TUBERCULOSIS RESEARCH CENTRE

CHETPUT MADRAS - 600 031

REPORT ON RESEARCH ACTIVITIES DURING 1986-87

INDIAN COUNCIL OF MEDICAL RESEARCH
NEW DELHI

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LABORATORY STUDIES

PREFACE

The research activities of the Centre continue to be focussed on developing suitable regimens of chemotherapy for tuberculosis which could be applied under the National Tuberculosis Control Programme. Keeping this in mind, fully oral regimens of shorter duration are being studied under controlled clinical trial conditions in pulmonary tuberculosis. These regimens are designed to enhance the efficacy in patients with initial drug resistance and prevent the emergence of further drug resistance. Furthermore, the regimens could be easily applied under programme conditions with all the existing operational constraints.

The SCC programme for tuberculosis in the 18 districts is being monitored and corrective measures are suggested wherever indicated. The results of the pilot study will be useful in evolving remedial measures for the lacunae in the effective implementation of the programme. Sociological studies were undertaken in a rural and a semi-urban area in North Arcot district to investigate the awareness of the disease among chest symptomatics in the community, and the utilisation of health facilities by them. Similar studies are being continued in the City of Madras. These studies will be able to throw light on community awareness and identify the socio-psychological aspects in patients with pulmonary tuberculosis.

Research activities to strengthen the biotechnological component are being continued so as to evolve simple and rapid diagnostic procedures in tuberculosis with special reference to smear negative pulmonary tuberculosis and pauci-bacillary extra pulmonary lesions. Efforts are being made to establish a molecular biology unit for application of genetic engineering techniques. DNA recombinant technology in mycobacterial research could form a basis for developing immunodiagnostic and immuno-prophylactic agents. Besides, the technology will offer a powerful approach for studying the genetics and physiology of *M. tuberculosis* including the study of potential targets of various anti-tuberculous drugs.

The expertise gained in the conduct of controlled clinical trials in pulmonary tuberculosis has been usefully harnessed in the conduct of similar studies in extra-pulmonary tuberculosis. Studies of tuberculous lymphadenitis in children, tuberculous meningitis, tuberculoma of brain and abdominal tuberculosis are in progress. These studies will enable us to evolve highly effective chemotherapeutic regimens of shorter duration and also diagnostic criteria. Controlled clinical studies are in progress in lymphatic filariasis with a new filaricidal drug Ivermectin, which has proved to be highly microfilaricidal with single dose oral administration. The results of the study will be highly beneficial in the strategy of control of filariasis which is highly endemic in certain parts of the country.

The Epidemiology Unit of the Centre has established two units for advanced training in clinical epidemiology at the Govt. General Hospital, Madras and the Institute of Child Health, Madras, for medical graduates and post-graduates in medicine and allied subjects. The advanced Centre is a collaborative effort of the ICMR and the Tamil Nadu Government in an attempt to strengthen the discipline of clinical epidemiology for future applications in health services and medical research. Further, the unit has been undertaking institutional and community-based epidemiological studies in acute respiratory infections in children and in filariasis. A case-control study of tuberculosis in children to evaluate the efficacy of BCG vaccination has been planned. Further, long term studies in the epidemiology of tuberculosis will be conducted in the BCG trial area in Chingleput district.

The main frame VAX computer system has been installed and data is being transferred from punch cards to magnetic tapes. The system has not become fully operational as yet, and it is hoped to utilise the system fully in a couple of months with terminals being installed in various departments.

The Scientific Advisory Committee met on 5-2-87 and stressed the greater importance of sociological studies in the context of the TB Control Programme and its effective implementation. The Centre, as stated earlier, has already made a beginning in this aspect and it is hoped that in-depth community studies will be undertaken in the coming years so as to operationalise the effective chemotherapy regimens that have been evolved at this Centre in the past.

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

The high standard of scientific research of the Centre has been possible by the unstinted efforts and devotion of the scientific, technical, paramedical and administrative staff of the Centre over the years which will be continued in the coming years.

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Director of Medical Education,
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CLINICAL STUDIES

STUDIES IN PROGRESS

Controlled clinical trial of 6-month intermittent regimens in Madras

Earlier studies at the Centre had shown that short-course regimens of 5 to 7 months' duration, with an initial daily phase of 2 to 3 months with streptomycin, isoniazid, rifampicin and pyrazinamide, bring about rapid sputum conversion (92% by 2 months and 96% by 3 months) and low relapse rates (0 to 5%) in patients with drug-sensitive bacilli initially. But the limitations are daily visits by the patients for 2 to 3 months and a high incidence of adverse reactions attributable to daily chemotherapy. The Centre, therefore, wanted to evolve regimens which will have less toxicity and require less frequent visits without compromising the efficacy. Fully intermittent regimens are usually less toxic than the daily regimens and are equally effective. Hence the Centre is currently carrying out a controlled clinical trial of 6-month intermittent regimens, which are fully supervised.

The regimens under study are as follows:-

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

(Regimens 4, 5 and 6:) Correspond to regimens 1, 2 and 3, respectively, except that the RSHZ is administered twice a week during the first 2 months (instead of thrice a week) and the dosage of pyrazinamide is 70mg/kg (instead of 50mg/kg).

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

Pyridoxine 10mg is incorporated in every dose of isoniazid throughout the 6-month period of chemotherapy. For rifampicin, isoniazid and pyrazinamide, a dosage schedule is used, the body-weight categories being less than 30.0 kg,

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*12 [illegible]

30.0—44.9 kg and 45.0 kg or more. All the anti-tuberculosis drugs are administered in the clinic under the close supervision of a clinic nurse.

The above-mentioned regimens are being investigated concurrently at Madras (Tuberculosis Research Centre), and in a semi-urban area around Tambaram, in collaboration with the Government TB Sanatorium, Tambaram (Superintendent: Dr. K. Jagannath, succeeded by Dr. Paramasivan).

The findings of 18 months of follow-up had been reported in the 1985-86 annual report. The findings of 30 months of follow-up are presented in this report.

In all, 960 patients were admitted to the study. After 74 exclusions, there remained 886 patients (725 with initially drug-sensitive bacilli, 161 with initially drug-resistant bacilli) for analyses of efficacy.

Of 725 patients with initially drug-sensitive bacilli, only 2 (0.3%) had an unfavourable response during chemotherapy. One developed miliary tuberculosis and the other had positive sputum cultures at 5 and 6 months.

Of 723 drug-sensitive patients with a favourable response at the end of chemotherapy, 715 were assessed for relapse. The bacteriological relapses requiring treatment during a follow-up of 30 months are presented in the table below.

Initial Rx. (2 months)	Continuation Rx (4 months)	Total assessed	Relapses requiring treatment							
			Total No.	%	Month of relapse after stopping drugs						
					1-3	4-6	7-9	10-12	13-18	19-24	25-30
RSHZ thrice weekly	SRHow	64	4	6	1	1	0	0	1	0	1
	RHow	68	1	1	1	0	0	0	0	0	0
	SRHtw	69	2	3	0	1	0	0	0	0	1
	RHtw	63	3	5	0	1	0	1	1	0	0
	SHtw	95	4	4	1	1	0	0	1	0	1
	Any	359	14	4	3	4	0	1	3	0	3
RSHZ twice weekly	SRHow	67	3	4	0	2	0	0	0	1	0
	RHow	65	6	9	4	2	0	0	0	0	0
	SRHtw	61	1	2	0	0	0	1	0	0	0
	RHtw	61	4	7	3	1	0	0	0	0	0
	SHtw	102	8	8	4	3	0	0	1	0	0
	Any	356	22	6	11	8	0	1	1	1	0

Fourteen (4%) of 359 thrice-weekly patients relapsed; the rates were similar and ranged from 1 to 6% for the various continuation regimens. Twenty two (6%) of the 356 twice-weekly patients relapsed during the same period; the rates ranged from 2 to 9% for the various continuation regimens. The relapse rate of 4% in the thrice-weekly series was not significantly different from that of 6% in the twice-weekly series ($P=0.2$).

Considering the patients who received rifampicin throughout 6 months, 10 (4%) of 264 thrice-weekly patients and 14 (6%) of 254 twice-weekly, patients relapsed. With respect to the number of drugs received in phase II the relapse rates in the thrice-weekly series were 6 (5%) of 133 patients who received streptomycin, isoniazid and rifampicin once or twice a week, 4 (3%) of 131 patients who received isoniazid and rifampicin once or twice a week and 4 (4%) of 95 patients who received streptomycin and isoniazid twice a week. The corresponding figures for the twice-weekly series were 4 (3%) of 128, 10 (8%) of 126 and 8 (8%) of 102, respectively. As regards the rhythm of administration in phase II, the relapse rates in the thrice-weekly series were 5 (4%) of 132 patients who received isoniazid and rifampicin (with or without streptomycin) once a week and 5 (4%) of 132 patients who received the above drugs twice a week; the corresponding figures for the twice weekly series were 9 (7%) of 132 and 5 (4%) of 122, respectively. None of the differences is statistically significant.

In all, there were 36 relapses. Of these, 28 (78%) occurred in the first year of follow-up, 5 (14%) in the 2nd year and 3 (8%) during the first half of the 3rd year. In the vast majority of cases, the bacilli were drug-sensitive at the time of relapse.

There were 161 patients with bacilli initially resistant to 1 or more drugs; 45 had bacilli resistant to streptomycin alone, 46 to isoniazid alone and 70 to both drugs. Of 45 patients with resistance to streptomycin alone, none had an unfavourable response and 6 relapsed. Of 46 patients with resistance to isoniazid alone, 7 (15%) had an unfavourable response and 3 relapsed. Of 70 patients with resistance to streptomycin and isoniazid, 11 (24%) of 45 who received rifampicin in the continuation phase and 19 (76%) of 25 who did not receive rifampicin had an unfavourable response ($P<.0001$).

(started: 1980; expected year of completion of 5 year follow-up: 1989).

(R. Parthasarathy, B. Janardhanam and N. Selvakumar).

Controlled clinical trial of fully oral short-course regimens in Madras

Earlier studies at this Centre had shown that short-course regimens of 5 to 7 months' duration are highly effective in the treatment of sputum-positive pulmonary tuberculosis. But these regimens may have only a limited application under programme conditions because they contain streptomycin and are fully supervised, requiring frequent visits to the clinic.

With a view to evolve regimens that will be applicable under programme conditions, the Centre is currently investigating 3 fully oral regimens of 6 or 8 months' duration with varying frequency of attendance and different methods of drug intake. Further, since eliciting the history of previous chemotherapy is not practicable under programme conditions, all patients, irrespective of the duration of previous chemotherapy, are admitted to the study.

The regimens investigated are as follows:

1. **$2EHRZ_7/6EH_7$(tm):** This is a fully self-administered daily regimen of 8 months' duration. During the first 2 months, 4 drugs, ethambutol 600mg, isoniazid 300mg, rifampicin 450mg and pyrazinamide 1.5g and during the next 6 months, 2 drugs, ethambutol 600mg and isoniazid 300mg are prescribed. The patients on this regimen collect the drugs once a week during the first 2 months and twice a month thereafter

2. **$2EHRZ_2/4EHR_2$(tw)** or **$2EHRZ_2/4EHR_2$(ow):** This is a twice-weekly regimen of 6 months' duration. During the first 2 months, four drugs, ethambutol 1200mg, isoniazid 600mg, rifampicin 450mg and pyrazinamide 2.0g and during the next 4 months, 3 drugs, ethambutol, isoniazid and rifampicin in the same dosages are prescribed. Half the patients, by random allocation, attend the clinic twice a week (tw) throughout and receive every dose under supervision. The other half attend once a week (ow) when that day's dose is administered under supervision and the other dose supplied to the patient for self-administration at home 3 or 4 days later.

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

One hundred and twenty-nine patients have been admitted so far and the intake is continuing. The patients will be followed up for 5 years.

(started: 1986; expected year of completion: 1993).

(This is a major study and all the senior scientific and technical staff members are involved in the planning and conduct of the study. However, the following scientists are mainly responsible for monitoring the study: Rema Mathew, M. S. Jawahar, T. Santha Devi, R. Parthasarathy, R. Prabhakar, B. Janardhanam, M. S. Krishnamurthy, P. R. Somasundaram, C. Alexander, C. N. Paramasivan, and G. Raghupati Sarma).

Collaborative controlled clinical trial of fully oral short-course regimens in Madurai

The fully oral short-course regimens investigated at Madras (see page 10) are also being investigated by the Centre's Unit at the Government Rajaji Hospital, Madurai, in collaboration with the Madurai Medical College (Dean: Dr. K. R. Jacob succeeded by Dr. S. C. Bose).

Patients are admitted on the basis of smear results 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.

One hundred and nineteen patients have been admitted so far and the intake is continuing.

(started: 1986; expected year of completion: 1993).

(K. Rajaram, S. Ramu, R. Parthasarathy, Victor Mohan, Fathima Rahman, P. R. Somasundaram, P. Venkataraman and C. N. Paramasivan).

Short-course chemotherapy under District Tuberculosis Programme

The pilot scheme on short-course chemotherapy (SCC) under the District Tuberculosis Programme (DTP) was continued and the Centre has been monitoring the progress of the scheme (see annual report 85-86). As mentioned in the earlier report, the short-course chemotherapy regimens are integrated in to the routine DTP in the 18 districts on a pilot scale to be operated by the staff responsible for implementing the programme. Three regimens under three policies of treatment are employed as follows:

1. *$2RHZ_2/4RH_2$:* Rifampicin 600mg plus isoniazid 600mg plus pyrazinamide 2.0g twice-weekly for 2 months, followed by rifampicin 600mg plus isoniazid 600mg twice-weekly for 4 months; all the doses are to be administered in the clinic under supervision.

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

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

Three policies of treatment are being followed:

Policy A: Regimen 1, with Regimen 2 as an alternative;

Policy B: Regimen 2;

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

The following table gives details regarding sputum examination and admission to the scheme.

S. No.	Policy	District	State	Total new smears examined	Smear pos. No.	%	Eligible for SCC	Put on SCC No.	%
1.	A	N. Arcot	T. Nadu	149804	8966	*6*	8966	5171	*58*
2.	A	Puri	Orissa	44816	1670	*4*	1429	1100	*77*
3.	A	Baroda	Gujarat	50496	7831	*16*	7746	3428	*44*
4.	A	Thane	Maharashtra	48803	3122	*6*	3122	2069	*66*
5.	A	Ujjain	M.P.	24334	1923	*8*	1816	823	*45*
6.	A	Dehra Dun	U.P.	25382	1975	*8*	1810	949	*52*
7.	B	Karnal	Haryana	29392	2336	*8*	2322	787	*34*
8.	B	Kanpur	U.P.	49452	5133	*10*	3798	851	*22*
9.	B	Nagpur	Maharashtra	75361	5846	*8*	5846	1647	*28*
10.	B	Rajkot	Gujarat	19612	1940	*10*	1329	850	*64*
11.	B	Raichur	Karnataka	44374	1720	*4*	1431	413	*29*
12.	B	Sagar	M.P.	15399	1578	*10*	866	354	*41*
13.	C	Pondicherry	U. Territory	59637	2738	*5*	2595	1150	*44*
14.	C	Vidisha	M.P.	15125	1341	*9*	1149	907	*79*
15.	C	Aurangabad	Maharashtra	18879	2291	*12*	2281	984	*43*
16.	C	Varanasi	U.P.	31820	1450	*5*	1182	690	*58*
17.	C	Sabarkantha	Gujarat	36194	2880	*8*	2780	1666	*60*
18.	C	W. Godavari	A.P.	47813	2352	*5*	2352	681	*29*

The smear positivity rates were between 4-9% in 13 districts, 10-14% in 4 4 districts and in 1 district it was 16%. The percentage of eligibles put on SCC regimens ranged between 20-39% in 5 districts, 40-59% in 8 districts, and 60-79% in 5 districts.

Teams from the Centre have made visits to the different districts for on-the-spot observations, and corrective measures suggested for any lacunae or deficiencies. In North Arcot and Pondicherry, laboratories for sputum culture have been set up with the Centre's expertise and specimens are collected in duplicate for processing at the district laboratory as well as the Centre's laboratory. In addition, the progress of the patients admitted to the SCC programme in these 2 districts is being monitored bacteriologically by examination of sputum by smear, culture and sensitivity tests in the Centre's laboratory. The two districts were chosen for monitoring bacteriology considering the operational convenience. Efforts are being made to set up laboratories to do culture work at the district level in more districts.

(started: 1983).

(N. M. Sudarsanam, T Santha Devi, R. Parthasarathy, M. S. Krishnamurthy, P. R. Somasundaram, C. Alexander, P. Venkataraman and R. Prabhakar).

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

In the National Tuberculosis Programme, case-finding is 'passive', and persons with chest symptoms are expected to seek diagnosis and treatment from the nearest health facility. For the success of the programme, it is, therefore, essential that chest symptomatics in the community are motivated enough to get their condition diagnosed. It was therefore felt that a study should be undertaken to identify such chest symptomatics in the community at large and to find out how many had sought medical advice and relief.

A sample survey was undertaken in a rural area (Chetpet block) and a semi-urban area (Tiruvannamalai town) in the North Arcot district.

In Tiruvannamalai town, 30 clusters were selected and in Chetpet block, 14 village clusters were chosen by simple random sampling. A detailed 2-stage questionnaire was employed (see annual report 85-86). The head of the household was contacted and details of the family composition were obtained by experienced census takers. A record was made at this stage of all individuals suffering from any episode of illness during the previous 4 weeks. From among those with illness, chest symptomatics as defined in the National Tuberculosis Programme (cough of 2 weeks' duration or more, with or without haemoptysis or chest pain) were subsequently interviewed by experienced medical social workers using Part-B of the questionnaire. The thrust of this interview was to obtain information about the action taken by the symptomatics for diagnosis and relief, the type of health facility made use of and the reason for the choice of the particular health

facility, knowledge about tuberculosis in general, its mode of spread in the community and its diagnosis. Two sputum specimens from each interviewee was obtained (1 supervised spot and 1 overnight collection) and processed by smear, culture and sensitivity and identification tests at the Centre's laboratory.

The numbers registered, number of symptomatics identified and number from whom samples were examined are shown in the table below:

	Tiruvannamalai	Chetpet
Total sample population registered	17409	18395
Symptomatics identified (⩾15 years)	1160(6.7%)	1064(5.8%)
No. interviewed	953(82.2%)	987(92.8%)
No. from who sputum samples collected	864(90.7%)	967(98.0%)

As can be seen, the coverage for interviews and sputum collection has been high. All the sputum positive symptomatics were given a referral slip to attend the Tiruvannamalai Govt. Chest Clinic (for those diagnosed in Tiruvannamalai) or the Primary Health Centre, Kommanandal (for those diagnosed in Chetpet) for further diagnosis and anti-tuberculosis treatment.

The data from the questionnaires are being analysed.

(started: 1986: completed: 1986)

(T. Santha Devi, N. M. Sudarsanam, M. S. Krishnamurthy, C. N. Paramasivan and Sudha Ganapathy).

Collaborative study of abdominal tuberculosis

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

The objectives of this study are:

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

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

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

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

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

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

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

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

So far, 126 patients have been admitted to the study; the intake is continuing The aim is to follow-up the patients for 5 years.

(started: 1983; expected year of completion of 5-year follow-up: 1992).

(Rani Balasubramanian, Paulin Joseph, Rajeswari Ramachandran, Vanaja Kumar, M. Nagarajan, Prema Gurumurthy and G. Raghupati Sarma).

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

As mentioned in a previous annual report (1985-86), the detailed findings of the first 3 studies (presented in the 1982 annual report) showed that the mortality was high, ranging from 20-24%. While this could have been due to

patients reporting late (87% had disease classified as Stage II or III on admission), a high prevalence of initial drug resistance contributing to failure of chemotherapy could not be ruled out. It was therefore decided to study more intensive regimens, with 5 drugs in an initial 2-month phase followed by 2 drugs twice weekly for 7 months.

Patients for the study are from those admitted to the Institute of Child Health, Madras (Director: Dr. T. Dorairaj), and diagnosed as having tuberculous meningitis on the basis of clinical findings such as fever, vomiting, irritability, apathy, refusal to play, anorexia and constipation in the early stages, followed by well-marked meningeal signs, mental confusion, neurological signs, coma and widespread paralysis. The CSF findings are also taken into consideration.

Patients found suitable for admission are randomly allocated after stratification according to clinical severity, in equal proportions to 2 regimens. The first regimen consists of initial treatment with streptomycin, ethambutol and isoniazid daily plus rifampicin and pyrazinamide thrice a week for 2 months, followed by rifampicin and isoniazid twice a week for 7 months ($2\ S_7H_7E_7R_3Z_3/7\ R_2H_2$). The second regimen consists of streptomycin, ethambutol and isoniazid daily plus rifampicin and pyrazinamide twice a week for 2 months, followed by rifampicin and isoniazid twice a week for 7 months ($2\ S_7H_7E_7R_2Z_2/7R_2H_2$).

The routine examinations and investigations include a detailed general examination with special reference to the nervous system, tuberculin test with 1 T.U., chest radiograph, biochemical and bacteriological examinations of cerebrospinal fluid, cell count in CSF, routine urine tests, haematological investigations and liver function tests.

All patients attend the Tuberculous Meningitis Clinic at the Institute of Child Health, after discharge from the ward. Patients who are discharged before completing the 2-month daily intensive phase of therapy attend daily till they complete the course and the others twice a week, once a week, or once in 15 days till they complete 9 months of treatment; thereafter they attend the clinic once a month till the 24th month, once in 3 months till the 36th month and once in 6 months till the 60th month.

So far, 215 patients have been admitted (108 to the first regimen and 107 to the second). Sixteen patients were excluded from the study after admission, for various reasons. Of the remaining 199 patients, 51 died, 23 were discharged against medical advice, 97 completed 9 months of treatment and 28 are still on treatment.

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

(Padma Ramachandran, Mahalakshmi*, Vanaja Kumar and N. Selvakumar).

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*Research Fellow

Study of brain tuberculoma

Brain tuberculoma is now being suspected much more often than in the past, partly due to increased awareness of the disease among medical personnel and also due to general availability of CT scan. No reports are available on the use of short-course treatment for brain tuberculoma, though there are reports to suggest that chemotherapy alone may suffice for large brain tuberculomas with or without increased intracranial tension, single or multiple lesions. A prospective study has been initiated to evaluate the efficacy of short-course chemotherapy in the management of brain tuberculoma.

Aims:

1. To investigate the efficacy of short-course regimens for brain tuberculoma.
2. To determine indications for surgery in brain tuberculoma.
3. To study CT scan appearance before, during and after anti-tuberculous treatment.
4. To undertake immunological studies of CSF and blood in patients with brain tuberculoma.
5. To estimate drug levels in blood, CSF and brain tissues.

CT scan criteria for diagnosis of tuberculoma: A circumscribed hyperdense lesion compared to the surrounding brain, with a minimum of 1000 cu.mm. in volume, enhancing with contrast and having adjacent oedema is taken as tuberculoma for selection purposes of this study. In case of multiple lesions at least one lesion should be 1000 cu. mm. or more in volume.

Regimens: Chemotherapy is prescribed for a period of 9 months. All cases admitted to the study are randomly allocated to one of the following 2 regimens.

Regimen-I: 3RHZ daily/6RH twice-weekly
Regimen-II: 3RHZ thrice-weekly/6RH twice-weekly

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

Steroids: In both the regimens, prednisolone is given during the first 6 weeks; for adults a dosage of 10mg thrice a day in the 1st week and twice a day in the 2nd week, 5mg thrice a day in the 3rd week, twice a day in the 4th

*The dosages employed are: Rifampicin 12mg/kg body weight (daily, thrice-weekly or twice-weekly); isoniazid 10mg/kg daily and 15mg/kg twice and thrice-weekly (incorporating 10mg of pyridoxine) and pyrazinamide 30mg/kg daily and 50mg/kg thrice weekly.

week and once a day in the 5th and 6th weeks is used. For children (< 12 years) the dosage is reduced by half. Decadron injections and anti-convulsants are added wherever indicated.

Investigations: The following investigations are done:

1. Hematological examinations
2. CT scan
3. Liver function tests
4. CSF culture
5. X-ray (chest and skull)
6. Mantoux
7. Culture of sputum and urine

CT scan is repeated after 1 month and once in 2 months thereafter.

Role of surgery: A biopsy of the mass is done for histopathology and culture examinations, if the size of the mass is more than 80% at the second monthly scan when compared to the '0' month scan.

So far, 12 patients have been admitted and the intake is continuing.

(started: 1986: expected year of completion of intake:1989).

(Rajeswari Ramachandran, R. Balambal*, S. Sivasubramanian and C.N. Paramasivan).

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

As mentioned in the previous annual report (1985-86) the Centre is undertaking a controlled clinical trial in the treatment of leprosy at the Government Royapettah Hospital, Madras. Interim findings up to five years on a larger number of patients are presented here.

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

Patients were randomly allocated to a standard regimen of dapsone plus clofazimine daily for 60 months (non-rifampicin regimen) or a 4-drug

*Research Associate

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

Patients in interim analyses: A total of 210 patients has been admitted to the trial. Of 177 patients who were considered for the present analyses, 20 were excluded. Six patients had died, 3 absconded (in the 1st, 3rd and 39th month), 4 patients discharged themselves against medical advice and 5 patients migrated, 4 at 6 months and 1 at 16 months; 2 patients developed tuberculosis, one at 12 months and the other at 17 months, and were treated with anti-tuberculosis drugs. Interim analyses have been undertaken on the remaining 157 patients (81 rifampicin, 76 non-rifampicin).

All but 20 of the 157 patients were males; 87 patients were under 30 years of age, 43 were aged 30-39 years, and the remaining 27 aged 40 years or more; the mean age was 29 years (range 12-58) and the mean weight 44.2 kg (range 21.3-75.8 kg). Of the 157 patients, 90 had had less than 1 year of previous chemotherapy, 43 had had 1-5 years of chemotherapy and 24 had had over 5 years of chemotherapy. The distributions in the 2 regimens were broadly similar.

Of 138 patients who had histopathology findings, 95 (69%) were classified as lepromatous, 17 (12%) as lepromatous indeterminate and 26 (19%) as borderline lepromatous.

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

% of Rx. received	Period (Months)					
	0-3		0-12		0-60	
	Rif.	Non-Rif.	Rif.	Non-Rif	Rif.	Non-Rif.
100	38	35	34	34	23	28
95–99	36	34	39	36	46	34
90–94	6	5	5	3	5	8
80–89	1	1	3	3	3	3
70–79	0	1	0	0	2	2
69 or less	0	0	0	0	2**	1*

**57% and 63% Rx. received; did not attend after 48m.

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

The regularity in the 2 regimens was similar. A total of 73 patients (46%) in the two regimens did not miss a single dose in the first 3 months, 70 (45%) received between 95 and 99% of the scheduled chemotherapy and the others received between 70 and 94%. The regularity continued to be high in subsequent periods also. However, 3 patients became uncooperative in the 5th year and stopped attending for drugs.

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

Progress	Period (Months)									
	0-12		0-24		0-36		0-48		0-60	
	Rif.	Non-Rif.	Rif.	Non-Rif.	Rif.	Non-Rif.	Rif.	Non-Rif.	Rif.	Non-Rif.
Improvement										
Marked	28	26	56	52	64	65	63	61	66	63
Moderate	32	35	19	16	9	7	13	14	5	6
Slight	19	13	5	6	5	2	3	0	1	2
No change	2	2	1	1	0	0	0	0	0	0
Deterio-ration	0	0	0	0	0	0	0	0	0	0

At 12 months, moderate or marked improvement was reported in 60 (74%) in the rifampicin regimen and 61 (80%) in the non-rifampicin regimen. The proportions were 92% and 90% respectively, at 24 months, 94% and 97% respectively, at 36 months, 96% and 100% respectively, at 48 months and 99% and 97% respectively, at 60 months. Thus, there was excellent clinical improvement in both series.

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

Regimens	BI	Months					
		0	12	24	36	48	60*
Rif.	Mean	4.3	3.6	2.6	1.9	1.3	1.1
(N =81)	Range	2.5-5.7	0.5-5.1	0.0-4.2	0.0-3.7	0.0-3.0	0.0-2.5
Non-Rif.	Mean	4.3	3.5	2.5	1.9	1.3	1.0
(N =76)	Range	2.7-5.3	0.3-4.8	0.2-4.5	0.0-3.8	0.2-3.5	0.0-2.8

Based on 75 rifampicin and 72 non-rifampicin patients, in both groups there was a steady and similar fall in the Bacterial Index values. The reduction over the 60-month period was similar in the 2 regimens, namely 3.2 and 3.3.

It can be seen from the distribution presented below that 3 patients from the rifampicin group and 4 patients from the non-rifampicin group had BI of 0.0 at 60 months (but none had BI values of 0.0 at 3 consecutive months), 27 patients in the rifampicin group and 31 patients in the non-rifampicin group had BI between 0.1 and 0.9, 40 patients (53%) from the rifampicin group and 28 (39%) from the non-rifampicin group had BI between 1.0 and 1.9; none had BI greater than 2.9.

BI	0.0	0.1—	0.5—	1.0—	1.5—	2.0—	2.5—2.9
Rif. (N=75)	3	8	19	24	16	4	1
Non-Rif. (N=72)	4	5	26	19	9	6	3

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

(started: 1977; expected year of completion: 1993).

(A.Thomas, B. Nagaraju, Lalitha Hari and M. Nagarajan).

Collaborative controlled clinical study of multi-drug chemotherapy in multi-bacillary leprosy

It is more than a decade since multi-drug therapy was introduced in the treatment of multi-bacillary leprosy. Drugs usually included were dapsone with rifampicin, clofazimine and thioamides. In spite of prolonged treatment, persisters were isolated.

Various studies in pulmonary tuberculosis have shown that pyrazinamide has sterilising action on intracellular organisms. Encouraged by the above findings a study to evaluate the role of pyrazinamide in multi-bacillary cases of leprosy was started.

Aim: To assess the relative efficacies of pyrazinamide and rifampicin in combination with clofazimine and DDS in the treatment of multi-bacillary leprosy.

Regimens: The following 3 regimens are being investigated.

I. *24* RCD: Rifampicin 12mg/kg body-weight once a month in addition to a daily dose of 12mg/kg body-weight for the first 14 days, clofazimine 300mg once a month and 50mg daily; and dapsone 100mg daily, for a total duration of 24 months.

II. *24 RZCD:* Rifampicin, clofazimine and dapsone as in regimen I, plus pyrazinamide 35mg/kg body-weight daily for the first 3 months, for a total duration of 24 months.

III. *24 ZCD:* As in regimen II but without rifampicin, for a total duration of 24 months.

If a patient develops major toxicity or reaction, chemotherapy may be modified or changed.

The study is being conducted at the Government Royapettah Hospital, Madras (Dean: Dr. G. Ananthasubramanian) and the patients are selected from among those attending leprosy clinics in and around Madras city.

So far, 12 patients have been admitted to the study. It is proposed to admit about 100 patients to each regimen.

(started: 1986; expected year of completion of intake: 1996)

(A. Thomas, K.C. Umapathy, Lalitha Hari, K. Thyagarajan and M. Nagarajan).

Study of Ivermectin, a new microfilaricidal drug

Ivermectin, a new antifilarial drug, was tested for its efficacy in bancroftian filariasis in collaboration with the Department of Clinical Pharmacology, Madras Medical College, Madras (Dr. V. Vijayasekaran). Forty adult, male, asymptomatic microfilaremics with moderate to heavy microfilaremia were treated with a single oral dose of ivermectin. Four dosage levels were used: 25, 50, 100 and 200 mcg/kg body weight. Microfilaria clearance was rapid and was completed by the 12th day in all groups. However, microfilariae reappeared as early as one month post-treatment and by 6 months microfilaremia had reached 15-30% of pre-treatment levels in all the groups. Nearly all patients had mild side effects (fever, headache, myalgia, giddiness/dizziness and sore throat) which were easily managed. Three patients developed postural hypotension which lasted for 12-72 hours but did not require any specific therapy. The intensity and the duration of the side effects were related to the initial parasite load and the lowest dose (25mcg/kg) was the least reactogenic. Ivermectin appears to be a promising agent in the treatment of lymphatic filariasis. The drug is currently being compared with diethylcarbamazine (DEC) in a double blind trial.

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

(V. Kumaraswami, R. Prabhakar and G. Raghupati Sarma).

Pulmonary function studies in healthy South Indian subjects

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

The investigations (clinical and pulmonary) that are being carried out have been mentioned in the 1985-86 annual report.

These are:

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

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

(1) Spirometry

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

(2) Lung volumes by gas dilution method

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

(3) Single breath diffusing capacity

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

So far 266 contacts have been admitted to the study and the study is being continued.

The age distribution of subjects admitted to the study is given in the following table.

Age (years)	Male	Female
< 20	41	13
20-40	75	89
> 40	24	24
Total	140	126

Analyses are being carried out to study the pattern of variation in the parameters. Multiple regression equations are being fitted to the parameters by entering age, height and weight as independent variables and the parameters as dependent variables, using the stepwise regression technique.

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

(V.K. Vijayan and K.V. Kuppu Rao)

Clinical and pulmonary function studies in tropical pulmonary eosinophilia

Tropical Pulmonary Eosinophilia (T.P.E.) patients are conventionally treated with Diethyl Carbamazine (D.E.C.) 6mg/kg body-weight daily for 3 weeks. Even though there is symptomatic improvement after 3 weeks' treatment in the majority of patients, a proportion of patients may develop chronic lung disease. Therefore T.P.E. patients being treated with D.E.C. for 3 weeks are being assessed for pulmonary function before treatment and at 1, 3, 6, 12, 24, 36, 48 and 60 months of follow-up. in order to understand the patho-physiology.

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

(1) Spirometry

(i) F.V.C.

(ii) $F.E.V._1$

(iii) $\frac{F.E.V_1}{F.V.C.}$ %

(iv) PEFR Flow 25%, Flow 50%, Flow 75%

(2) Lung Volumes

(i) T.L.C.

(ii) F.R.C.

(iii) R.V.

(iv) $\frac{\text{R.V.}}{\text{T.L.C.}}$ %

(3) Single breath diffusing capacity

(i) T.L.C.O.

(ii) K.C.O.

In all, 88 patients have been admitted to the study so far. The coverages for follow-up examinations after completion of treatment are shown in the table below:

Month of examination	Due	Examined	
		No.	%
6	73	70	*96*
12	64	56	*88*
24	36	27	*75*
36	10	8	*(80)*

The study is being continued.

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

(K.V. Kuppu Rao and V.K. Vijayan).

Broncho-alveolar lavage studies in tropical pulmonary eosinophilia

The current concept of the pathogenesis of Tropical Pulmonary Eosinophilia suggests that it begins with a lung parenchymal inflammation in persons highly sensitised immunologically to filarial parasites. It is hypothesised that the abnormalities in the lower respiratory tract in TPE are mediated by the inflammatory cells that accumulate in the lung parenchyma. Fibreoptic bron-

choscopy and broncho-alveolar lavage are utilised to sample the inflammatory cells present in the lower respiratory tract of patients with TPE and to define the inflammatory process.

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

Lavages are done before treatment and at 1, 6, 12, 24, 36, 48 and 60 months of follow-up. So far 29 patients have been admitted to this study; the intake to the study is being continued.

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

(V.K. Vijayan).

LABORATORY STUDIES

STUDIES COMPLETED

Salivary levels of isoniazid and rifampicin in tuberculous patients

Estimation of drug concentrations in saliva has been employed to monitor compliance, for calculation of pharmacokinetic variables and even for therapeutic drug monitoring. Most drugs enter saliva by passive diffusion and the rate of diffusion is determined by several physicochemical characteristics such as the molecular weight and spatial configuration, lipid solubility and in the case of weak electrolytes, the degree of ionisation at physiological pH, and binding to plasma proteins. Use of saliva, where applicable, will have obvious practical advantages, particularly in young children as it avoids venipuncture. Moreover, salivary concentrations reflect the unbound physiologically active 'free' concentrations of the drug. The concentration of the unbound drug in equilibrium with extravascular tissues, is difficult to measure accurately by separation of the relevant plasma moiety and the concentration of certain drugs in saliva has been shown to be related to their tissue concentrations.

Information on the diffusion of anti-tuberculous drugs into saliva is scarce; investigations were therefore undertaken to determine the concentrations of the more commonly used drugs such as isoniazid, rifampicin, pyrazinamide and ethambutol simultaneously in serum and saliva.

Isoniazid 300mg and rifampicin 12mg/kg were administered to 19 patients with pulmonary tuberculosis and 13 patients with abdominal tuberculosis. Of these, 19 were slow and 13 rapid acetylators of isoniazid. Blood and saliva were collected at 1, 2, 3, 6 and 8 hours after drug administration. Serum was separated from the blood samples and stored at -20° C; saliva samples were frozen by keeping overnight at -20° C; thawed, centrifuged and the supernatants stored at -20° C (the residue containing mucoproteins was discarded). Concentrations of isoniazid employing a spectrophotometric method involving condensation with vanillin, and rifampicin by the plate diffusion assay employing *Staph. aureus*, were determined in the serum and saliva samples after randomisation. The mean isoniazid concentrations at the different time-points and the means of some pharmacokinetic variables calculated from these concentrations are presented in the table on page 29.

The concentrations of isoniazid at each of the time-points (except at 8 hrs. in rapid acetylators) are in general similar in saliva and serum suggesting that isoniazid diffuses very readily into saliva and that equilibrium is attained within one hour. Isoniazid is not bound to plasma proteins and it is likely that there is very little ionisation of the drug. These results demonstrate that several pharmacokinetic variables of the drug such as exposure (AUC) and half-life can be calculated on the basis of the salivary concentrations instead of plasma or serum concentrations. It should

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Hours after drug administration	Mean isoniazid concentrations (μg/ml)			
	Slow acetylators		Rapid acetylators	
	Saliva	Serum	Saliva	Serum
1	7.5	7.5	5.6	5.4
2	6.4	7.1	4.8	4.7
3	5.2	5.8	3.1	3.3
6	2.5	2.7	0.76	0.94
8	1.6	1.8	0.28	0.68
Mean peak concentration (μg/ml)	8.0	8.3	6.0	6.0
Exposure (μg/ml.hrs)	38	41	17	19
Half-life (hrs)	3.2	3.1	1.6	1.8
No. of patients	19		13	

also be possible to determine the acetylator phenotype on the basis of salivary concentrations of the drug. There are very few reports on the pharmacology of isoniazid in infants and young children and the feasibility of employing salivary concentrations should make it possible to undertake detailed studies in this age group.

Investigations were also undertaken to study if detection of isoniazid or its metabolites (acetylisoniazid and isonicotinic acid) in saliva would be more advantageous than in urine, to monitor ingestion of the drug. Several tests were undertaken in paired samples of saliva and urine collected up to 72 hours from 5 slow and 5 rapid acetylators following ingestion of isoniazid 300mg. Results (not presented here) showed that urine was a far better fluid than saliva with any of the tests undertaken for this purpose.

The mean values for rifampicin in saliva and serum (based on a total of 27 patients) together with the saliva to serum ratios are presented in the table on page 30.

Techniques employing equilibrium dialysis and ultra-centrifugation have demonstrated that about 90% of circulating rifampicin is bound to plasma proteins. The saliva to serum ratios, ranging from 0.08 to 0.14, confirm these findings. A correlation co-efficient of 0.71 was obtained between serum and salivary estimations on the basis of the 135 pairs of observations.

Hours after drug administration	Mean rifampicin concentrations (μg/ml)		Saliva: serum ratio
	Saliva	Serum	
1	0.33	4.6	0.08
2	0.73	7.6	0.10
3	0.94	7.6	0.12
6	0.61	5.2	0.12
8	0.48	3.5	0.14

An interesting observation employing TLC (thin-layer chromatography) on silica gel was that rifampicin was found in the form of rifampicin quinone in saliva. The concentrations of rifampicin quinone, a minor metabolite of the drug, are very low in serum. It is possible that rifampicin is being oxidised to rifampicin quinone after it enters saliva, either through atmospheric oxidation or the action of some oxidases present in saliva. Further investigations to elucidate this point as well as to determine the activity of rifampicin quinone in relation to that of rifampicin against both *Staph. aureus* and *M. tuberculosis* are in progress. Investigations are also in progress to determine the concentrations of pyrazinamide and ethambutol in paired samples of serum and saliva, the former using a HPLC technique and the latter with a plate diffusion assay employing *M. smegmatis*.

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

(Prema Gurumurthy, G. Raghupati Sarma, Fathima Rahman and A. S. L. Narayana).

Assay of anti-tuberculous drugs stored under field conditions

Short-course chemotherapy of 6 or 8 months' duration, in newly diagnosed smear-positive cases of pulmonary tuberculosis, was introduced under DTP conditions in North Arcot district in 1983 and in 17 other districts in India subsequently. The drugs most commonly used are rifampicin, isoniazid and pyrazinamide. The storage conditions of these drugs in the various centres participating in the programme are far from ideal, with the drugs being exposed to extremes of temperature and humidity. It is essential to obtain information on the stability of the potency of these drugs kept stored for long periods of time under unsatisfactory conditions, and if necessary, to take remedial action by either restricting the supply or improving conditions of storage.

These investigations were undertaken on the drugs issued to North Arcot district. This district covers an area of about 12650 sq.km. with a population of 4.5 million and there are 87 health facilities taking part in the programme. The tem-

perature fluctuates between a low of 20°C to a high of 43°C and the relative humidity ranges from 60% to 90%.

The anti-tuberculous drugs namely, rifampicin (150 and 300mg), isoniazid (300mg) and pyrazinamide (500mg) were assayed for their content by standard procedures before issue, and after storage for periods ranging from 6 to 30 months. The results are presented in the following table:

Period of storage (months)	RMP 150mg		RMP 300mg		INH 300mg		PZA 500mg	
	Sample size	Mean value (mg)	Sample size	Mean value (mg)	Sample size	Mean value (mg)	Sample size	Mean value (mg)
0	5	157	5	295	14	297	5	491
3	11	157	11	299	11	299	5	486
6	16	152	20	292	20	297	34	469
12	9	150	9	292	9	298	12	498
20	10	150	12	286	12	290	9	485
30	12	154	14	297	14	297	13	495

RMP: Rifampicin; INH: Isoniazid; PZA: Pyrazinamide.

These results clearly demonstrate that there has been no loss in the potency (as assessed by the content of the capsules/tablets) of any of the drugs investigated even when stored up to 30 months, under conditions far from favourable.

(started: 1985; completed: 1986).

(G. Raghupati Sarma, A. S. L. Narayana, M. S. Krishnamurthy and N. M. Sudarsanam).

Absorption of anti-tuberculosis drugs in patients with abdominal tuberculosis

No investigations have so far been undertaken to study the gastro-intestinal absorption of anti-tuberculous drugs in patients with abdominal tuberculosis. The general impairment of absorption, especially in those with intestinal involvement, could lead to a decrease in absorption of anti-tuberculosis drugs administered orally, and effective serum concentrations may not be attained. Xylose absorption test is normally used to study the degree of malabsorption occurring, particularly in the jejunal portion of the small intestine. An investigation was therefore undertaken to compare the absorption of isoniazid, rifampicin and D-Xylose in patients with abdominal (intestinal) tuberculosis with that in patients with pulmonary tuberculosis.

Thirteen patients with abdominal tuberculosis (with intestinal involvement) were admitted to the study. Four of these patients had resection of the diseased portion of the intestine and were excluded from the main analysis. Of the remaining 9 patients, 8 were slow acetylators and 1 rapid acetylator. A total of 17 patients with pulmonary tuberculosis (controls) were admitted to the study; of these, 9 were slow and 8 were rapid acetylators. Both groups of patients received on an empty

stomach, a uniform dose of isoniazid 300 mg plus rifampicin 10 mg/kg, and half an hour later, a uniform dose of D-Xylose 5 g. Blood was collected at 1,2,3,6 and 8 hours and urine over the periods 0-½ h and then at two-hourly intervals up to 8½ hours after administration of anti-tuberculosis drugs. Serum separated from the blood samples and aliquots of the urine samples were stored at −20°C till assay. Concentrations of isoniazid and rifampicin were determined employing standard techniques. D-Xylose was estimated in the protein-free filtrate prepared from the blood sample collected at 2 hours (i.e. 1½ hours after the dose of D-Xylose). The proportion of dose of isoniazid excreted as isonicotinyl compounds (i.e. isoniazid, acetylisoniazid, isonicotinic acid and isonicotinyl glycine) was estimated after acid hydrolysis of the urine samples by which all isonicotinyl compounds were converted to isonicotinic acid. D-Xylose was estimated in urine excreted over the periods ½ to 2½, 2½-4½, 4½-6½ and 6½-8½ hours after administration of anti-tuberculous drugs (i.e. 0-2, 2-4, 4-6 and 6-8 hours after administration of D-Xylose). All estimations were undertaken after randomising the samples.

The mean serum concentrations of isoniazid at 1,2,3.6 and 8 hours and the means of some of the pharmacokinetic variables calculated on the basis of these concentrations for slow acetylators with abdominal or pulmonary tuberculosis (8 in each group) are presented in the following table. The mean body-weight was 38.3 kg for the slow acetylators with abdominal tuberculosis and 42.6 kg for those with pulmonary tuberculosis; the mean dosages of isoniazid administered were 8.3 and 7.2 mg/kg, respectively in the two groups.

Hours after administration	Mean serum isoniazid concentrations (μg/ml)	
	Slow acetylators	
	Abdominal TB	Pulmonary TB
1	9.2	5.9
2	8.0	5.9
3	6.3	5.0
6	2.7	2.5
8	1.9	1.7
Mean peak concentration (μg/ml)	9.7	6.9
Exposure (μg/ml. hrs)	44	37
Half-life (hrs)	2.8	3.4
No. of patients	8	8

The mean concentrations at each of the time-points, the mean peak concentration and the exposure are higher and the mean serum half-life slightly lower in patients with abdominal tuberculosis than in those with pulmonary tuberculosis. It is only the difference in mean peak concentrations which attains statistical significance ($P = 0.04$).

There was only one rapid acetylator (body-weight 37.5kg) with abdominal tuberculosis. Serum isoniazid concentrations in this patient were 5.0, 5.5, 4.0, 0.98 and 0.71 μg/ml at 1,2,3.6 and 8 hours, respectively; the exposure was 21 μg/ml. hrs and isoniazid was eliminated with a half-life of 1.6 hours. In comparison, the mean values for 9 rapid acetylators with pulmonary tuberculosis (mean body-weight 38.0 kg) were 5.5, 4.9, 3.5, 0.92 and 0.65 μg/ml; the exposure was 19 μg/ml. hrs and the serum half-life was 1.7 hours.

It has been demonstrated earlier that slow and rapid acetylators excrete similar proportions of the dose of isoniazid administered as isonicotinyl compounds. Amalgamating the findings in the two phenotypes, it was observed that the mean proportion of the dose of isoniazid excreted as isonicotinyl compounds in urine collected over the period 0-8½ hours was 50% in patients with abdominal tuberculosis and 55% in those with pulmonary tuberculosis ($P > 0.2$). All these findings suggest that there is no impairment of the absorption of isoniazid in patients with abdominal tuberculosis with intestinal involvement.

The serum levels and a few pharmacokinetic variables of rifampicin in the two groups of patients are presented in the following table. While all 9 patients with abdominal tuberculosis are included in the analysis, the mean values of only 9 patients with pulmonary tuberculosis are included (the assays have not been completed in the remaining 8 patients). The mean body weight was 38.2 kg in patients with abdominal tuberculosis and 42.1 kg in those with pulmonary tuberculosis; the mean dosages of rifampicin administered were 10.1 and 10.2 mg/kg respectively in the two groups.

Hours after dosage	Mean serum rifampicin concentrations (μg/ml)	
	Abdominal TB	Pulmonary TB
1	5.2	4.9
2	8.9	8.1
3	8.4	7.2
6	5.3	4.5
8	3.6	3.0
Mean peak concentration (μg/ml)	10.0	8.8
Exposure (μg/ml. hrs)	84	69
Half-life (hrs)	4.1	4.7
No. of patients	9	9

The mean rifampicin concentrations are similar in the two groups, and the differences in exposure and half-life were not significant. This suggests, that as with isoniazid, the absorption of rifampicin is as good in patients with abdominal tuberculosis as in patients with pulmonary tuberculosis.

No information is available on the exact site of absorption of either isoniazid or rifampicin; it is possible that these two drugs are absorbed rapidly even from the stomach and the duodenum. Such an assumption could explain the similarity of the findings in the two grouos of patients.

The mean blood levels of xylose at 1½ hours and the mean proportion of the dose excreted in urine over the period 0-8 hours following administration of D-Xylose 5g are presented in the following table:

	Abdominal TB	Pulmonary TB
Blood xylose (mg/dl)	15.9	14.8
Proportion of the dose of xylose excreted in urine (0-8 hrs)	34%	41%
No. of patients	9	17

The similarity of both the mean blood levels and the proportion of dose excreted in urine in patients with abdominal tuberculosis and those with pulmonary tuberculosis suggests that the jejunal portion of the small intestine is not affected in the former.

As mentioned earlier, 4 patients with abdominal tuberculosis (3 slow and 1 rapid) were excluded from the main analysis as these patients had a part of the diseased portion of their small intestine resected. The mean concentrations of isoniazid and rifampicin in these patients, together with those of 9 patients (8 slow and 1 rapid) who did not have any surgical intervention are presented in the first table on page 35. (For convenience, the findings of the slow and rapid acetylators have been amalgamated).

The serum concentrations of both isoniazid and rifampicin were broadly similar in the 2 groups. Further, the blood xylose concentrations were 20.0 mg/dl and 15.9 mg/dl at 1½ hours and the proportion of dose of xylose excreted in urine over the period 0-8 hours (after an oral dose of D-xylose 5 g) were 35% and 34% respectively in patients who had resection and in those who did not. These findings suggest that removal of part of the small intestine has not affected the absorption of isoniazid, rifampicin or D-Xylose.

Hours after dosage	Mean concentrations (μg/ml) of			
	Isoniazid		Rifampicin	
	Resection	No resection	Resection	No resection
1	6.4	8.7	4.9	5.2
2	7.1	7.7	8.1	8.9
3	5.8	6.0	8.7	8.4
6	2.8	2.5	6.8	5.3
8	1.5	1.7	3.9	3.6
No. of patients	4	9	4	9
Mean dosage(mg/kg)	9.0	8.3	9.0	10.1

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

(Prema Gurumurthy, G. Raghupati Sarma, Rajeswari Ramachandran, Rani Balasubramanian, Fathima Rahman and A S. L. Narayana).

Hydrogen peroxide producing potential of monocytes in pulmonary tuberculosis

There are several direct and indirect evidences to suggest the role of H_2O_2 mediated killing of intracellular pathogens. Therefore, the *in vitro* H_2O_2 producing capacity of monocytes from 7 patients with bacteriologically proved tuberculosis and 7 healthy controls was tested. It appears that the monocytes from both controls and patients are equally capable of producing H_2O_2 *in vitro* as shown in the following tables.

H_2O_2 production by peripheral blood monocytes*	
Control	Patients
8.0	13.6
8.8	12.8
10.0	13.6
8.8	8.8
12.0	10.0
10.4	17.8
10.8	18.0
9.8 ±1.4**	13.5 ±3.5**

*n mol/0.4 $\times 10^6$ monocytes. **Mean ±S.D.; $P > 0.2$

H_2O_2 production by peripheral blood monocytes*	
Control	Patients
7.9	8.5
10.8	17.9
12.4	16.8
8.9	14.9
19.8	12.4
26.5	43.5
15.2	29.9
14.5 ±6.6**	20.5 ±12.1**

*n mol/0.1 $\times 10^6$ monocytes; after correcting for the number of monocytes by non-specific esterase staining.

** Mean ± S.D.; $P > 0.2$

(started: 1985; completed: 1986).

(Rajiswamy, P. R. Narayanan, G. S. Acharyulu and R. Prabhakar).

The possible role of soluble material from macrophages in cell mediated immunity in pulmonary tuberculosis

Macrophages have been shown to produce, *in vitro*, a soluble material (SM) capable of inhibiting the blastogenic response of normal T-cells to mitogens and antigens in chronic fungal infection. However, not all T-cells were equally susceptible to its suppressive effect. Only the short-lived, low density T-cells (PBMC) could be suppressed by SM. Hence, incubation of PBMC for seven days in culture fluid could result in a functional deletion of the short-lived low density T-cells and thereby yield high density T-cells whose reactivity cannot be suppressed by SM. The possible existence of such a suppressive SM was tested in 12 bacteriologically confirmed pulmonary tuberculosis patients and 12 healthy blood bank donors. Proliferative response of those individuals' lymphocytes to mitogen (PHA 5 μg/ml) and

antigen (PPD 50 μg/ml) were assayed on days, 0, 2 and 7 of PBMC culture. The results are presented in the table below.

Day	Stimulation Index (mean ± S.D.) with			
	PHA (5μg/ml)		PPD (50 μg/ml)	
	Patients (12)	Controls (12)	Patients (12)	Controls (12)
0	1.53 ±0.09	1.53 ±0.17	1.12 ±0.11	1.13 ±0.12
2	1.56 ±0.12	1.51 ±0.13	1.13 ±0.08	1.10 ±0.11
7	1.37 ±0.13	1.37 ±0.18	1.16 ±0.15	1.18 ±0.18

Also, the supernatant of these cultures collected on days 2 and 7 were tested for their suppressive effect on the proliferative response of fresh PBMC from two healthy volunteers; the results are shown in the table below.

No	Stimulation Index (mean ± S.D.) for PHA (5 μg/ml)			
	Day 2		Day 7	
	Patients SN (10)	Control SN (10)	Patients SN (10)	Control SN (10)
1.	1.33 ±0.11	1.37 ±0.05	1.38 ±0.06	1.39 ±0.09
2.	1.26 ±0.09	1.33 ±0.09	1.32 ±0.09	1.32 ±0.06

Numbers in parantheses indicate number of individuals tested.

SN = Supernatant.

The results indicated that the patients' and control lymphocytes showed similar response; the culture supernatants also did not show any difference. We conclude that the defect in CMI in tuberculosis is unlikely to be due to a soluble material from macrophages.

(started: 1985; completed: 1986).

(Rajiswamy, P. R. Narayanan and R. Prabhakar).

STUDIES IN PROGRESS

Kinetics of delayed hypersensitivity response in tuberculous guinea pigs

To try and demonstrate the interrelationship betweer. tuberculous and non-tuberculous mycobacteria (NTM), and between mycobacteria and BCG, groups of guinea pigs were inoculated subcutaneously with (i) High virulent South Indian variant of *M. tuberculosis*, (ii) Low virulent South Indian variant, (iii) $H_{37}Rv$, (iv) *M. bovis*, (v) BCG, (vi) *M. avium* and (vii) *M. scrofulaceum*. One group (uninoculated) was left as controls. Skin tests were done for each guinea pig with sonicates of NTM, PPD-S and saline control. Sub-groups of guinea pigs were superinfected sub-cutaneously with the organisms mentioned above. At the end of 12 weeks after infection/superinfection each animal was sacrificed and the extent of infection assessed by visual scoring and viable count in spleen. The viable count has been set up for the last group of animals. The data are being analysed.

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

(Daniel Herbert, C. Alexander and C. N. Paramasivan).

Sero-epidemiology of influenza A and B in Koppur village children

Sero-epidemiological studies are important in establishing the behavioural pattern of influenza A and B viruses and to trace the epidemiologic course followed by the influenza viruses in tropical regions. The present study in Koppur village near Tiruvallur (Chingleput district) is based on a limited population aged 1-5 years. During November-December 1986, 350 sera were collected and information regarding the presence or absence of any form of acute respiratory infection in the children at the time of collection or within the previous one month were obtained. The anti-haemagglutinin antibody level to the epidemic strains of influenza A H1N1, A H3N2 and 2 influenza B virus subtypes are being carried out with the sera of these children. The anti-haemagglutinin antibody level to influenza viruses will give evidence to recent as well as past infection, because of the heterologous nature of antibody response to various influenza virus subtypes in persons who are infected with one of the subtypes in their life time.

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

(K. Sivadasan*, C. N. Paramasivan, P.G. Gopi, R. S. Vallishayee, Manjula Dutta and R. Prabhakar).

In vitro susceptibility of *M. tuberculosis* isolated in South India against tuberactinomycin

Tuberactin (synonym: Tuberactinomycin or Enviomycin) is an antituberculous drug obtained from *Streptomyces griseoverticillatus* var *tuberacticus*. It has been shown from clinical studies conducted in Japan, that tuberactin, as a secondary anti-tuber-

*Research Fellow.

culous drug, was good in retreating cases of refractory tuberculosis, particularly cases resistant to primary anti-tuberculous drugs. (Fuji et al: *IRYO* (1981) *35*. 434-439). It was therefore planned to investigate the *in vitro* susceptibility of *M. tuberculosis* isolated from South India, in order to get some information on the prevalence of drug resistance to the drug.

A total of 139 strains of *M. tuberculosis* have been studied so far. The indirect sensitivity test was performed, using L-J medium with different concentrations of tuberactin. The distribution of the minimum inhibitory concentration (MIC) of tuberactin for the 139 strains is presented in the table below:

Tuberactin MIC (mcg/ml)	10	25	50	100	Total
No. of strains	13	104	18	4	139

It can be seen that 117 of the 139 strains were inhibited at a concentration of 25 mcg/ml or less. The standard strain, *M. tuberculosis* $H_{37}Rv$ also showed an MIC of 25 mcg/ml on each of 15 occasions tested. The proportion sensitivity test (PST) is in progress to precisely assess the *in vitro* tuberactin resistance.

(started: 1986; expected year of completion: 1988).

(N. Selvakumar, Vanaja Kumar, C. N. Paramasivan, Fathima Rahman and G. S. Acharyulu).

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

As described in the 1985-86 annual report, the study of colonial morphology in South Indian strains of *M. tuberculosis* was continued. The primary isolate was plated out on OAA agar and incubated. Colonies were observed for morphological appearance under the microscope. Single colonies were picked out and subcultured on LJ medium.

A total of 395 cultures from 284 patients admitted to studies at Madras and Madurai were examined. 70% of the cultures had a rough morphology (see table below).

Centre	Rough		Smooth		Total tested
	No.	%	No.	%	
Madras	137	*68*	65	*32*	202 (100%)
Madurai	140	*73*	53	*27*	193 (100%)
Total	277	*70*	118	*30*	395 (100%)

One patient (Madras) gave both rough and smooth types of colonies in the same culture on both the pre-treatment isolates tested. The different type of colonies did not differ in their drug sensitivity pattern (all were sensitive to isoniazid) and the guinea pig virulence (all were highly virulent; mean root index of virulence (RIV.): 1.31, range 1.16-1.46).

In order to study whether any difference was observed in the guinea pig virulence between the two types of morphology, a sample of 25 strains (13 rough and 12 smooth) were tested. It was observed that the proportion of high and low virulent strains was similar in the two types of colonial morphology, as can be seen from the table below.

Morphology	RIV ≤ 1.0		RIV > 1.0		Total
	No.	%	No.	%	
Rough	6	*46*	7	*54*	**13**
Smooth	7	*58*	5	*42*	12
Total	13	*52*	*12*	*48*	25

(started: 1985; completed: 1987).

(P. Venkataraman and R. Prabhakar).

Bacteriocin typing of mycobacteria

Rapidly growing non-tuberculous mycobacteria (NTM) obtained from South Indian patients were screened for the production of mycobacteriocin in order to find out new and suitable indicator strains for bacteriocin typing. One hundred strains of NTM, after identification up to species level, had been selected for this purpose. Each one of these strains was screened against the other 99 strains by the 'streak plate' method as described earlier (annual report, 1983).

Based on the inhibition patterns, 9 indicator strains were selected which could group all the 100 rapid growers into 10 major types. These major types could be further subdivided into several subtypes. The untypable group has now been reduced from 23% (annual report, 1985-86) to 15%. The strains are being classified according to Tsukamura's classification which could help in finding out inter– and intra–species differentiation, if any.

This screening for the production of bacteriocin has also provided us with a group of 18 promising bacteriocin producing strains.

As a continuation of this study, screening of typical *M. tuberculosis* strains with the 9 indicator strains has been started. So far, 50 strains have been screened and it is planned to screen a total of 200 strains.

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

(Vanaja Kumar, N. Selvakumar and C. N. Paramasivan).

Bacteriology of broncho-alveolar lavage fluid

In a preliminary bacteriological study consisting of 40 samples of broncho-alveolar lavage fluid (BALF) collected from 26 patients with tropical pulmonary eosinophilia (TPE), 6 samples yielded a mixture of organisms indicating probable contamination of BALF with normal bacterial flora of the upper respiratory tract (annual report 1985-86). Hence, another study was undertaken to look into the bacteriological profile of BALF and nasal swab of the patients simultaneously. So far, 9 patients with active pulmonary tuberculosis, 7 patients with inactive pulmonary tuberculosis, 12 patients with non-tuberculous chest diseases and 6 patients with TPE have been studied. All the 9 patients in the active tuberculosis group were smear negative and culture positive for *M. tuberculosis*. The patients included in the inactive TB group were those who had undergone treatment for tuberculosis elsewhere and were smear and culture negative on examination at the Centre but the X-ray showing healed lesions. In all, 44 samples of BALF and nasal swabs were collected. The summary of the culture results is presented in the table below:

Culture results	Group				Total
	Active TB	Inactive TB	Non-TB	TPE	
NS and BALF with same type of growth	0	4	2	2	8
NS positive, BALF sterile	5	1	3	5	14
NS sterile, BALF positive*	2	0	1	1	4
NS with one type/group, BALF with a different type/group*	2	2	6	8	18
Total	9	7	12	16	44

NS: Nasal swab; BALF: Broncho-alveolar lavage fluid

*Pure growth profile of BALF when the corresponding NS was sterile or yielded a mixture of organisms other than the one yielded by BALF.

Of the 44 BALF specimens cultured, 8 (18.2%) showed a mixture of organisms similar to that obtained from the corresponding nasal swabs thereby showing contamination of BALF with the upper respiratory tract flora.

Nasal swabs yielded either pure growth of *Staph. aureus* or *Staph. albus* or a mixture of *Neisseria, Staphylococcus, Streptococci*, gram negative bacilli and diphthe-

roids. BALF alone yielded *Pneumococci* from 2 patients and *H. influenzae* from 7 patients.

It is intended to study at least 50 patients in each group.

(started: 1986; expected year of completion: 1988).

(K. Sivadasan*, C. N. Paramasivan and V. K. Vijayan).

Determination of the acetylator phenotype employing salivary concentrations of isoniazid

Salivary concentrations of isoniazid have been shown to be similar to those in serum following administration of isoniazid 300 mg in both slow and rapid acetylators of the drug (see page 28). It is possible that salivary levels of isoniazid could discriminate between slow and rapid acetylators and tests employing saliva for determining the acetylator phenotype would have obvious practical advantages particularly in children from whom it is not easy to obtain blood and timed urine collections. Prior to undertaking the investigations in adult patients and in children, a pilot study was undertaken in healthy adult volunteers whose acetylator phenotype had been determined earlier by standard methods, to obtain information on the most suitable time-point for collection of saliva after oral administration of a uniform test dose of isoniazid 300 mg.

Five *known* slow and 5 *known* rapid acetylators were administered a uniform dose of 300 mg of isoniazid orally and saliva samples at 2,3,4,5 and 6 hours were collected. The saliva samples were stored at -20°C for a day and after thawing centrifuged to remove mucoproteins, and supernatants separated. The supernatants were processed for the quantitative estimation of isoniazid by a standard spectrophotometric method.

The mean salivary isoniazid concentrations of the 5 slow and 5 rapid acetylators are presented in the following table:

Time of collection (hrs.)	Mean (A.M.) salivary concentrations of isoniazid (μg/ml)		Index of discrimination*
	Slow	Rapid	
2	5.96	2.50	4.1
3	4.96	1.30	6.6
4	3.62	0.78	6.2
5	2.52	0.52	4.8
6	2.34	0.36	5.7

*The ratio of the difference in mean between slow and rapid acetylators to its standard error.

*Research Fellow.

The mean body weights of the 5 slow and 5 rapid acetylators were 56.5 kg (range: 44.5-63.0 kg) and 63.1 kg (range: 57.6-70.0 kg) respectively and the mean dosages of isoniazid were 5.4 and 4.8 mg/kg respectively. The pilot study suggests that it is possible to determine the acetylator phenotype by employing the salivary concentrations of isoniazid. It also appears that adequate discrimination is likely to be obtained on the basis of isoniazid concentrations in saliva collected at 3 or 4 hours.

Before undertaking the investigation in children, it was decided to carry out the study in adult subjects who had been classified earlier as slow or rapid acetylators on the basis of the ratio of acetylisoniazid to isoniazid in urine collected over the period 24-25 hours following an oral dose of matrix isoniazid 30 mg/kg. A total of 82 patients comprising of 42 slow and 40 rapid acetylators were admitted to the study. They were administered a uniform oral dose of isoniazid 300 mg and concentrations of isoniazid in saliva collected at 3, 4 and 5 hours were determined. The mean body–weight of these patients was 44.2 kg (range:25.7-63.1 kg) and the mean dosage of isoniazid administered was 6.8 mg/kg (range: 4.8-11.7mg/kg.).

Results (not presented) showed that the distributions according to concentrations of isoniazid in saliva collected at 3, 4 and 5 hours were bimodal; however, it was difficult to determine the concentration for best discrimination due to a considerable overlap. It is possible that a dose of isoniazid 300 mg to the patients with a lower body-weight is too high for this purpose. Hence, an investigation to classify patients as slow or rapid acetylators on the basis of salivary concentrations at 2, 3, 4 and 5 hours following a uniform oral test dose of isoniazid 100 mg, has been started. This dose would be similar to the 3 mg/kg i.m. dose employed successfully earlier for the determination of the acetylator phenotype.

(started: 1987; expected year of completion: 1988).

(S. Kailasam, G. Raghupati Sarma and A.S.L. Narayana).

Mouse peritoneal and tissue macrophage activity during infection with different doses of *M. tuberculosis* and *M, bovis*

An investigation has been initiated to study the bactericidal activity of the macrophage in tuberculous infection in order to understand the mechanism of its action (whether oxygen-dependent or independent) and to postulate a model for pharmaco-regulation of its activity. The investigations have been started with peritoneal and spleen tissue macrophages in mice infected with tubercle bacilli; it is later proposed to undertake *in vitro* investigations for easier manipulation.

Pyrazinamide is a powerful anti-tuberculosis drug and it has been established that its action is restricted to bacilli in an acid environment such as prevalent inside the macrophage. The mechanism of pyrazinamide action has not been delineated so far and it is possible that pyrazinamide might exert its bactericidal action indirectly through the activation of the macrophage. It was decided to undertake a preliminary investigation to study the effect of infecting

mice with different dose levels of *M. tuberculosis* (pyrazinamide-sensitive strain) and *M. bovis* (a naturally resistant strain to pyrazinamide) on the release of hydrogen peroxide, and the activities of lysozyme, and three other lysosomal hydrolases, namely acid phosphatase, β-glucuronidase and cathepsin-D.

Swiss albino mice were infected intravenously with 0.1 ml containing 10^6, 10^5 and 10^4 organisms of *M. tuberculosis* and *M. bovis* in 7H9 liquid medium. Six control mice (uninfected) and 8 mice each from the low (10^4), medium (10^5) and high (10^6) dose infected groups were sacrificed on day 3, 7, 14 and 21 after infection.

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

Analysis of data (not presented) indicated no significant differences between the inoculation doses in either the release of hydrogen peroxide or the activities of the lysosomal hydrolases with both *M. tuberculosis* and *M. bovis*. The findings with the three different doses have therefore been amalgamated and the mean values are presented in the tables given below. (Each value, therefore, represents the arithmetic mean of 12 experiments and each experiment is based on the pooled peritoneal exudate cells of the spleen tissue of 2 animals).

Non-specific esterase staining of the peritoneal exudate cells revealed that about 50% of the cells (range: 48-54%) were macrophages and the proportions were similar between species and between different inoculation doses.

The mean spleen viable counts (V.C.) and the hydrogen peroxide (H_2O_2) released by the peritoneal exudate cells (PEC) on the different days following infection with *M. tuberculosis* or *M. bovis*. are presented in the following table:

Days after infection	*M. tuberculosis*		*M. bovis*	
	Spleen V.C. (log units)	H_2O_2 released (n moles/ 10^6 PEC)	Spleen V.C. (log units)	H_2O_2 released (n moles/ 10^6 PEC)
0 (Control)	0	0.05	0	0
3	4.88	0.84	2.92	0.62
7	5.22	1.31	4.14	1.47
14	5.68	1.17	3.56	1.40
21	4.50	1.66	3.79	2.31

With both species, infection was established by the 3rd day. With *M. tuberculosis*, there was a gradual increase in the spleen viable counts up to the 14th day and then a slight fall by the 21st day, the difference between the 3rd day's and 14th day's counts being significant ($P<0.02$). With *M. bovis*, it appears that the peak infection occurs by the 7th day, with little change thereafter, none of the differences being statistically significant. The mean of log viable counts (mean of log viable counts obtained on days 3, 7, 14 and 21) was 5.07 with *M. tuberculosis* and 3.60 with *M. bovis*, a significant difference ($P<0.001$), suggesting that *M. tuberculosis* is more virulent than *M. bovis* in the mouse. Ingestion of the bacilli appears to have triggered the release of hydrogen peroxide, the differences between the mean values in the controls and those on any of the days after infection being highly significant ($P<0.001$). There is approximately a 2-4 fold increase in the release of hydrogen peroxide between the 3rd and 21st days with both *M. tuberculosis* ($P=0.09$) and *M.bovis* ($P<0.01$). The release of hydrogen peroxide by the macrophages appears to be less with the more virulent *M. tuberculosis* than with less virulent *M. bovis*; the difference, however, was not statistically significant. These findings are in agreement with previously published work demonstrating a negative correlation between the virulence of the infecting organism and the release of hydrogen peroxide by the macrophages.

The mean activities of the lysosomal hydrolases in the peritoneal exudate cells following infection with *M. tuberculosis* are presented in the following table:

Enzyme	Days after infection with *M. tuberculosis*				
	0 (Control)	3	7	14	21
Acid phosphatase (n moles/mg protein)	11.87	12.35	12.56	13.85	15.75
β-glucuronidase (n moles/mg protein)	15.02	20.78	16.24	18.57	15.87
Cathepsin-D (units/mg protein)	0.31	0.21	0.16	0.18	0.27
Lysozyme (μg/mg protein)	3.06	1.57	5.82	6.33	5.43

While the activity of β-glucuronidase was fairly constant, there was a slight but steady increase in that of acid phosphatase and a decrease (up to the 14th day) in the activity of cathepsin-D; none of the differences was however statistically significant. The mean lysozyme value on the 3rd day of infection was lower than that in the control group; there was a steady increase

thereafter up to the 14th day, the difference between the mean values on the 3rd and the 14th day being significant ($P<0.02$). The lysosomal enzyme levels in spleen cells following infection with *M. tuberculosis* are presented in the following table:

Enzyme	Days after infection with *M. tuberculosis*				
	0 (Control)	3	7	14	21
Acid phosphatase (n moles/mg protein)	44.72	42.76	47.02	49.89	56.34
β-glucuronidase (n moles/mg protein)	96.09	78.79	89.26	91.34	111.6
Cathepsin-D (units/mg protein)	0.96	0.76	0.80	0.58	0.54
Lysozyme (μg/mg protein)	25.70	21.70	23.26	41.72	34.58

The mean activities of all the enzymes on the 3rd day after infection were slightly lower than in the controls though none of the differences was significant. With an increase in the number of days after infection, there was a significant increase in the activities of acid phosphatase, β-glucuronidase and lysozyme and an appreciable decrease in that of cathepsin-D. The peak activities of acid phosphatase and β-glucuronidase were observed in the 21st day, while peak levels of lysozyme were observed on the 14th day. The mean activities of acid phosphatase and β-glucuronidase were 32% and 42% higher on the 21st day than the respective mean values on the 3rd day ($p<0.05$ and <0.001, respectively). The mean lysozyme activity on the 14th day was about 92% higher than that on the 3rd day ($p<0.02$). The mean cathepsin activity on the 21st day was about 44% lower than that in the controls ($P<0.01$) and about 30% lower than on the 3rd day ($P<0.05$).

The results of the activities of the lysosomal hydrolases in both the peritoneal exudate cells and the spleen cells following *M. bovis* infection are being analysed.

Ingestion of mycobacteria by the macrophages appears to activate both the oxygen-dependent and the oxygen-independent mechanisms of the bactericidal action of this cell. There is an appreciable increase in the release of hydrogen peroxide by the macrophages after infection and the degree of release appears to be slightly lower with *M. bovis* than with *M. tuberculosis*. There is an increase in the activities of the lysosomal hydrolases investigated, with the exception of cathepsin-D, the changes being more prominent in the tissue

macrophages (spleen cells) than in the peritoneal exudate cells. The reasons for the unexpected decrease in the activities of cathepsin-D need to be elucidated. Further, the changes in the activities of the hydrolases as well as the release of hydrogen peroxide appear to broadly correlate with the viable counts of the bacilli in the spleen. Investigations are in progress to study the effect of both pyrazinamide and pyrazinoic acid, alone and in combination with other anti-tuberculosis drugs, on the activity of the macrophage as expressed by the release of hydrogen peroxide and the secretion of the lysosomal hydrolases. The activity of pyrazinamide deamidase (the enzyme that catalyses the conversion of pyrazinamide to pyrazinoic acid) in the peritoneal exudate cells as well as spleen tissue macrophages is also being determined to study the degree of conversion of the drug to its primary metabolite within the cell.

(started: 1985; expected year of completion: 1989).

(M. Kannapiran, G. Raghupati Sarma, C.N. Paramasivan, Fathima Rahman and A. S. L. Narayana).

Production of monoclonal antibodies to *M. tuberculosis* H_{37} Rv and South Indian strain-7219

Spleen cells of BALB/C mice immunised with sonicates of *M. tuberculosis* $H_{37}Rv$ and South Indian strain-7219 were fused with Sp2/O myeloma cells for the production of monoclonal antibodies to these antigens. Nine fusions each have been done so far for $H_{37}Rv$ and 7219. From the $H_{37}Rv$ fusions, 213 hybridoma clones were obtained and were screened by RIA and by ELISA for antibody production. Of the 213 clones, 2 have been repeatedly positive for antibody production against $H_{37}Rv$ sonicate antigen by ELISA. For one of them, two limiting dilutions have been carried out for purification. Supernatant from the purified clone was subjected to specificity assay against 14 different mycobacterial antigens and one non-mycobacterial antigen—*E. coli*, by ELISA. It was found that the monoclonal antibody specifically reacted with the mycobacterial antigens tested. The other antibody producing clone against $H_{37}Rv$ is being purified by limiting dilutions. From the fusions of the strain 7219, a total of 159 hybridoma clones were obtained and screened for antibody production. Of them, 5 have been positive for antibody production against strain-7219 in the initial screening by ELISA. The consistency of antibody production is being checked by repeat ELISA. Simultaneously, limiting dilutions are being carried out to purify these clones. Further fusions will be done for the production of more clones against both $H_{37}Rv$ and 7219.

On the completion of limiting dilution and specificity assay, those monoclonal antibodies which show specificity to mycobacterial antigens will be concentrated in mouse ascitis and partially purified by precipitation with ammonium sulphate. The immunoglobulin class will be determined by isotyping with the aid of immuno-fluorescence. Further purification of these antibodies will be done by protein-A affinity column chromatography and cyanogen bromide activated sepharose column chroma-

tography. Sonicated antigen preparations of *M. tuberculosis* $H_{37}Rv$, 7219 and non-tuberculous mycobacteria will be separated by SDS-PAGE. The separated antigens will be analysed against the monoclonal antibodies by Western blotting, to identify species specific antigens which will be characterised later.

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

(Daniel Herbert and C. N. Paramasivan).

Development of an assay for studying mycobacterial properties of human peripheral blood monocytes derived macrophages (MDM)

An *in vitro* experiment to assess the ability of macrophages to kill intracellular mycobacteria is under standardization. Adherent monocytes from peripheral blood were allowed to mature in culture into macrophages. Lymphocytes from the same individual were stimulated with 1 μg/ml PPD for four days. The culture supernatant containing the lymphokines were used to stimulate the macrophages; 0, 10, 20 and 30% lymphokines were added to the monocyte cultures from day 4 and maintained till day 7. On day 7 single cell suspension of *M. microti* was used as the phagocytic challenge for the MDM. The ratio of MDM to *M. microti* was 1:10. After phagocytosis, extracellular bacteria were removed. The viability of the intracellular bacteria was tested immediately after phagocytosis (T0) and 48 hours after phagocytosis (T48), by culturing the lysed macrophages in 7H11 agar. The CFUs were counted one week later. The difference in the number of CFUs between T0 and T48 indicates the mycobactericidal capacity of the macrophages.

Since multiplication of *M. tuberculosis* inside the macrophage is the cardinal pathological feature of tuberculosis, the possibility of impaired mycobactericidal function of the macrophage as a cause for the defect in cell mediated immunity in tuberculosis is being explored with the above test.

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

(Rajiswamy and P. R. Narayanan).

Human leucocyte antigen (HLA) typing

As mentioned in the earlier annual report (1985), steps to procure HLA A, B and DR sera are still continuing and a definitive start of HLA typing work can be made only when the collection of relevant sera is completed.

In the mean time, the role of Ia antigen (Immune region associated antigen—HLA, —DR, —DQ and —DP) on immune functions of the broncho-alveolar lavage cells of pulmonary tuberculosis patients (sputum negative and treated) have been started. Hydrogen peroxide, known for its microbicidal effect, is released by monocyte/macro-

phage and has been used here as a parameter of macrophage function. The present preliminary study reveals that broncho-alveolar lavage cells produce less hydrogen peroxide when compared to the production by blood monocytes. Among the broncho-alveolar lavage cells, Ia negative macrophages produce more H_2O_2 than the Ia positive macrophages. The investigation is being continued.

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

(P. Selvaraj, P. R. Narayanan, V. K. Vijayan and R. Prabhakar).

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

Characterisation of mycobacterial antigen by Western blotting and immunological screening is being continued. The antigenic component around 14K and other antigenic components which are prominently recognised by the sera of tuberculosis patients are being further characterized by using affinity purification and peptide mapping.

Affinity purification: The mycobacterial antigenic components on the nitrocellulose paper (NCP) are incubated with serum from tuberculosis patients and volunteers separately. After incubation, the NCP is washed with PBST thrice and the antibody bound to the antigen is eluted by using glycine HCl buffer of low pH. The eluted antibody, which is the affinity purified antibody is again used to recognise the mycobacterial antigenic components transferred on to a new NCP. The clarity of the recognition pattern is improved and the antigenic components of importance can be picked up.

Peptide mapping: This is one of the widely accepted methods for establishing relatedness between different proteins, and also in the further characterization of purified proteins. Essentially, it entails the partial enzymatic digestion of proteins in the presence of sodium dodecyl sulphate and the analysis of the digest by SDS-Poly acrylamide gel electrophoresis. (SDS-PAGE).

The polypeptides of interest are located by brief statining and destaining after SDS-PAGE, then cut out and the gel slices are directly loaded on to the secondary peptide mapping gel and then overlaying each slice with protease. The secondary gel has larger sample wells and larger stacking gel. The digestion of the polypeptide by the protease is carried on in the stacking gel. After this proteolysis, the polypeptides are electrophoresed as usual. This procedure is being standardised using proteases like Chymotrypsin, Papain and Trypsin.

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

(Sujatha Narayanan and P. R. Narayanan).

Evaluation of Antigen-5 in immunodiagnosis of pulmonary and extra-pulmonary tuberculosis

Development of techniques which can rapidly identify and measure specific mycobacterial components in clinical specimens is an area of importance in tuberculosis research.

A double antibody sandwich ELISA, employing antisera against the whole mycobacteria in two different animal species, for detection of total mycobacterial antigens has been developed at the Centre. Also, a competitive inhibition ELISA using monospecific goat antiserum for detection of Antigen-5 has been employed. These assays work with a sensitivity of 5 ng/ml.

Detection of antibodies to Antigen-5 in serum and CSF has proved to be a better discriminatory test between disease and control samples than antibodies to crude mycobacterial extracts.

It is proposed to apply the technique of ELISA for detection of total antigen and specific antigens like Antigen-5 in serum, sputum, cerebrospinal fluid, bronchotalveolar lavage and ascitic fluid samples obtained from patients with pulmonary and extra-pulmonary tuberculosis. Antibodies to Antigen-5 also will be detected in these samples. In addition, attempts are under way for modifications of the ELISA, like use of monoclonal antibody for trapping the antigen as well as for screening.

(started: 1986; expected year of completion: 1988).

(Alamelu Raja and P. R. Narayanan).

Studies in characterisation and purification of filarial antigens

The aim of this project has been to isolate and characterise filarial antigen(s) which can be used subsequently in immunoassays that are aimed at diagnosis or understanding the immune response of the human host. One of the antigens of *S. digitata* with a mol. wt. of 21,000 which was identified in immunoblot with sera from *W. bancrofti* infected patients (see annual report 1985-86) was taken for further analysis. This protein was found to be a glycoprotein by periodic staining.

This protein was further purified by using a Con-A affinity column. The alpha-methyl mannoside elutable glycoprotein fraction had two proteins of mol.wt. 21KD and 43KD. The antibody titres against these two antigens were of the order TPE> chronic> asymptomatic mf carrier> endemic. The antibody level against these two antigens decreased after treatment in TPE patients (as seen by decreasing intensity of the bands in the immunoblots). Further, in the immunoblots, characterization of these two antigens revealed that the antigenic determinant was present in protein moiety and the two proteins were found to have different antigenic determinants. The two proteins were heat and acid resistant. These two antigens did not share homology with ascariasis antigen. The two antigens were also not recog-

nised by sera of TB patients, North American sera and sera from patients with ascariasis infection.

Attempts are still being made to purify this protein to homogeneity before its usefulness in the immunoassays can be evaluated.

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

(C. R. Vanamala and P. R. Narayanan).

Antigen detection assay for circulating antigens in filariasis

In the previous annual report (1985-86) the generation of monoclonal antibodies against filarial parasites has been reported. They were developed with the aim of developing monoclonal antibody based assay for the detection of microfilaremic subjects in the population.

Hence, all available monoclonal antibodies were tested in two-site ELISA for their capacity to recognize the filarial antigens.

The following table shows the mean optical density (OD) obtained with 10 non-endemic serum samples (2nd column). The mean +2 SD values in the third column of the table shows the threshold O.D. An O.D. above mean +2 S.D. was considered indicative of circulating antigen being recognized by monoclonal antibody.

Monoclonal Ab	Mean O.D.	Mean O.D.+2S.D.
M1b	0.101	0.185
M3a	0.845	0.951
M5a	0.309	0.373
4-5	0.158	0.230
4-8	0.146	0.210
b7	0.138	0.194
5BC5	0.263	0.357
7AE8	0.391	0.479
CA16	0.133	0.205
CA86	0.225	0.321
CA90	0.182	0.278

The results obtained with 10 sera from normal healthy individuals from the Centre's laboratory are presented in the following table. The results are expressed as positive or negative for the antigen in circulation.

Monoclonal antibody	Control subjects									
	1	2	3	4	5	6	7	8	9	10
M1b	—	+	—	—	—	—	+	+	+	+
M3a	—	—	—	—	—	—	+	—	+	—
M5a	—	+	—	—	—	—	+	—	—	—
4-5	—	—	—	—	—	—	—	—	—	—
4-8	—	—	—	—	—	+	+	—	—	—
5BC5	—	+	—	—	—	—	+	—	—	—
7AE8	—	—	+	—	—	+	+	—	—	—
b7	—	+	—	—	—	—	+	—	—	—
CA16	—	—	—	—	—	+	+	—	+	—
CA86	—	—	—	—	—	—	+	—	—	—
CA90	—	—	—	—	—	—	+	—	—	—

The same monoclonal antibodies were tested against 10 sera from proven microfilaremic individuals. The results are presented below:

Monoclonal antibody	Microfilaremics									
	1	2	3	4	5	6	7	8	9	10
M1b	—	+	—	+	+	+	+	+	+	+
M3a	—	+	—	—	—	—	—	—	+	—
M5a	—	+	—	—	+	+	+	+	+	+
4-5	—	+	—	—	—	+	+	+	—	—
4-8	—	+	—	—	+	+	+	+	+	—
5BC5	—	+	—	+	+	+	+	+	+	+
7AE8	+	+	+	+	+	+	+	+	+	+
b7	—	+	—	—	+	—	—	—	—	—
CA16	+	+	+	+	+	+	+	+	+	+
CA86	—	+	—	+	+	+	+	+	+	+
CA90	—	+	—	+	+	+	+	+	+	+

From the above table, it is evident that monoclonal antibodies CA16 and 7AE8 recognise the circulating filarial antigen in all microfilaremic individuals tested. To examine whether CA16 and 7AE8 recognize the same epitopes, an attempt was made to block reaction of biotinylated CA16 and *B. malayi* antigen using monoclonal antibody 7AE8 and vice versa. The table on page 53 shows the results obtained.

Blocking Ab	Reacting Ab	O.D.	Blocking Ab	Reacting Ab	O.D.
—	7AE8	0.511	—	CA16	0.550
7AE8	7AE8	0	CA16	CA16	0
CA16			7AE8		
1/20	"	0.077	1/20	"	0
1/40	"	0.071	1/40	"	0
1/80	"	0.136	1/80	"	0
1/160	"	0.157	1/160	"	0.001
1/320	"	0.189	1/320	"	0.014
1/640	"	0.234	1/640	"	0.032
1/1280	"	0.279	1/1280	"	0.065
1/2560	"	0.350	1/2560	"	0.039
1/5120	"	0.421	1/5120	"	0.047
1/10240	"	0.474	1/10240	"	0.077

The results indicate that 7AE8 and CA16 cross react; while 7AE8 is able to block CA16 completely, CA16 can block 7AE8 only partially. This result is suggestive of either partial identity of the epitopes recognized by the antibodies or extreme difference in the affinities of the two antibodies to the same epitope.

The utility of these two monoclonal antibodies is further being assessed for the detection of antigen in circulation. However, the applications of these monoclonal antibodies may be limited as they recognize phosphorylcholine epitopes.

Hence, more monoclonal antibodies are being generated for application in immunodiagnosis.

(started: 1984; expected year of completion: 1989).

(R. S. Paranjape).

EPIDEMIOLOGICAL STUDIES

STUDIES COMPLETED

BCG prophylaxis in tuberculosis

The final round of the 15-year follow-up was concluded. During the year under report, 44,858 registration were made and 21,459 individuals needed X-ray examinations. Of these, 19,316 (90%) were X-rayed and 131 sputum positive cases were detected from among the 1961 persons subjected to sputum examination.

During the period under report, in the Project's central clinic at Tiruvallur and at Periapalayam PHC in the study area, 2553 persons were X-rayed and 272 sputum positive cases were detected.

Treatment of these patients had been the responsibility of the staff of the Epidemiology Unit. With effect from December, 1986, treatment cards for 589 patients who were currently on treatment were duplicated and handed over to the District Tuberculosis Officer, Kanchipuram, for continuation of treatment.

The detailed data analyses is being carried out.

(started: 1968; completed: 1986).

(R. V. S. N. Sarma, Manjula Datta, R. S. N. Rao, D. S. Anantharaman, B. N. Appegowda, D. L. S. Rao, V. Venkatesha Prasad, P. G. Gopi, M. P. Radhamani, R. S. Vallishayee, N. Shivaramu, R. Subramani, A. M. Diwakara, B. N. Gopalan and C. N. Paramasivan).

BCG prophylaxis in leprosy

In the BCG prophylaxis study in leprosy, the 15 year follow-up was concluded. During the period under report, 1, 23, 717 registrations were made in 50 panchayats. Out of 93,861 due for examination, 84,946 (91%) persons were examined for evidence of leprosy and 2,091 new cases of leprosy (1787 definite and 304 suspected) were diagnosed. The data are being analysed.

(started: 1973; completed: 1986).

(Mohan Nataraj, K. Sadacharam, V. Balasubramaniam, C. Kolappan, B. Nagaraju, K. R. Bhima Rao, R. Selvaraj, R. S. Vallishayee, R. Subramani and A. M. Diwakara).

Resurvey of filariasis in two endemic villages

In two endemic villages with an approximate population of about 3000, a resurvey was conducted after an interval of 3 years employing standard methodology.

The objectives of the resurvey were (1) to compare the incidence rates of clinical filarial disease in the groups with and without initial microfilaraemia (2) to find out the incidence rate of microfilaraemia and (3) to find out the fate of initial microfilaraemics and those with clinical disease.

Incidence of disease: The table below gives the incidence of disease:

Status at baseline survey (1982-83)	Status at resurvey (1985-1986)		
	Number exam.	No. of cases	Rate (%) (over 3 years)
Apparently normal	1575	56	3.56 ±0.91*
Microfilaria positive	232	5	2.16 ±1.87

*95% Confidence Interval

The incidence rates of disease were similar (P>0.25) in those with and without initial microfilaraemia (mf). However, these observations need to be substantiated further on a larger population base. The population base is being expanded from 3000 to 15,000 in endemic villages (see page 59).

Incidence of microfilaraemics: Out of 608 males and 823 females who were initially 'normal', 39 and 57 respectively, showed mf at the end of 3 years. Thus the overall mf incidence rate, over 3 years, was 6.7%; 6.4% in males and 6.9% in females.

Persistence of microfilaraemia: In 149 (71%) of the 210 initially mf +ve subjects, the microfilaraemia was seen to be persistent at the end of 3 years. The rate of persistence was similar in the two sexes; males 74%, females 68%.

(started: 1985; completed: 1986).

(R. V. S. N. Sarma, D. S. Anantharaman, A. Venkatesha Reddy and M. P. Radhamani).

Standardization of physicians in clinical examination of filariasis

The objectives, study design and methodology of this study were described in detail in the 1985-86 annual report. The data on the interphysician agreement have been analysed in detail, using appropriate statistical procedures, and the overall interphysician agreement comparing all the 3 physicians obtained. The table on page 56 shows the overall Kappa values (measure of inter-observer agreement), considering all 3 physicians, for each of the clinical manifestations before and after standardization.

Clinical Manifestation	Before standardization		After standardization	
	P	Kappa	P	Kappa
1. Lymphangitis	0.96	0.57	0.92	0.57
2. Lymphadenitis (femoral)	0.59	0.16	0.76	0.48
3. Lymphoedema (leg)	0.79	0.62	0.91	0.65
4. Lymphoedema (foot)	0.77	0.58	0.92	0.63
5. Lymphoedema of scrotum	0.73	0.42	0.95	0.57
6. Chronic inflammation of cord	0.67	0.18	0.76	0.52
7. Chronic inflammation of testes	0.63	0.23	0.89	0.35
8. Hydrocele	0.81	0.62	0.84	0.65

P = Overall agreement.
Kappa = Chance-adjusted agreement.

It can be clearly seen that for some clinical signs like lymphadenitis (femoral) and scrotal lymphoedema, the disagreement is markedly reduced and K-values improved after standardization, while the K-values for some clinical signs with high initial agreement such as lymphangitis, lymphoedema of leg and foot and hydrocele remained the same.

(started: 1986; completed: 1986).

(K. C. Umapathy, R. V. S. N. Sarma, V. Kumaraswami, D. S. Anantharaman, R. S. Nagabhushana Rao, B. N. Appegowda, A. Venkatesha Reddy, M. P. Radhamani and R. S. Vallishayee).

STUDIES IN PROGRESS

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

This study was started, in collaboration with the Institute for Research in Medical Statistics (Madras Chapter), with the objective of obtaining information on two short-course chemotherapy regimens with respect to their feasibility, acceptability, toxicity and effectiveness under programme conditions in the 12 Primary Health Institutions in the BCG trial area, in Tiruvallur, Chengalpattu district.

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

In all, 510 patients have been admitted to the study, 156 to the twice-weekly regimen and 354 to the daily regimen. The drug compliance rates for the two regimens are shown below:

Drug compliance rate (%)	Twice-weekly*		Daily (self-administered)	
	Number	%	Number	%
100	58	*37*	192	*54*
80–	21	*14*	31	*9*
60–	19	*12*	24	*7*
40–	7	*5*	19	*5*
20–	17	*11*	35	*10*
<20	33	*21*	53	*15*
Total	155	*100*	354	*100*

* One patient changing over from twice-weekly to daily excluded.

It can be seen that 51% and 63% of patients in the twice-weekly and daily regimens respectively had collected 80% or more of the required doses of chemotherapy. An interim analysis has shown that of 53 twice-weekly and 127 daily regimen patients, who had collected at least 80% of the required doses and in whom a 24-month sputum collection has been made, 50 (94%) and 117 (92%) were culture negative at the end of 18 months of follow-up. The follow-up of the patients is continuing and information on bacteriological status, drug sensitivity and symptomatology is being systematically collected and recorded.

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

(K. C. Umapathy, R. V. S. N. Sarma, Manjula Datta, B. N. Appegowda, R. S. Vallishayee, B. N. Gopalan and C. N. Paramasivan).

Studies in childhood tuberculosis

Childhood tuberculosis has been designated as one of the priority areas for research by the Epidemiology Unit of the Centre. It is proposed to take up a study on the protective efficacy of BCG in childhood tuberculosis very shortly. It was decided to review the information on childhood tuberculosis that is already available in the project, and to undertake a few pilot studies in order to decide upon and develop the most suitable methodology for this study. One of these is described below.

Childhood tuberculosis in a rural area: A descriptive study was undertaken in order to obtain information on the different forms of childhood tuberculosis treated in the study area, the basis on which the diagnosis was made, the treatment particulars and the current health status of the patients.

All children aged 0-12 years (born after the BCG vaccination in the trial area was over), for whom anti-tuberculosis treatment was initiated in the past 5 years were listed. Information on their disease status, and basis for diagnosis was extracted from their treatment cards. Each child was visited and information regarding their treatment status and current health status was obtained by a medical officer. Children who had some disability attributable to tuberculosis were visited by a second medical officer, who confirmed the post-tuberculosis disability.

Anti-tuberculosis treatment had been initiated for 204 children during the period January 1980 to December 1984. The manifestations seen are given in the table below.

Diagnosis	No. of children
Primary pulmonary complex	47
Progressive primary disease	42
Tuberculous lymphadenitis	48
Tuberculous meningitis	14
Miliary tuberculosis	2
Tuberculous abdomen	5
Bone and joint tuberculosis	7
Ocular tuberculosis	4
Not available	35

109 of these children have been visited, of whom 14 had died, 81 were alive and completely healthy, and the remaining 14 were alive and sick; 39 of the 95 children alive have completed treatment. The study is in progress.

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

(Manjula Datta, K. Sadacharam and R. Selvaraj).

Natural history studies in filariasis

Epidemiological information available on filariasis is mostly based on single point surveys of an endemic population. In order to understand the dynamics of disease transmission, the determinants of filarial infection and disease, and to plan control strategies that will be congruent with the natural history of the disease, it is essential to undertake longitudinal studies. In the trial area of the epidemiology unit, several endemic foci have been identified and population studies launched on a large scale.

So far, baseline studies in 12 endemic villages with a population of about 11,500 have been completed and the survey is being extended to some more endemic villages in order to have a population base of 15,000 by the end of June 1987. Detailed follow-up of this endemic population (over a period of 5 years) is planned, which includes periodic resurveys for microfilaraemia and clinical disease, close surveillance for acute disease manifestations and study of new borns in the endemic areas. Information on risk factors, socio-demographic and environmental aspects related to filariasis will also be prospectively collected in the endemic villages. The clinical disease rate in the population (so far analysed) ranges from 9-14% while the microfilaraemia rate ranges from 7-13% in the different villages. In these endemic villages, tropical pulmonary eosinophilia has not been observed.

(started: 1986; expected year of completion: 1991).

(R. V. S. N. Sarma, R. S. Nagabhushana Rao, D. L. Sathyanarayana Rao, A. Venkatesha Reddy and M. P. Radhamani).

Immunodiagnostic study in filariasis

Monoclonal antibodies CA 101 and CA 16 were employed in antigen detection by ELISA technique on 42 serum samples (see 1985-86 annual report). The results of this initial assay suggested that the assay is recognizing all the carriers and a considerable proportion of the patients with clinical filariasis. This promising assay is being further evaluated among subjects with asymptomatic microfilaraemia and manifest clinical filariasis. Endemic normals and non-endemic normals are also being studied.

The study subjects are drawn from the endemic filarial villages and non-endemic villages of the trial area, and the monoclonal antibody assay is being carried out in the immunology laboratory of the Centre.

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

(Ramesh Paranjape, R. V. S. N. Sarma and R. S. Vallishayee).

Studies in acute respiratory infections (ARI)

Studies in ARI were continued during the year under report. The main objective of the project was to study the epidemiological features of ARI and to establish the causative organisms responsible.

An algorithm had been developed for primary care management of ARI, as reported in the 1985-86 annual report. This algorithm was also used for the operational classification of ARI into life threatening forms, severe forms, moderately severe forms and mild forms, in the epidemiological studies.

The following studies are in progress:

Surveillance for epidemiological features of ARI: The surveillance instituted in Koppur (1985-86 annual report), with a population of 1864 was continued. There were 313 children 0-5 years old at the start of the surveillance. Subsequently 246 children (57 new born, 35 settlers, 154 visitors) were added, making a total of 559 children. Of these, 160 have left the village and 6 (4 neonatal and 2 postneonatal) died leaving 393 children available for examination.

After a complete house-to-house census to identify eligible children, each child is seen once in 15 days to collect information (on precoded forms) on the presence or absence of ARI. Those children with ARI are seen again after a week and information on their clinical condition, compliance status and preference for other types of treatment collected. Children who developed ARI in between rounds (surveillance or follow-up) were encouraged to come for treatment to a central clinic in the village, and their morbidity status was recorded. Complete information on socio-economic status and the knowledge, attitudes and practices of the population with respect to ARI were also collected.

During the year under report, 26 surveillance rounds and 26 follow-up rounds were completed and on an average 317 children examined in each round. In all, there were 4891 episodes of ARI (of which 59% were mild, 40% were moderately severe requiring antibiotics and 1% were severe requiring referral to a hospital). The mean proportion of children suffering from ARI in a round was 59% (range being 40%–76%). Simple rhinorrhoea contributed to at least 44% of the burden of illness at each round.

One child with congenital heart disease succumbed to a life-threatening broncho-pneumonia, this being the only death attributable to respiratory infections.

Compliance with treatment given continued to be over 95% at each round. On an average 41% (range 17%–61%) had recovered or improved. Of those who had not improved, the majority were cases of rhinorrhoea.

Each child had, on an average, 15 episodes of ARI (both upper and lower) over a period of 1 year and the average duration of each episode, by the time the child was seen by the physician, was 7 days.

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

(Manjula Datta, B. N. Appegowda and P. G. Gopi.)

Persistent respiratory infections

During the surveillance for ARI, it was found that a proportion of children continued to have symptoms referrable to ARI at each round. It was decided to label this phenomenon, "Persistent respiratory infection" and to study the extent of the problem and the factors associated with the risk of a child developing this condition. A child was considered to be suffering from persistent respiratory infection if a single episode of respiratory infection "persisted" for 30 days or longer. It was found that out of 296 children who had been repeatedly seen over 12 surveillance rounds, 173 (58%) had episodes of respiratory infection lasting for 30 days or more. Factors attributable to the child, like nutrition, anaemia, previous history of measles and whooping cough, worm infestation, immunisation status and vitamin-A deficiency, and factors attributable to the household, like family size, socio-economic status and type of household were assessed as risk factors in a cross sectional study. The prevalence of each of these risk factors in the population is given in the table below:

Risk factor	Total children	No. in whom factor present	%
Vit. A deficiency	296	61	*20.6*
History of measles	296	47	*15.9*
History of whooping cough	296	40	*13.5*
Mantoux positive	283	10	*3.5*
BCG not given	290	263	*90.7*
Triple antigen not given	295	166	*56.3*
Polio vaccination not given	295	175	*59.3*
Weight inadequate for age	296	55	*18.6*
Worm infestation	289	178	*61.6*
Haemoglobin low(6-10 gms%)	289	121	*41.9*
Mother illiterate	294	187	*63.6*
Cooking inside only	296	171	*57.8*
Low socio-economic status	296	195	*65.9*

However, in a preliminary analysis, no significant risk ratios were obtained for any of these conditions, except previous history of whooping cough, as can be seen from the table below.

Factors*	OR	CI	RR	AR	EF
1. Vit. A deficiency	1.03	0.58-1.83	1.01	0.01	0.00
2. History of measles	1.46	0.76-2.81	1.16	0.14	0.02
3. History of whooping cough	3.26	1.45-7.36	1.45	0.31	0.06
4. Mantoux>12mm	0.71	0.20-2.50	0.85	0.17	0.01
5. BCG not given	0.96	0.43-2.14	0.98	0.02	0.02
6. Triple antigen not given	1.01	0.63-1.61	1.01	0.01	0.00
7. Polio drops not given	1.00	0.62-1.60	1.00	0.00	0.00
8. Weight inadequate for age	0.99	0.54-1.79	0.99	0.01	0.00
9. Worm infestation	0.62	0.38-1.01	0.82	0.22	0.12
10. Haemoglobin low(6-10gms%)	1.40	0.87-2.25	1.15	0.13	0.06
11. Illiterate mother	0.88	0.43-1.81	0.95	0.05	0.03
12. Cooking inside only	1.83	0.92-3.64	1.28	0.22	0.14
13. Low socioeconomic status	1.06	0.51-2.19	1.02	0.02	0.02
14. Low per capita space	0.95	0.49-1.88	0.98	0.02	0.01

* OR = Odds ratio; CI = Confidence interval; RR = Relative risk; AR = Attributable risk; EF = Etiologic fraction.

It may be noted that the prevalence of both risk factors and cases was high in this population. The data are being analysed further.

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

(Manjula Datta, B. N. Appegowda and P. G. Gopi).

Study of causative organisms in ARI

A descriptive study was undertaken in the Institute of Child Health to identify the organisms responsible for causing pneumonia in children between 0-5 years. Children admitted to the wards of the Institute of Child Health, with a radiological abnormality, were identified as study subjects. Laryngeal swabs, throat swabs and

nasal swabs were collected for both bacteriology and virology. Blood was collected under aseptic conditions for both culture and serological studies. Information on clinical status, diagnosis, X-ray findings and other socio-economic factors were also collected from these children. Lysozyme and C-reactive protein levels were also done in a sample of these children. In all, 197 children have been admitted to the study. The study is planned for one year and is continuing.

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

(Manjula Datta, K. Sivadasan*, C. N. Paramasivan, P. G. Gopi and R. S. Vallishayee).

AIDS surveillance cell

The AIDS surveillance cell was constituted in July 1986 with the objective of studying the mode of spread of AIDS, particularly in high risk groups. Since all the infected were prostitutes, the study was confined to prostitutes in the Vigilance Home who were awaiting final judgement and court orders, under the Immoral Traffic Act. A social worker interviewed these women and obtained information on clinical history, socio-economic factors, sexual habits, number and type of partners and other personal habits. These interviews were conducted in strict privacy and confidence and included both infected and uninfected women. Except in a few early instances, the interviews were completed before the infection status become known.

In all, information was obtained on 214 out of 257 women who were remanded to the Vigilance Home for some reason or other; 33 of these women were found to be infected with AIDS as detected by the ELISA method.

The study is being continued.

(started: 1987)

(Sundari Viswanathan and Manjula Datta.)

ELECTRONIC DATA PROCESSING

Main frame computer

During the year, a main frame computer was installed in the Centre. The main configuration includes a two megabyte CPU memory, 121 megabyte hard disk with Winchester drive, two 9-track tape drives (1600 bpi) and dual floppy drives, each capable of handling 8" floppy diskettes (1 megabyte capacity).

The software includes VAX/VMS operating system, an editor, a sort/merge utility, a macro assembler, document formatting and a few other utilities. The system supports C, BASIC, FORTRAN and PASCAL programming languages. The data management package available is Datatrieve. At present, there are

*Research Fellow.

four alphanumeric video terminals one of which is expected to be installed shortly in the statistics department. Further, a card reader with a speed of 600 cards per minute has also been installed. (A printer is to be installed soon.)

Terminals will be utilised for feeding programmes and processing the data. The punch cards for different studies will be copied on to tapes using the card reader. It is expected that the data generated from randomised controlled clinical trials for tuberculosis and extra-pulmonary tuberculosis conducted at Madras and other Centres, the data generated from field studies conducted in the 18 districts, from community awareness studies at North Arcot and Madras will all be stored and processed using the main frame computer.

Microcomputer

The statistics department acquired a 16-bit IBM-PC compatible microcomputer in June 1986. The important features consist of 512 kilobytes of CPU memory, a 21 megabyte hard disk and two 5¼" floppy disk drives and a bidirectional dot matrix printer with a speed of 180 characters per second. The PC has a wide range of software support.

The computer is used for storing in floppy diskettes several hundreds of weekly smear and culture results for different studies which are recorded along with patient details in culture registers. Routine lists are prepared by processing the above data and some of the lists are sent for use in North Arcot and Madurai and also to London. The computer is also used extensively for detailed statistical analyses of many laboratory experiments, and exploratory graphics.

(A. M. Diwakara, R. Subramani, R. S. Vallishayee, G. S. Acharyulu and M. S. Krishnamurthy).

APPENDICES

TRAINING PROGRAMMES

WHO fellows

Mr. Viliami, Ika, Tongo, from 7-4-86 to 18-4-86.

Dr. Miss. Aziza Abdulla, Afghanistan, for 2 weeks from 26-5-86.

Mrs. Mahani Mahwood, Malaysia, from 25-8-86 to 29-8-86.

Trainees

The following underwent training in different departments as follows:

Bacteriology

Sri A. Nithanam, from Ramalingam Tuberculosis Sanatorium, Perundurai, for 1 month from 7-4-86.

Dr. Rishendra Verma, from Indian Veterinary Research Institute, Izatnagar (UP), from 17-9-86 to 4-10-86.

Miss. Ch. de Severy, from Sacred Heart Leprosy Centre, Kumbakonam, from 15-1-87 to mid February, 1987.

A Laboratory Technician from Bhilai Steel Plant, Bhilai, from 15-3-87 to 15-4-87.

A Senior Laboratory Technician from Government Chest Clinic, Pondicherry, from 16-3-87 to 28-3-87.

Epidemiology

Miss Allysan Armstrong Brown, Medical Student, University of Newcastle, Australia, from 28-10-86 to 30-12-86.

General

Mr. Greg Berg, medical student, University of Manitoba, Canada, from 8-4-86 to 14-4-86.

Five senior staff members from Christian Medical College Hospital, Vellore, to observe and get the structural details of our TB inoculation hoods in September, 1986.

Ms. Fateema Jetha, medical student, Canada, from 5-1-87 to 15-2-87.

Dr. Mani and Mrs. Aleyamma Jacob from Neyveli Lignite Corporation, Neyveli, for training in Cardio Pulmonary Medicine (including fibreoptic bronchoscope and broncho-alveolar lavage) from 5-1-87 to 10-1-87.

Mr. Fraser Norrie and Miss Karin Goddard, final year medical students, University of Manitoba, Canada, from 5-2-87 to 13-2-87.

Mr. C. Arun Peter, final year medical student from Andhra Medical College, Vizag, from 3-3-87 to 19-3-87.

A batch of 10 DPH students from the Madras Medical College, Madras, from 9-12-86 to 12-12-86.

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

Christian Medical College, Vellore—1 batch
Thanjavur Medical College, Thanjavur—1 batch

Post-graduate students

MD (TB) students from the S.V. Medical College, Tirupati, Andhra Pradesh.

MD (TB) students from the Andhra University, Andhra Pradesh.

MD (TB) students from Kurnool Medical College, Kurnool, Andhra Pradesh.

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

MD/DTCD students from Medical College, Beach Hospital, Calicut.

MD (TB) students from Rangaraya Medical College, Kakinada, Andhra Pradesh.

A MD (TB) student from Osmania Medical College, Hyderabad, Andhra Pradesh.

Nursing and para-medical students

Medical Social Worker students from the Department of Social Work, Loyola College of Social Sciences, Trivandrum, Kerala.

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

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

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

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

Students undergoing the Sanitary Inspectors' Course at Gandhigram Rural Institute, Ambathurai.

STAFF DEVELOPMENT PROGRAMME

1. Dr. Rani Balasubramanian was awarded a Commonwealth Medical Fellowship to undergo training in Upper Gastrointestinal endoscopy, sigmoidoscopy and colonoscopy at the Academic Unit of Gastroenterology, London Hospital, London, for one year from April, 1986.

2. Mrs. Sudha Ganapathy was awarded a 4-month W.H.O. Fellowship to undergo training in sociological aspects in the management of tuberculosis at the National Tuberculosis Centre, Jalan Pahang, Kuala Lumpur, Malaysia, Department of Tuberculosis Control, Tan Took Seng Hospital, Singapore, and Department of Chest Diseases, Siriraj Hospital, Bangkok, during April-August, 1986.

3. Dr. K. Rajaram underwent the one year post-graduate Diploma Course in Tuberculosis and Chest Diseases (DTCD) at the Institute of Tuberculosis and Chest Diseases, Madras, from June, 1986.

4. Dr. Paulin Joseph underwent the one year post-graduate Diploma Course in Dermatology (DD) at the Govt. General Hospital, Madras, from June, 1986.

5. Dr. Reetha Vijayan underwent the one year post-graduate Diploma Course in Child Health (DCH) at the Govt. Stanley Medical College & Hospital, Madras, from June, 1986.

6. Mrs. Alamelu Raja, was awarded a 6-month W.H.O. Fellowship to undergo training in "Monoclonal anti-bodies to enable isolation of pure antigens from *M. tuberculosis*" at the Department of Medicine, University Hospitals, Cleveland, Ohio, U.S.A., during April-September, 1986.

7. Mrs. Sujatha Narayanan participated in an international workshop on Molecular Biology Techniques applicable to the study of Tropical Diseases Pathogens, at the National University of Singapore, during November, 17-29, 1986.

8. Six staff members (Mr. P.A. Bhaskar, Mr. Arjunan, Mr. Parandhaman, Mr. Jacob, Mr. Janakiraman and Mr. Kalyanaraghavan) were trained in Nutritional Anthropometry at the Institute for Research in Medical Statistics, Madras, in February, 1987.

9. Mrs. Sujatha Narayanan was awarded the Ph. D. degree in Immunology by the University of Madras in February, 1987.

10. Mrs. Alamelu Raja was awarded the Ph. D. degree in Immunology by the University of Madras in March, 1987.

PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

Name of conference, venue and date	Title of paper	Name of staff member
IV International Clinical Epidemiology net work meeting, Shanghaii, China, 2-14 April, 1986	Development of algorithm for primary care management of ARI	Dr. Manjula Datta
-do-	A study on interphysician's agreement in clinical filariasis	Dr. R.V.S.N. Sarma
Indo-UK symposium on Leprosy, Central Jalma Institute, Agra, 7-11 April, 1986	Cultivation of *M.leprae*— An overview	Dr. R. Prabhakar
-do-	Sensitisation pattern of tuberculosis patients and volunteers to various mycobacterial antigens by ELISA and LTT	Dr. C.N. Paramasivan
First National Update on Flexible Fibreoptic Bronchoscopy, Bombay, 27 April, 1986	Alveolitis of Tropical Pulmonary Eosinophilia	Dr. V.K. Vijayan
Annual Conference of National Council for International Health, Washington D.C. U.S.A 11-13 June, 1986	Circulating antigens in immunodiagnosis of filariasis	Dr. R. S. Paranjape
National Conference of the Indian Association of Medical Microbiologists, Bangalore, 20-22 October, 1986	Non-fermenters in acute respiratory infection in children	Dr. C.N Paramasivan
-do-	Bacteriological investigation of acute respiratory infections in children	Mr. K. Sivadasan*

*Recognised Fellow

41st National Conference on Tuberculosis and Chest Diseases, Hyderabad, 25-28 October, 1986	Short Course Chemotherapy under DTP conditions	Dr. T. Santha Devi
-do-	Monitoring Short Course Chemotherapy under DTP conditions—a review	Mr. M.S. Krishnamurthy
-do-	Rifampicin-induced release of hydrazine from isoniazid: a possible cause of hepatitis during treatment of tuberculosis with regimens containing isoniazid and rifampicin	Dr. G.R. Sarma
-do-	Laboratory diagnosis of tuberculosis—an overview (Wander-TAI Oration)	Dr. R. Prabhakar
XXVI IUAT World Conference on Tuberculosis and Respiratory Diseases, Singapore 4-7 November, 1986	Fully intermittent 6-month regimens for pulmonary tuberculosis in South India	Dr. R. Prabhakar
Fourth Annual Conference of the Indian Society for Medical Statistics, Bangalore, 24-26 November, 1986	Collection of accurate and complete data relating to controlled clinical trials in tuberculosis	Mr. P. R. Somasundaram
-do-	Use of Kappa statistic in measuring observer agreement among multiple readers for categorical data	Mr. R. S. Vallishayee
XIII Annual Conference of the Indian Immunology Society, New Delhi 6-8 December, 1986	Modulation of eosinophil function	Dr. V. Kumaraswami
"	Immunological investigations in tuberculous sclera	Dr. Rajiswamy

XIII Annual Conference of the Indian Immunology Society, New Delhi, 6 - 8 December, 1986	Monoclonal antibody based antigen detection assay for diagnosis of bancroftian filariasis	Dr. R.S. Paranjape
-do-	Defective Con-A induced suppression in bancroftian filariasis	Miss. C. R. Vanamala
-do-	Characterisation of filarial antibody response by immunoblot technique	-do-
36th Annual Congress of Neurological Society of India, New Delhi, 12-15 December, 1986	Short Course Chemotherapy in the treatment of Pott's paraplegia	Dr. Rajeswari Ramachandran
Sixth National Congress on Respiratory Diseases, Bombay, 13 December, 1986	Causes of hypoxemia in Tropical Pulmonary Eosinophilia	Dr. V. K. Vijayan
XXXII Annual Conference of Association of Physiologists and Pharmacologists of India, Hyderabad, 27-29 December, 1986	Single breath carbon monoxide transfer factor in pulmonary tuberculosis patients treated with short-course chemotherapy	Mr. K. V. Kuppu Rao
42nd Joint Annual Conference of the Association of Physicians of India, Madurai, 25-26 January, 1987	Diffuse interstitial lung diseases	Dr. V. K. Vijayan
-do-	Experience with 200 broncho-alveolar lavages	-do-
Fifth International Clinical Epidemiology net work meeting, Mexico, 26-31 January, 1987	Factors associated with persistent and recurrent respiratory infections	Dr. Manjula Datta

87th Annual Meeting of the American Society for Microbiology, Atlanta, Ga. U.S.A., 1-6 March, 1987	Characterisation of epitopes of *Mycobacterium tuberculosis*. *Antigen*-5 using monoclonal and polyclonal antibodies (Slide presentation)	Mrs. Alamelu Raja
-do-	Specific detection of *Mycobacterium tuberculosis* in radiometric cultures by immunoassay (Poster presentation)	-do-

LIST OF PUBLICATIONS

Papers published

1. Raghupati Sarma, G., Chandra Immanuel, Kailasam, S., Narayana, A.S.L. and Venkatesan, P. Rifampin-induced release of hydrazine from isoniazid: A possible cause of hepatitis during treatment of tuberculosis with regimens containing isoniazid and rifampin. *American Review of Respiratory Disease*, 1986, *133*, 1072-1075.

2. Tuberculosis Research Centre, Madras and National Tuberculosis Institute, Bangalore. A controlled clinical trial of 3- and 5- month regimens in the treatment of sputum-positive pulmonary tuberculosis in South India. *American Review of Respiratory Disease*, 1986, *134*, 27-33.

3. Parthasarathy, R., Raghupati Sarma, G., Janardhanam, B., Padma Ramachandran, Santha, T., Sivasubramainan, S., Somasundaram, P.R. and Tripathy, S.P. Hepatic toxicity in South Indian patients during treatment of tuberculosis with short-course regimens containing isoniazid, rifampicin and pyrazinamide. *Tubercle*, 1986, *67*, 99-108.

4. Rani Balasubramanian, Rajeswari Ramachandran and Prabhakar, R. A case of late generalised tuberculosis with normal chest radiograph. *Indian Journal of Tuberculosis*, 1986, *33*, 136-137.

5 Paramasivan, C.N., Daniel Herbert, Narayana, A.S.L., Prabhakar, R. and Somasundaram, P.R. Evaluation of homogenising substances in estimating the viability of tubercle bacilli. *Indian Journal of Medical Microbiology*, 1986, *4*, 177-180.

6. Radha Madhavan, Sanjeevi, C.B., Narayanan, P.R. and Chandrasekaran, A.N. Comparison of ELISA and tube agglutination for the detection of anti-Yersinia enterocolitica antibodies among arthritis patients. *Indian Journal of Pathology and Microbiology*, 1986, *29*, 9-14.

7 Thiruvengadam, K.V., Kumaraswami, V. and Krishnan, S. Immunology of occupational lung diseases caused by dust: An overview. *Indian Journal of Tuberculosis*, 1986, *33*, 171-174.

8. Spry, C.J.F., Kumaraswami, V. and Tai, P.C. The effect of nedocromil sodium on secretion from human eosinophils. *European Journal of Respiratory Diseases*, 1986, *69* (suppl. 147), 241-243.

9. Kumar, S., Moudgil, K.D., Band, A.H., Narayanan, P.R., Gupta, S.K., Sharma, A.K. and Talwar, G.P. A dot enzyme immunoassay for detection of IgM antibodies against phenolic glycolipid-I in sera from leprosy patients. *Indian Journal of Leprosy*, 1986, *58*, 185-190.

10. Vijayan, V.K. Changes in pulmonary functions in victims of Bhopal tragedy. *Indian Journal of Tuberculosis*, 1986, *33*, 87.

11. Kuppu Rao, K.V., Vijayan, V.K., Venkatesan, P. and Prabhakar, R. Single breath carbon monoxide transfer factor in pulmonary tuberculosis patients treated with short course chemotherapy. *Indian Journal of Physiology and Pharmacology*, 1986, *30*, (suppl. I), 26.

12. Monga, J.L. Role of para-medical personnel in National Tuberculosis Programme. *Indian Journal of Tuberculosis*, 1986, *33*, 91.

13. Sujatha Narayanan, Paramasivan, C.N., Prabhakar, R. and Narayanan, P.R. Effect of oral exposure of *Mycobacterium avium intracellulare* on the protective immunity induced by BCG. *Journal of Biosciences*, 1986, *10*, 453-460.

14. Acharyulu, G.S., Narayanan, P.R., Krishnamurthy, P.V. and Prabhakar, R ELISA: Its reproductibility and its use in tuberculosis case finding. *Indian Journal of Tuberculosis*, 1987, *34*, 17-21.

15. Rani Balasubramanian, Alexander, C., Prabhakar, R., Paramasivan, C.N., Rajaram, K., Somasundaram, P.R. and Thyagarajan, K. Kanamycin plus rifampicin plus ethambutol in the retreatment of patients with tubercle bacilli resistant to isoniazid and streptomycin. *Indian Journal of Tuberculosis*, 1987, *34*, 12-16.

16. Rathnasabapathy, S.V., Paramasivan, C.N., Shanmugasundaram, N. and Jagannath, K. Secondary bacterial flora in patients with pulmonary tuberculosis. *Lung India*, 1987, 5, 43-46.

17. Rajaram, K., Ramu, S., Prabhakar, R., Deivanayagam, R., Muktar Ahmed and Swaminathan, T. Saccharin test of mucociliary clearance in Kartagener's syndrome. *Lung India*, 1987, *5*, 33-35.

18. Prabhakar, R., Venkataraman, P., Vallishayee, R.S., Reeser, P., Musa, S., Hashim, R., Kim, Y., Dimmer, C., Wiegeshaus, E., Edwards, M.L. and Smith, D.W. Virulence for guinea-pigs of tubercle bacilli isolated from the sputum of participants in the BCG trial, Chingleput District, South India. *Tubercle*.

Papers accepted for publication

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

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

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

4. Sarma, R.V.S.N., Vallishayee, R.S., Mayurnath, S., Narayanan, P.R., Radhamani, M.P. and Tripathy, S.P. A prevalence survey of filariasis in two villages in Chingleput District of Tamil Nadu. *Indian Journal of Medical Research.*

5. Wallace Fox, Prabhakar. R., Sudha Ganapathy and Somasundaram, P.R. An enquiry into the coloration of the urine by rifampicin and South Indian patients' attitudes to it. *Tubercle.*

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JOURNAL CLUB

Meetings of the Journal Club were held each week. At these meetings, scientific articles of interest, comprising a wide range of subjects, were reviewed by different staff members by turn. This was followed by a discussion about the matter presented, and its relevance to the Centre's activities where indicated. In all, 42 staff members reviewed a total of 50 articles during the year.

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

GUEST LECTURE

Dr. S. Radhakrishna, Director, Institute for Research in Medical Statistics (Madras Chapter) gave a talk entitled "A review of present tuberculosis control activities in Sri Lanka and a proposed survey design to determine the current prevalence of tuberculosis and tuberculous infection".

LECTURES BY VISITING SCIENTISTS

Subject	Speaker
Metabolism in *M.leprae*—problems and possibilities	Dr. R.P. Wheeler, University of Hull, U.K.
Cloning of mycobacterial DNA	Dr. Ian Lamb, National Institute of Medical Research, London, U.K.
Acute renal failure due to rifampicin therapy	Dr. Muthusethupathy, Nephrology Unit, Royapettah Hospital, Madras
New anti-tuberculosis drugs	Prof. D.A. Mitchison, Royal Post-graduate Medical School. London, U.K.

DISTINGUISHED VISITORS

1. Prof. Philippe H. Lagrange, Pasteur Institute, Paris, France.

2. Dr. Sonia Hurd, Director, Division of Lung Diseases, National Heart, Lung and Blood Institute, National Institute of Health, Bethesda, Maryland and Dr. Sonia Buist, Professor of Medicine, Oregon Health Science Unit, Oregon, USA.

3. Dr. E.A. Ottesen, Chief, Section of Clinical Parasitology, National Institutes of Health, Bethesda, Maryland, USA.

4. Dr. Bosko Popovic, WHO Representative to India, Ministry of Health and Family Welfare, Government of India.

5. Dr. Donald Smith, Professor, Department of Medical Microbiology, Medical Sciences Centre, University of Wisconsin Medical School, USA.

6. Dr. Philip Schambra, US Embassy, Mr. David E, Hohman, Director of the Secretary's Office of International Affairs, Mr. Horace M. Lukens, Director of the Secretary's Scheduling Office, Mrs. Linda Vogel, Associate Director of the Office of International Health, Mr. Manmohan Saxena, US Embassy, New Delhi.

7. Dr. P.R. Wheeler, Department of Biochemistry, University of Hull, Hull, U.K.

8. Dr. Pranab Kumar Das, Dermatoen Histo-Immunologist, Department of Pathology, University of Amsterdam, The Netherlands.

9. Dr. A.R.M. Coates, Sr. Lecturer, London Hospital Medical College, London.

10. Dr. Roger W. Doyon, NSF Coordinator for Science and Technology initiative, Mr. Shinaishin and Mr. K. R. Subramanian from US. Embassy, New Delhi.

11. Dr. T.B. Nutman, Dr. Freedman and Ms. Renu Lal, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, Maryland, U.S.A.

12. Dr. R.H. Morrow, Prof. V.I. Mathan, Dr. C.P. Ramachandran, Dr.J. Putrali and Dr. Ladouceur, Tropical Diseases Research/Research Strengthening Group, W.H.O. (SEARO).

13. Dr. Roger Beasley, Health & Nutrition, USAID, American Embassy, New Delhi.

14. Dr. Kenneth S. Warren, Rockefeller Foundation, New York.

CONSULTANTS

During the period under review, the following scientists visited the Centre as Consultants:

1. Prof. D.A. Mitchison, Royal Postgraduate Medical School, London.

2. Dr. T.W. Meade, Director, MRC Epidemiology and Medical Care Unit, Northwick Park Hospital, Middlesex, U.K.

PRIZES AND AWARDS RECEIVED BY STAFF MEMBERS

1. Dr. Padma Ramachandran was awarded the "R.C. Garg Memorial Award" for the best article published in 1986 in the Indian Journal of Tuberculosis for the paper entitled "Three Chemotherapy Studies of Tuberculous Meningitis in Children".

2. Dr. T. Santha Devi was awarded the "Dr. R. Krishna Memorial Award" for the best paper presented at the 41st National Conference on Tuberculosis and Chest Diseases held at Hyderabad in October, 1986 for the paper entitled "Short Course Chemotherapy under District Tuberculosis Programme".

3. Dr. R. Prabhakar was nominated to deliver the Wander-TAI Oration on "Laboratory Diagnosis of Tuberculosis—an Overview" at the 41st National Conference on Tuberculosis and Chest Diseases held at Hyderabad in October, 1986.

STAFF MEMBERS ON ADVISORY COMMITTEES OF OTHER INSTITUTIONS

Staff member	Name of committee
Dr. R. Prabhakar	Scientific Advisory Committee of the Regional Medical Research Centre,ICMR, Port Blair, Andamans
Dr. R. Prabhakar	Planning and Research—Medical Research Committee of the University of Health Sciences, Vijayawada
Dr. G. Raghupati Sarma	Scientific Advisory Committee, Institute of Genetics, Osmania University, Hyderabad
Dr. V.K. Vijayan	Project Advisory Committee for Epidemiological and Clinic Studies on Methyl Icsoyanate (MIC)—exposed people of Bhopal, Indian Council of Medical Research

ADDENDUM TO PAGE 6:

SCIENTIFIC ADVISORY COMMITTEE

	Member
Prof.S.K.Jain	Head, Dept.of Cardio-respiratory Physiology, Vallabhai Patel Chest Institute, New Delhi.

ACKNOWLEDGEMENT

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