update in Tropical Eosinophilia" at the 16th Andhra Pradesh TB & Chest Diseases Workers' Conference held at Warangal on 4-5 February, 1989.

ACKNOWLEDGEMENT

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