# TUBERCULOSIS RESEARCH CENTRE

CHETPUT MADRAS - 600 031

# REPORT ON RESEARCH ACTIVITIES DURING 1988

INDIAN COUNCIL OF MEDICAL RESEARCH

NEW DELHI

# TUBERCULOSIS RESEARCH CENTRE

**CHETPUT MADRAS - 600 031**

# REPORT ON RESEARCH ACTIVITIES DURING 1988

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

# CONTENTS

**CLINICAL STUDIES**—(*Contd.*)



**LABORATORY STUDIES**

**Studies Completed**



**Studies in Progress**

**LABORATORY STUDIES**—(*Contd.*)

**APPENDICES** (*Contd.*)

# PREFACE

During the year under review, the activities of the Centre were mainly focused on operational research concerning the District Tuberculosis Programme (DTP) with the accent on improving its efficiency. Accordingly, the feed-back obtained from monitoring the programme in 18 districts, where Short Course Chemotherapy (SCC) has been implemented, was utilised to identify the lacunae in the various components of the DTP. Training and reorientation programmes were organised for the medical and paramedical staff in some of these districts, in order to refresh their knowledge of DTP procedures. Detailed plans of action were drawn up to improve certain important components of the DTP, such as case finding, case holding and documentation. A study was initiated to obtain accurate addresses from patients in semi-urban and rural areas, by utilising an "address card", to improve the efficiency of retrieval of defaulters by posting reminder letters.

A survey to assess the awareness of tuberculosis among chest symptomatics in the community, in rural, semi-urban and urban areas, and the action taken by chest symptomatics to get relief, was completed. Epidemiological studies of the prevalence of tuberculosis in the community and the extent of drug resistance in patients with tuberculosis have been initiated in one district. The surveys would be extended to other districts also, to provide base-line data of the disease and to help assess the impact of SCC on the epidemiological profile of the disease. Two workshops were conducted under the joint auspices of the Council and the World Health Organisation, In which the District and State Tuberculosis Officers as well as experts in the field of tuberculosis participated in panel discussions on various aspects of the programme.

Recombinant DNA technology has been established, which is another milestone in the advancement of the laboratory. This is being utilised for the development of specific immuno-diagnostics in tuberculosis, which would be of special value for pauci-bacillary pulmonary and extra-pulmonary forms of the disease. Simpler methods for determining drug levels in body fluids by utilising saliva instead of serum have been standardised; this will be of immense help for conducting pharmacological studies, especially in children. Dose kinetic studies of anti-tuberculosis drugs in patients with renal insufficiency of different grades are being undertaken, with a view to establish the optimum dosage of the drugs in such patients who have tuberculosis. In-depth studies to characterise various antigenic fractions of *M. tuberculosis*, using immunoblot techniques for possible isolation of highly specific immuno-diagnostic agents, are being carried out. Studies have been undertaken for the histopathological grading of tuberculous lymphadenitis, using immunochemical and histochemical techniques.

The Scientific Advisory Committee met on 21-5-1988 under the Chairmanship of Prof. K. V. Thiruvengadam, and gave valuable guidelines for the conduct of future research at the Centre.

Dr. K. Jagannath, Director, Institute of Thoracic Medicine, Madras, Prof. K. V. Krishnaswami, Former Director, Institute of Tuberculosis and Chest Diseases, Madras, Prof. K. V. Thiruvengadam, former Professor of Medicine, Madras Medical College, Madras, Dr. S. Radhakrishna, Director, Institute for Research in Medical Statistics (Madras Chapter) and Dr. T. S. Nataraian, Tribal Research Centre, Tamil University, Uthagamandalam, continued to act as consultants. Dr. I. Kandaswamy, Govt. General Hospital, Madras continued as consultant in Radiology and Prof. N. S. Venugopal and Dr. S. Thyagarajan continued as consultants in Ophthalmology.

# STAFF MEMBERS AS ON 31-12-1988

### Director

R. Prabhakar, M.D.

### Division of Chemotherapy

Padma Ramachandran, B.Sc., M.D., D.C.H.
T. Santha Devi, M.B.B.S., D.T.C.D.
V.K. Vijayan, M.D., D.T.C.D.
A. Thomas, M.D., Dip. in Leprosy
N.M. Sudarsanam, B.Sc., M.B.B.S., D.T.C.D.
Rani Balasubramanian, M.D., D.G.O.
V. Kumaraswami, M.D., M.N.A.M.S.
Rajeswari Ramachandran, M.D., D.M. (Neuro.)
M.S. Jawahar, M.D.
K. Rajaram, B.Sc., M.B.B.S., D.T.R.D.
Rema Mathew, M.B.B.S., D.C.H.
A.M. Reetha, M.B.B.S., D.C.H.
Paulin Joseph, M.B.B.S., D.D.
K. Palanimurugan, M.B.B.S.
R. Balambal, M.D.
K. C. Umapathy, M.B.B.S.
Usha Ramanathan, M.B.B.S., D.P.M.
M. Parvathy Raghavan, R.N., R.M., C.P.H.
Leelavathy Aaron, B.Sc. (Hons.) (Nursing)
Ambujam Ganesh, R.N., R.M., C.P.H.
Rajamanohari Dason, R.N., R.M., C.P.H.
B. Venkatasubbamma Chalapathi Rao, B.Sc. (Nursing)
Jayalakshmi Vadivelu, B.Sc. (Nursing)
G. Arumaikkannu, B.Sc. (Hons.)(Nursing)
Sudha Ganapathy, M.A.
K. N. Gopilingam, C.R.A.
K.V. Kuppu Rao, M.Sc.
S. Achuthan, R.H.V., R.M.
R. Kamala, R.N., R.M.
Susheela Kalyanasundaram, R.N.

### Division of Bacteriology

C.N. Paramasivan, Ph.D.
N. Selvakumar, Ph.D.
Vanaja Kumar, Ph.D.
P. Venkataraman, B.Sc., A.I.C.
C. Alexander, B.Sc., C.L.T.
B.N. Gopalan, B.Sc., D.M.T.
Sara Mathew, B.Sc.
Lalitha Hari, M.Sc.
M. Naseema, Ph.D.
Alamelu Narayanan, B.Sc., D.M.T.
A. Palos, C.L.T.
Santhamma Chacko, C.L.T.
R. Adimulam, B.A., C.L.T.

### Division of Biochemistry

G. Raghupati Sarma, Ph.D.
Prema Gurumurthy, Ph.D.
S. Kailasam, B.Sc., A.I.C.
M. Kannapiran, M.Sc.
Chandra Immanuel, M.Sc.
K. Jayasankar, M.Sc.
Lalitha Victor, M.Sc.

### Division of Immunology

P.R. Narayanan, Ph.D., D.I.I.Sc.
Rajiswamy, M.D.
Ramesh Shivaram Paranjape, Ph.D.
A. Ravoof, B.Sc.
Alamelu Raja, Ph.D.
Sujatha Narayanan, Ph.D.
P. Selvaraj, Ph.D.
S. Ramanujam, B.Sc.

### Division of Pathology

V.D. Ramanathan, M.B.B.S., Ph.D.

## Division of Epidemiology

Manjula Datta, M.D., D.C.H., M.Sc.(D.M.E.)
R.V.S.N. Sarma, M.D., M.Sc. (D.M.E.)
C. Kolappan, M.B.B.S.
V. Balasubramaniam, M.B.B.S.
K. Sadacharam, M.B.B.S.
D.L. Sathyanarayana Rao, B.Sc.
B.N. Appe Gowda, B.Sc.
A.M. Diwakara, M.Sc.
M.P. Radhamani, M.Sc.
P.G. Gopi, M.Sc.
R. S. Nagabhushana Rao, B.Com.
R. Selvaraj, M.Sc.
R. Subramani, M.Sc.
N. Ravi, D.E.E.
K.R. Bhima Rao, B.Sc.
N. Shivaramu, B.Sc.
A. Venkatesh Reddy, B.Sc.
V. Venkatesh Prasad, B.Sc.
D.S. Raghavendra Rao

## Division of Statistics

P.R. Somasundaram, B.A., Stat. Dip. (I.S.I.)
R.S. Vallishayee, M.Sc.
M.S. Krishnamurthy, M.A. (Stat.), M.A. (Econ.)
G.S. Acharyulu, M.A. (Maths.), M.Stat.
P.V. Krishnamurthy, M.Sc. (Stat.), M.Sc. (D.M.E.)
B. Janardhanam, B.A.
A.S.L. Narayana, B.Sc.
S. Sivasubramanian, B.A.
D. Rajappa, B.Sc., Dip. Stat.
Fathima Rahman, B.Sc., Stat. Dip. (I.S.I.)
M. Nagarajan, B.Sc., B.G.L.
Victor Mohan, B.Sc.
N. Raghuraman, B.Sc.
K. Thyagarajan, B.Sc.

## Library

Kursheed Ara Begum, B.A. (Hons.), B.Lib.Sc.

## Administration

K.C. Valsarajan, M.A., D.S.S.
J.N. Tandon, B.A.
V. Lakshminarayanan, B.Com., D.Com., A.C.S.
Sakunthala Sundar
S. N. Sankaralingam, B.E.
V. Sankaranarayanan Nair
C.J. Arunachalam
D. Dakshinamurthy, M.A.
K. Venkatarama Devi
Ranajit Sankar Sen
Vijayalakshmi Gopalan
M.R. Srinivasan
P. Prabhakar Rao, B.Sc. (Epid. Unit)
M. Subramaniam, B.Com -do-
V. Adhikesavan, B.A. -do-
M. Vijayalakshmi -do-
P.K. Srinivasan, B.Sc. -do-

# SCIENTIFIC ADVISORY COMMITTEE

**Chairman**

| | |
|---|---|
| Prof. A.S. Paintal | Director-General, Indian Council of Medical Research, New Delhi |

**Members**

| | |
|---|---|
| Dr. G.V.J. Baily | Director, National Tuberculosis Institute, Bangalore |
| Prof. C.S. Bhaskaran | Director of Medical Education, Government of Andhra Pradesh, Hyderabad |
| Prof. J.S. Guleria | Professor and Head, Department of Medicine, All India Institute of Medical Sciences, New Delhi |
| Dr. K. Jagannath | Director, Institute of Tuberculosis and Chest Diseases, Madras |
| Prof. S.K. Jain | Head, Department of Cardio-respiratory Physiology, Vallabhai Patel Chest Institute, New Delhi |
| Dr. K.C. Mohanty | Professor, Department of TB & Respiratory Diseases, Grant Medical College and Sir J.J. Group of Hospitals, Bombay |
| Prof. M.G. Muthukumaraswami | Director of Medical Services, Government of Tamil Nadu, Madras |
| Dr. T.S. Natarajan | Tribal Research Centre, Tamil University, Uthagamandalam |
| Prof. G. Padmanabhan | Professor of Biochemistry, Indian Institute of Science, Bangalore |
| Dr. S. Radhakrishna | Director, Institute for Research in Medical Statistics (Madras Chapter), Madras |
| Dr. (Mrs.) Ramani Sivaraman | Director of Medical Education, Government of Tamil Nadu, Madras |
| Prof. A.M. Selvaraj | Honorary Professor of Clinical Medicine, Madras Medical College, Madras |
| Prof. R.N. Srivatsava | Principal, B.R.D. Medical College, Gorakhpur |
| Dr. A.K. Sur | Adviser-in-Tuberculosis, Government of India, New Delhi |
| Prof. K. V. Thiruvengadam | Professor of Medicine (Retd.), Madras Medical College, Madras |
| Dr. S.P. Tripathy | Additional Director-General, Indian Council of Medical Research, New Delhi |
| Dr. R. Prabhakar (member-secretary) | Director, Tuberculosis Research Centre, Madras |

# ETHICAL COMMITTEE

**Chairman**

Prof. M.V. Chari,
Consultant Physician,
V.H.S. Hospital, Madras

**Members**

Prof. K.N. George,
Director,
Madras School of Social Work,
Madras

Dr. (Mrs.) Lalitha Kameswaran,
Vice-Chancellor,
Dr. M.G.R. Medical University,
Madras

# OPERATIONAL RESEARCH STUDIES

## Short-course chemotherapy under District Tuberculosis Programme

As reported in earlier annual reports (1983 onwards), the Centre had been given the responsibility of monitoring the working of the Short Course Chemotherapy (SCC) programme under the District Tuberculosis Programme (DTP) that had been introduced in 18 districts in India on a pilot scale. In 1987, a mandate was given to the Centre to concentrate on improving the functioning and efficiency of the DTP, and undertake field studies to identify the several problems that confront the programme and suggest corrective measures. Accordingly, there was a major reorientation in the thrust of the Centre's activities.

Extensive analyses of the data have been undertaken and are presented here. The regimens that are being prescribed are:

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

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

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

**Policies:** Three policies of treatment, one each in 6 districts, 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.

**Eligibility:** Patients satisfying the following criteria are eligible to be prescribed SCC:

1. Newly diagnosed sputum smear positive.
2. Aged 15 years or more.
3. No previous specific anti-tuberculosis treatment for more than 2 months

The programme of SCC is integrated with the District Tuberculosis Programme; hence the implementation and running of the programme is the responsibility of the staff of the District Tuberculosis Centre and the Peripheral Health Institutions (PHI). This Centre has been given the responsibility of monitoring the programme and helping the district staff to identify and set right the lacunae observed and run the programme efficiently.

**Smear examination and intake to SCC:** The table on page 11 gives details regarding sputum smear examination and admission to SCC up to December, 1988.

The smear positivity rates were 3% in Puri, 11% in Aurangabad, 14% in Baroda, and between 5-9% in the remaining 15 districts. The percentage of eligible patients who were put on SCC regimens ranged between 44-75% in the Policy A districts, between 24-64% in the Policy B districts and between 36-78% in the Policy C districts. Considering all 18 districts, the admissions to SCC ranged between 20-39% in 5 districts, between 40-59% in 6 districts and between 60-78% in the remaining 7 districts.

Considering the proportion of patients who were prescribed the main regimens in Policies A and C ($2RHZ_2/4RH_2$ and $2RHZ/4RH_2$, respectively), there is wide variation between the districts (see last column of table on page 11), namely 20%–92% and 3%–98%, respectively.

| District | State | Population (00,000s) | No. of PHIs | When started (M/Y) | Total new smears examined | Positive | | Eligible for SCC No. | Put on SCC | | I reg* % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | No. | % | | No. | % | |
| **Policy A** | | | | | | | | | | | |
| N. Arcot | T. Nadu | 45.0 | 94 | 3/83 | 222752 | 12387 | *6* | 12145 | 7132 | *59* | — |
| Puri | Orissa | 29.2 | 80 | 10/83 | 81433 | 2757 | *3* | 2361 | 1780 | *75* | *64* |
| Baroda | Gujarat | 25.6 | 73 | 4/84 | 83563 | 12012 | *14* | 11435 | 4978 | *44* | *26* |
| Thane | Maharashtra | 33.5 | 92 | 12/84 | 108470 | 6051 | *6* | 5857 | 3918 | *67* | *4* |
| Ujjain | M.P. | 11.2 | 55 | 1/85 | 40405 | 3446 | *9* | 3050 | 1365 | *45* | *53* |
| Dehra Dun | U.P. | 7.6 | 26 | 1/85 | 41633 | 3362 | *8* | 2988 | 1965 | *66* | *5* |
| **Policy B** | | | | | | | | | | | |
| Karnal | Haryana | 13.2 | 21 | 3/84 | 45673 | 3337 | *7* | 3155 | 1124 | *36* | — |
| Kanpur | U.P. | 37.4 | 26 | 3/84 | 89061 | 7384 | *8* | 5706 | 1368 | *24* | — |
| Nagpur | Maharashtra | 25.9 | 110 | 3/84 | 129791 | 9268 | *7* | 8979 | 3188 | *36* | — |
| Rajkot | Gujarat | 20.9 | 60 | 1/85 | 37947 | 3590 | *9* | 2503 | 1469 | *59* | — |
| Raichur | Karnataka | 17.8 | 72 | 1/85 | 62720 | 3398 | *5* | 2872 | 1128 | *39* | — |
| Sagar | M.P. | 13.2 | 42 | 1/85 | 31733 | 2748 | *9* | 1475 | 948 | *64* | — |
| **Policy C** | | | | | | | | | | | |
| Pondicherry | U. Territory | 4.4 | 58 | 3/84 | 93633 | 4444 | *5* | 4123 | 1844 | *45* | *99* |
| Vidisha | M.P. | 7.8 | 26 | 10/84 | 28776 | 2339 | *8* | 1985 | 1557 | *78* | *21* |
| Aurangabad | Maharashtra | 24.3 | 46 | 12/84 | 37836 | 4121 | *11* | 3998 | 1676 | *42* | *14* |
| Varanasi | U.P. | 37.0 | 33 | 12/84 | 66011 | 3232 | *5* | 2491 | 1509 | *61* | *75* |
| Sabarkantha | Gujarat | 15.0 | 44 | 1/85 | 73991 | 5118 | *7* | 4834 | 2994 | *62* | *2* |
| W. Godavari | A.P. | 28.7 | 57 | 3/85 | 81398 | 4262 | *5* | 4129 | 1470 | *36* | *95* |

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

**Year by year comparison:** The table below gives a comparison of smear positivity rates and intake to SCC, year by year, from the inception of the programme, in each of the 18 districts.

| District | Percentage sputum positive | | | | | | Percentage put on SCC | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 |
| North Arcot | 8 | 6 | 6 | 5 | 5 | 4 | 53 | 52 | 66 | 61 | 68 | 54 |
| Puri | — | 4 | 3 | 4 | 3 | 3 | 43 | 87 | 88 | 64 | 79 | 66 |
| Baroda | — | 18 | 15 | 15 | 13 | 12 | — | 28 | 64 | 49 | 44 | 43 |
| Thane | — | 7* | 6 | 7 | 6 | 4 | — | 8* | 72 | 64 | 64 | 71 |
| Ujjain | — | — | 8 | 9 | 8 | 10 | — | — | 46 | 43 | 47 | 43 |
| Dehra Dun | — | — | 10 | 7 | 8 | 9 | — | — | 43 | 49 | 86 | 89 |
| Karnal | — | 8 | 8 | 8 | 6 | 6 | — | 24 | 36 | 38 | 31 | 52 |
| Kanpur | — | 22 | 10 | 9 | 6 | 5 | — | 57 | 26 | 12 | 18 | 36 |
| Nagpur | — | 6 | 9 | 8 | 7 | 6 | — | 41 | 16 | 32 | 33 | 63 |
| Rajkot | — | — | 11 | 10 | 10 | 8 | — | — | 75 | 59 | 51 | 52 |
| Raichur | — | — | 4 | 3 | 7 | 10 | — | — | 27 | 30 | 39 | 58 |
| Sagar | — | — | 13 | 9 | 7 | 7 | — | — | 44 | 37 | 79 | 98 |
| Pondicherry | — | 5 | 4 | 5 | 5 | 5 | — | 26 | 45 | 52 | 48 | 45 |
| Vidisha | — | 8 | 10 | 9 | 7 | 7 | — | 56 | 82 | 80 | 76 | 81 |
| Aurangabad | — | 5* | 16 | 10 | 7 | 13 | — | 45* | 39 | 46 | 48 | 36 |
| Varanasi | — | 11* | 5 | 5 | 4 | 6 | — | 13* | 58 | 64 | 62 | 67 |
| Sabarkantha | — | — | 10 | 7 | 7 | 5 | — | — | 60 | 60 | 59 | 64 |
| W. Godavari | — | — | 4 | 6 | 6 | 6 | — | — | 24 | 30 | 40 | 50 |

*December only

It is seen that as far as smear positivity rates are concerned, in most of the districts, there has been no significant variation over the years. However, there has been a steady decline in Baroda (18% to 12%), Kanpur (22% to 5%), Rajkot (11% to 8%), Sagar (13% to 7%) and Sabarkantha (10% to 5%). In Aurangabad, there has been wide fluctuation (5%, 16%, 10%, 7% and 13%). More detailed analyses, taking into account the number of specimens examined, the number of microscopy centres and the population of the district are in progress.

Considering intake to SCC of eligible patients, most of the districts have shown improvement over the years, the most striking being that of Dehra Dun (from 43% in 1985 to 89% in 1988), Karnal (from 24% in 1984 to 52% in 1988), Raichur (from 27% in 1985 to 58% in 1988), Sagar (from 44% in 1985 to 98% in 1988) and Vidisha (from 56% in 1984 to 81% in 1988). Kanpur shows fluctuations with a low intake of only 12% of eligibles in 1986. In Rajkot, the intake has dropped from 75% in 1985 to 52% in 1988.

**Cohort analysis of treatment completion rates:** The number and percentage of patients who completed or collected at least 80% of their scheduled chemotherapy and of those 'lost' from treatment, are presented in the table below by regimen.

| Regimen | District | No. of patients in cohort | Completed 80% or more of Rx | | Lost | |
|---|---|---|---|---|---|---|
| | | | No. | % | No. | % |
| $2RHZ_2/4RH_2$ | N. Arcot | 5280 | 2394 | *45* | 2511 | *48* |
| | Puri | 828 | 557 | *67* | 234 | *28* |
| | Baroda | 878 | 490 | *56* | 341 | *39* |
| | Thane | 75 | 45 | *60* | 27 | *36* |
| | Ujjain | 413 | 199 | *48* | 202 | *49* |
| | Dehra Dun | 90 | 64 | *71* | 17 | *19* |
| 2RHZ/6TH | Karnal | 740 | 334 | *45* | 330 | *45* |
| | Kanpur | 887 | 559 | *63* | 274 | *31* |
| | Nagpur | 1971 | 1411 | *72* | 478 | *24* |
| | Rajkot | 949 | 481 | *51* | 425 | *45* |
| | Raichur | 486 | 188 | *39* | 276 | *57* |
| | Sagar | 377 | 80 | *21* | 245 | *65* |
| | Puri | 249 | 123 | *49* | 99 | *40* |
| | Baroda | 2273 | 1216 | *53* | 1015 | *45* |
| | Thane | 1350 | 1001 | *74* | 250 | *19* |
| | Ujjain | 360 | 166 | *46* | 178 | *49* |
| | Dehra Dun | 1025 | 749 | *73* | 150 | *15* |
| | Vidisha | 713 | 255 | *36* | 422 | *59* |
| | Aurangabad | 611 | 245 | *40* | 303 | *50* |
| | Varanasi | 45 | 36 | *80* | 9 | *20* |
| | Sabarkantha | 1834 | 599 | *33* | 1095 | *60* |
| | W. Godavari | 37 | 35 | *95* | 2 | *5* |
| $2RHZ/4RH_2$ | Pondicherry | 1244 | 865 | *70* | 338 | *27* |
| | Vidisha | 167 | 71 | *43* | 78 | *47* |
| | Aurangabad | 116 | 92 | *79* | 21 | *18* |
| | Varanasi | 685 | 442 | *65* | 215 | *31* |
| | Sabarkantha | 36 | 31 | *86* | 5 | *14* |
| | W. Godavari | 679 | 315 | *46* | 249 | *37* |

It is seen that the completion rates ranged between 45–71% with $2RHZ_2/4RH_2$, 21–95% with 2RHZ/6TH and 43–86% with $2RHZ/4RH_2$ (mean rates: 50%, 55% and 62% respectively).

The table below gives the treatment completion rates for 3 different cohorts (admissions since inception of SCC to October 1985, November 1985 to June 1986, and July 1986 to June 1987, respectively). In the case of Baroda, Thane, Dehra Dun, Vidisha and West Godavari, separate data for Cohort I are not available and the data are amalgamated with Cohort II. Of the remaining 13 districts, 4 districts have shown an increase of 5% or more between Cohort I and Cohort II including 1 with an increase of 10-19%, while 4 districts have shown a decrease of 5% or more including

| District | Cohort I** | | | Cohort II | | | Cohort III | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of patients | Rx completed | | No. of patients | Rx completed | | No. of patients | Rx completed | |
| | | No. | % | | No. | % | | No. | % |
| **Policy A** | | | | | | | | | |
| N. Arcot | 3291 | 1481 | *45* | 774 | 361 | *47* | 1215 | 553 | *46* |
| Puri | 666 | 457 | *69* | 89 | 55 | *62* | 322 | 168 | *52* |
| Baroda | — | — | — | 2347 * | 1231 | *52* | 804 | 405 | *50* |
| Thane | — | — | — | 324 * | 218 | *67* | 1101 | 828 | *75* |
| Ujjain | 282 | 131 | *46* | 221 | 116 | *52* | 270 | 118 | *44* |
| Dehra Dun | — | — | — | 345 * | 239 | *69* | 770 | 574 | *75* |
| **Policy B** | | | | | | | | | |
| Karnal | 260 | 131 | *50* | 169 | 51 | *30* | 311 | 152 | *49* |
| Kanpur | 435 | 228 | *52* | 150 | 105 | *70* | 302 | 226 | *75* |
| Nagpur | 635 | 456 | *72* | 434 | 300 | *69* | 902 | 655 | *73* |
| Rajkot | 273 | 125 | *46* | 278 | 139 | *50* | 398 | 217 | *55* |
| Raichur | 73 | 27 | *37* | 131 | 44 | *34* | 282 | 117 | *41* |
| Sagar | 99 | 28 | *28* | 82 | 4 | *5* | 196 | 48 | *24* |
| **Policy C** | | | | | | | | | |
| Pondicherry | 470 | 381 | *81* | 379 | 218 | *58* | 417 | 281 | *67* |
| Vidisha | — | — | — | 539 * | 181 | *34* | 358 | 92 | *26* |
| Aurangabad | 282 | 127 | *45* | 245 | 114 | *47* | 200 | 96 | *48* |
| Varanasi | 145 | 86 | *59* | 258 | 170 | *66* | 327 | 222 | *68* |
| Sabarkantha | 683 | 217 | *32* | 350 | 136 | *39* | 837 | 276 | *33* |
| W. Godavari | — | — | — | 460 * | 209 | *45* | 256 | 141 | *53* |

**Cohort I—from inception of SCC programme up to October 1985;
Cohort II—November 1985 to June 1986; Cohort III—July 1986 to June 1987
*Combined data for Cohorts I and II.

3 with a decrease of more than 20%. Between Cohort II and Cohort III, 9 districts showed an increase by 5% or more including 3 with an increase of 10-19%, while 4 districts have shown a decrease by 5-10%. Also, among the 13 districts with data on all 3 cohorts, Kanpur and Rajkot have shown a steady increase in the completion rates.

**Defaulter retrieval:** For defaulting patients (i.e., those failing to attend on the due date), first action, either by posting a letter or making a home visit, is to be done on the *same* day or the next day. If the patient fails to turn up within 7 days, second action is to be taken. However, in many districts, such defaulter retrieval procedures are not being followed routinely or regularly.

**'Lost' cases:** For the purpose of the SCC programme, a 'lost' case is defined as a patient who defaults continuously for one month or more. Considering the proportion of 'lost' cases, the ranges were 19-49% for the $2RHZ_2/4RH_2$, 5-65% for the 2RHZ/6TH and 14-47% for the $2RHZ/4RH_2$ regimen (see table on page 13). It is to be noted that with the 2RHZ/6TH regimen, in 5 districts, 50% or more were 'lost' although this is an unsupervised regimen and patients have to come to the treatment centre only once in 15 days for collecting their drugs. This aspect needs to be studied in detail.

**Operational & Field studies:** In order to understand the problems involved in patients dropping out from treatment and also to understand the psychosocial aspects, other studies have been undertaken; these are reported elsewhere (see page 16)

(started: 1983)

## Training Programmes

As mentioned above (see page 9), the Centre's activities were re-oriented towards SCC under DTP. Hence more of the staff of the Centre had to be involved in the monitoring activities, for which giving adequate training to the staff of the Centre in the DTP procedures and acquainting them with related problems was found necessary. Senior staff members who had been involved in the DTP over a number of years undertook the training of fellow staff members by giving lectures and arranging panel discussions. This was followed by visits to the adjoining North Arcot district to get first-hand knowledge of the working of the Programme.

As part of the strengthening of DTP activities, training programmes were also arranged at the district level for the DTC and PHI staff by this Centre. These training programmes were arranged at 2 or 3 places in each district. At this time, several PHIs were also visited, to identify the thrust areas for the training. Such programmes were undertaken in Raichur and West Godavari districts during the year. After the training programme for the district personnel, follow-up visits were made to the 2 districts for visiting more PHIs, which were selected after careful scrutiny of

their performance recorded in the monthly/quarterly reports. It is proposed to have training-cum-visit programmes in more districts in a phased manner. Corrective actions were initiated by the Centre's visiting team to rectify any deficiencies noted, so that the efficiency of the programme could be improved. Also, defective microscopes were attended to during these visits.

In keeping with the change in the direction of the Centre's thrust area of research, the DTP functioning is being monitored more intensively and defects are being communicated to the local authorities for necessary remedial action. In a phased manner, more intensive activities are being planned for the 18 districts and areas requiring operational and field research inputs are being identified.

(started: 1988)

**Introduction of address card system in North Arcot District**

The 'Address card system' has been proved to be an efficient and inexpensive means of obtaining accurate addresses of patients in large towns in Tamil Nadu. In order to try out the effectiveness of this system in small towns and villages, a study was undertaken in North Arcot district. The study was conducted in two large hospitals, five primary health centres and a panchayat union dispensary; the local staff of these health facilities were briefed by the Centre's staff regarding the procedure.

In all, 379 patients were given the address cards at their respective treatment centres and were requested to get their complete postal address written on the address cards by the postman or a neighbour or friend. The patients were instructed to post the completed address cards to the Centre's field unit; 94% of the address cards were returned to the field unit with completed address. Reply paid letters were posted to all these patients to the addresses obtained through the address cards (Type A letter) to check their accuracy. The addresses of these patients as recorded on the treatment cards were compared with the addresses obtained through the address cards. Whenever there was any discrepancy, another letter was posted to the address recorded on the treatment card (Type B).

In all, 316 Type A letters and 233 Type B letters were posted. The data are being analysed. More information is being obtained regarding the receipt or non-receipt of the letters through home visits.

(started: 1988; expected year of completion: 1989)

**Influence of motivation on caseholding in two centres in North Arcot district**

In order to assess the influence of motivation in improving patient compliance under the DTP, a study was initiated in 2 treatment centres (the District TB Centre (DTC) and a large hospital in North Arcot District). At the DTC, all newly-diagnosed smear-positive and X-ray positive patients initiated to treatment are motivated by a

medical social worker of the Centre at the start of treatment, in the presence of an accompanying family member. Flash cards are used as an aid in motivation. At the large hospital, in addition to the initial motivation, the patients are also motivated at 1, 2 and 5 months after the start of treatment.

The study was started in October, 1988 and is still in progress. A total of 308 patients (169 on Short Course Chemotherapy and 139 on standard regimens) at the DTC and 126 patients (83 on Short Course Chemotherapy and 43 on standard regimens) at the hospital have been admitted to the study.

The regularity of attendance and the proportion of defaults are being analysed.

(started: 1988; expected year of completion: 1989).

**Bacteriological investigations for Short Course Chemotherapy under DTP in two districts**

As mentioned in the 1987 annual report, sputum specimens are being collected from patients admitted to short course chemotherapy regimens in North Arcot and Pondicherry, on admission, at the end of treatment, and at 9 and 12 months wherever possible. The specimens are being collected from the patients attending the District Tuberculosis Centre and major centres contributing a high proportion of cases in North Arcot and mostly from the District Tuberculosis Centre at Pondicherry. The specimens are transported to the Centre's laboratory at Madras through messengers for examination by smear, culture, sensitivity and identification tests. An analysis of the results obtained so far are presented below.

Of the 3148 pretreatment sputum specimens processed from North Arcot, 76% were positive by culture for *M. tuberculosis*, 16% were negative and 7% of specimens were lost due to contamination. Non-tuberculous Mycobacteria (NTM) was isolated from 2% of the specimens (see table below).

| Culture result | % of specimens | |
|---|---|---|
| | North Arcot | Pondicherry |
| Positive | *76* | *92* |
| Negative | *16* | *3* |
| Contaminated | *7* | *3* |
| NTM | *2* | *2* |
| Total No. of specimens | 3148 | 1554 |

Of the 1554 specimens from Pondicherry, 92% were culture positive, 3% were culture negative, 3% were lost due to contamination and NTM were isolated from 2% of the specimens.

All positive cultures were tested for their sensitivity to streptomycin, INAH and rifampicin. The findings among the pre-treatment specimens from North Arcot is given in the table below.

Of 2375 cultures from North Arcot, 1748 (74%) were fully sensitive to streptomycin, isoniazid and rifampicin; 90 (4%) were resistant to streptomycin, 317 (13%) to INAH and 178 (7%) to both streptomycin and INAH. Besides, there were 42 strains (2%) resistant to rifampicin, either alone or in combination with other drugs.

| Sensitivity result | No. | % |
|---|---|---|
| Sensitive to S,H & R | 1748 | *74* |
| Resistant to: S | 90 | *4* |
| H | 317 | *13* |
| SH | 178 | *7* |
| R | 1 | *2* |
| SR | 1 | |
| HR | 16 | |
| SHR | 24 | |
| Total | 2375 | *100* |

The drug sensitivity test results of Pondicherry cultures (see top table on page 19) show that, out of 1393 cultures tested, 1201 (86%) were sensitive to all three drugs tested. Resistance to streptomycin was seen in 55 (4%), to INAH in 88 (6%) and to both drugs in 44 (3%). The incidence of rifampicin resistance was very low, one being resistant to rifampicin alone, two to INAH and rifampicin, and two to all three drugs.

| Sensitivity result | | No. | % |
|---|---|---|---|
| Sensitive to S, H & R | | 1201 | *86* |
| Resistant to: | S | 55 | *4* |
| | H | 88 | *6* |
| | SH | 44 | *3* |
| | R | 1 | *0.4* |
| | HR | 2 | |
| | SHR | 2 | |
| Total | | 1393 | *100* |

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

| On admission | Total | Results at 6 months | | |
|---|---|---|---|---|
| | | Culture positive | | Resistant to S,H or SH |
| | | No. | % | |
| Sensitive | 272 | 37 | *14* | 31 |
| Resistant | 81 | 34 | *42* | 30 |
| Total | 353 | 71 | *20* | 61 |

Culture results were available for 353 patients. Of these, 71 patients had a positive culture at the end of treatment; 37 had initially had drug-sensitive organisms and 34 had initial resistance to one or more drugs. Of the former, 31 had developed resistance to streptomycin, isoniazid or both drugs. Of the latter 34 patients, 30 continued to excrete drug-resistant organisms. Of these, 7 were resistant to rifampicin, including 2 who had shown resistance to rifampicin on admission also.

The table on page 20 shows the corresponding results from Pondicherry. Of 548 patients who had received 80% or more of their treatment and for whom results were available, 510 (93%) were culture negative at 6 months. Of 38 patients with a positive culture, 23 had drug sensitive organisms initially. Of these, 10 had

acquired resistance to one or more drugs but none had resistance to rifampicin. Of the 15 patients who had had initial resistance to one or more drugs, 13 continued to excrete organisms resistant to one or more drugs; 8 of these had resistance to rifampicin, including 2 who had shown resistance to rifampicin on admission also.

| On admission | Total | Results at 6 months | | |
|---|---|---|---|---|
| | | Culture positive No. | % | Resistant to S.H or SH |
| Sensitive | 467 | 23 | *5* | 10 |
| Resistant | 81 | 15 | *19* | 13 |
| Total | 548 | 38 | 7 | 23 |

Of the patients who had received 80% or more of the prescribed chemotherapy, as many as 80% from North Arcot and 93% from Pondicherry became culture negative at the end of treatment under programme condition, which is a very encouraging finding. Even among patients with drug resistance on admission, a high proportion had a favourable response. However, the overall efficacy of these regimens will depend upon the relapse rates in these patients, and specimens are being collected at different periods of follow-up for bacteriological investigation.

(started: 1983)

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

As mentioned in the 1986-87 annual report, the Centre undertook a sample survey of awareness of symptoms in Komrnanandal village, a rural area in N. Arcot district. The details of the sample and the methodology of the survey have already been described. In brief, a sample of 18395 persons was chosen, a complete census of each household was done and chest symptomatics with the following symptoms were identified: (*a*) cough of 2 weeks and more, (*b*) haemoptysis at any time, and/or (*c*) chest pain at any time. A medical social worker of the Centre interviewed the symptomatics, using a detailed questionnaire, to find out their knowledge about tuberculosis and the action taken by them for diagnosis and relief of symptoms.

The table on page 21 gives the basic information regarding the sample size and the coverages achieved.

The main findings on the awareness of the disease and action taken for diagnosis and treatment are presented here. Of the 987 symptomatics, 598 (60.6%) were illiterate. Of those who were illiterate, 71.7% had sought medical advice and of the rest, 78.9% had done so. Considering the type of diagnostic facility utilised, 61.8% of the illiterates, and 61.2% of the literates had utilised a private practitioner,

| | No. | % |
|---|---|---|
| Total sample population registered | 18395 | |
| Symptomatics identified (15 years or more) | 1064 | *5.8* |
| Interviewed | 987 | *92.8* |
| Sputum samples collected | 967 | *98.0* |

private hospital or nursing home, compared to 54.3% and 54.4% respectively, who had been to a Govt. Hospital or clinic (some symptomatics had attended more than one health facility). Of those who went to a private hospital or nursing home, 60.9% said they did so because of good care while among those who attended a Govt. agency, only 31.3% gave this as the reason. On the other hand, only 19.8% of the group attending a Govt. agency said that they did so because of proximity.

Of the 257 who had not sought medical advice, 83 (31.3%) attributed lack of severity of symptoms as one of the reasons. Pressure of work was given as a reason by 37.4%. As high a proportion as 88% had heard of tuberculosis, including 83% of the illiterates.

The data are being extensively analysed, including the sample surveys undertaken in semi-urban and metropolitan areas.

(started: 1986; completed: 1988).

# CLINICAL STUDIES

## STUDIES COMPLETED

### Screening for symptoms and signs of abdominal tuberculosis in patients under assessment for pulmonary tuberculosis

From an earlier study of abdominal tuberculosis undertaken in our Centre, the most common combinations of symptoms and signs were obtained. The clinical profile in 126 patients of abdominal tuberculosis. confirmed either by histopathological examination or bacteriology is as follows:

Pain abdomen in association with alteration in bowel habits or tenderness of abdomen or borborygmi or distension of abdomen was observed in 75% of abdominal tuberculosis patients. Loss of weight in association with alteration in bowel habits or distension of abdomen or anorexia was observed in an additional 15% of abdominal tuberculosis patients. Hence, this algorithm was applied to screen for probable cases of abdominal tuberculosis among patients with pulmonary tuberculosis attending the Centre. A proforma was used to note the presence or absence of symptoms suggestive of abdominal tuberculosis with duration and the signs. Details of alcohol consumption and smoking were also elicited and recorded. A score of one was given for each symptom and sign and the total score was recorded.

A total of 323 patients have been screened for symptoms and signs. Out of this, data were incomplete in 10 patients and hence 313 cases were analysed. The table below shows the number and percentage of patients with symptoms and signs suggestive of abdominal tuberculosis:

| Symptoms and signs | No. | % |
|---|---|---|
| Pain abdomen | 54 | 17 |
| Distension of abdomen | 3 | 1 |
| Borborygmi | 20 | 6 |
| Fever | 151 | 48 |
| Nausea and vomiting | 33 | 11 |
| Alteration in bowel habits | 12 | 4 |
| Anorexia | 114 | 36 |
| Loss of weight | 128 | 41 |
| Doughy feeling | 5 | 2 |
| Ascites | 1 | 0·3 |
| Palpable liver | 8 | 3 |
| Palpable lump | 1 | 0·3 |
| Visible peristalsis | 1 | 0·3 |
| Tenderness of abdomen | 22 | 7 |
| Others | 1 | 0.3 |

Of the 313 patients, 68% had a total score of 2 or less and 29% had a score of 3-5, whereas in 2 patients, the score was more than 6.

Chest X-ray was suggestive of pulmonary tuberculosis in 83%, and 53% had a sputum culture positive for *M. tuberculosis*.

By applying the algorithm, it was found that 90 (29%) of 313 patients had presented with these combinations of signs and symptoms.

In order to find out the specificity of this symptomatology, this algorithm was applied to 119 patients who were investigated for abdominal tuberculosis, but considered as not suffering from abdominal tuberculosis after investigating them completely. The data of the clinical profile of these patients were analysed. The proportions in this group were similar to those in abdominal tuberculosis patients with confirmed (either histopathological or bacteriological) disease. Hence, this algorithm (symptomatology) does not seem to be specific for abdominal tuberculosis and so cannot be used as a method of screening for abdominal tuberculosis.

(started: 1987; completed: 1988).

## STUDIES IN PROGRESS

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

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

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

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

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

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

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

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

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

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

Of the 1023 patients with drug-sensitive bacilli, only 2 (0.2%) had an unfavourable response during chemotherapy; one developed miliary tuberculosis and the other had positive sputum cultures at 5 and 6 months. Of the 1021 drug-sensitive patients with a favourable response at the end of chemotherapy, 962 were assessed for relapse. The bacteriological relapses requiring treatment during a follow-up of 42 months are presented in the table below.

| Initial Rx (2m) | Continuation Rx (4m) | Total patients | Relapses requiring treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total | | Month of relapse after stopping Rx. | | | | | | |
| | | | No. | % | 1–6 | 7–12 | 13–18 | 19–24 | 25–30 | 31–36 | 37–42 |
| RSHZ thrice weekly | SRHow | 101 | 6 | *6* | 2 | 0 | 1 | 0 | 2 | 1 | 0 |
| | RHow | 100 | 2 | *2* | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | SRHtw | 92 | 3 | *3* | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| | RHtw | 92 | 5 | *5* | 1 | 0 | 1 | 1 | 1 | 0 | 1 |
| | SHtw | 95 | 5 | *5* | 2 | 0 | 1 | 0 | 1 | 0 | 1 |
| | Any | 480 | 21 | *4* | 8 | 0 | 3 | 1 | 5 | 2 | 2 |
| RSHZ twice weekly | SRHow | 104 | 7 | *7* | 4 | 0 | 0 | 2 | 0 | 1 | 0 |
| | RHow | 98 | 7 | *7* | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| | SRHtw | 89 | 3 | *3* | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| | RHtw | 89 | 6 | *7* | 5 | 0 | 0 | 0 | 0 | 1 | 0 |
| | SHtw | 102 | 10 | *10* | 7 | 0 | 1 | 0 | 0 | 1 | 1 |
| | Any | 482 | 33 | *7* | 23 | 1 | 2 | 2 | 1 | 3 | 1 |

Twenty-one (4%) of 480 thrice-weekly patients relapsed; the rates were similar for the various continuation regimens and ranged from 2 to 6%. Thirty-three

(7%) of 482 twice-weekly patients relapsed during the same period; the rates ranged from 3 to 10% for the various continuation regimens. The relapse rate of 4% in the thrice-weekly series was not significantly different from that of 7% in the twice-weekly series ($P = 0.2$).

Of the 54 relapses, 32 (59%) occurred in the first year of follow-up, 8 (15%) in the 2nd year, 11 (20%) in the 3rd year and 3 (6%) during the first half of the 4th year. In the vast majority of cases, the bacilli were drug-sensitive at the time of relapse.

There were 223 patients with bacilli initially resistant to 1 or more drugs; 62 had bacilli resistant to streptomycin alone, 64 to isoniazid alone and 97 to both drugs. Of 62 patients with resistance to streptomycin alone, one had an unfavourable response and 7 relapsed. Of 64 patients with resistance to isoniazid alone, 11 (17%) had an unfavourable response and 3 relapsed. Of 97 patients with resistance to streptomycin and isoniazid, 22 (30%) of 73 who had received rifampicin in the continuation phase and 18 (75%) of 24 who did not receive rifampicin had an unfavourable response ($P < 0.001$). Bacteriological relapse occurred in 6 of 51 patients who received rifampicin throughout and 2 of 6 who did not receive rifampicin.

The patients are being followed up till 5 years.

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

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

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

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

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

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

3. *$2HRZ_2/4HR_2$(tw) or $2HRZ_3/4HR_2$(ow):* This is similar to regimen 2, but without ethambutol.

Four hundred and thirty patients have been admitted to the study so far and the intake is continuing. The patients will be followed up till 5 years.

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

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

The fully oral short-course regimens investigated at Madras (see above) are also being investigated by the Centre's unit at the Government Rajaji Hospital, Madurai (Dean: Dr. R. Ramamurthy).

Patients are admitted on the basis of smear examinations 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.

Four hundred and thirteen patients have been admitted so far. The intake is continuing.

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

**Collaborative clinical trial of tuberculous lymphadenitis in children—follow-up of patients up to 36 months after stopping treatment**

The follow-up of patients admitted to the short course chemotherapy study of tuberculous lymphadenitis (see 1987 annual report) is being continued. This is a clinical trial conducted by the Centre in collaboration with the pediatric surgery departments of the Institute of Child Health and Hospital for Children, and the Government Stanley Hospital, Madras (annual report 1985-86). The subjects were children aged 1-12 years with lymph node tuberculosis confirmed by histopathology or culture.

Patients admitted to the study were treated with a fully supervised intermittent regimen for 6 months, consisting of streptomycin, isoniazid, rifampicin and pyrazinamide thrice a week for 2 months, followed by streptomycin and isoniazid twice a week for 4 months. At the end of chemotherapy, a repeat lymph node biopsy was done in those with significant residual lymphadenopathy (exceeding 10 mm). Patients were assessed at regular intervals at the Centre and at the referral hospitals.

In all, 197 patients were admitted to the study. After 27 exclusions, 170 are available for analyses. Repeat lymph node biopsy was done in 54 patients at the end of treatment; in 2, the lymph node culture was positive for tubercle bacilli. These were considered as failures of chemotherapy. Six patients had histopathological evidence of tuberculosis but negative cultures.

In all, 160 have completed 36 months of post-treatment follow-up. During the follow-up period, 3 patients required additional chemotherapy for tuberculosis, 1 each for relapse of lymph node tuberculosis (2 months post-treatment), abdominal tuberculosis (4 months post-treatment) and tuberculous meningitis (36 months post-treatment). The patient with relapse of lymph node tuberculosis had histological evidence of tuberculosis in his post-treatment repeat lymph node biopsy. Five other patients who had a similar finding in the post-treatment biopsy during the follow-up period showed no clinical evidence of reactivation of the disease. Thus, 157 patients have successfully completed 36 months of post-treatment follow-up.

To summarise, only 5(3%) of 160 patients have required retreatment for tuberculosis by the end of 36 months of follow-up. The others have done well. A 6-month intermittent regimen therefore seems to be very effective in the treatment of tuberculous lymphadenitis in children. The patients are being followed up till 5 years.

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

**Collaborative clinical trial of tuberculous lymphadenitis in children at Madurai**

A controlled clinical trial of tuberculous lymphadenitis in children has been in progress since June 1988. This study is being carried out in collaboration with the Paediatric Surgery department (Dr. A. J. Thiruthuvathas, Paediatric Surgeon) of the Government Rajaji Hospital, Madurai.

Children aged 1-12 years, with or without history of previous chemotherapy, are considered for the study, provided the clinical diagnosis is confirmed by either histopathology or culture of lymph node biopsy. The histopathology slides are read by Dr. Ananthalakshmi, Professor of Pathology, Madurai Medical College succeeding Dr. Thilagavathy, and bacteriological investigations are done at the Centre in Madras.

Patients admitted to the study are treated as out-patients, either with a 6-month daily self-administered regimen of rifampicin and isoniazid ($6RH_7$) supplied twice a month, or with a 6-month fully supervised twice-weekly regimen of rifampicin, isoniazid and pyrazinamide for 2 months followed by rifampicin and isoniazid for 4 months ($2RHZ_2/4RH_2$).

The patients are assessed clinically at regular intervals at the Unit and by the paediatric surgeon. At the completion of chemotherapy, the patients are assessed by an independent observer, who recommends a repeat lymph node biopsy if the patient has significant residual lymphadenopathy. So far, 17 patients have been admitted and the intake is continuing.

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

**Collaborative study of abdominal tuberculosis**

As mentioned in previous annual reports (1985-86; 86-87; 1988) the Centre is carrying out a collaborative study of abdominal tuberculosis.

The objectives of this study are as follows:

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

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

A subsidiary objective is to develop, from the findings of this study, satisfactory criteria for diagnosis, assessment of progress, and identification of relapse in abdominal tuberculosis.

The study is being conducted in collaboration with the Departments of Medicine and Medical and Surgical Gastro-enterology of the Government General Hospital, Madras, and the Institute of Thoracic Medicine, Madras.

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

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

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

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

Between September 1983 and December 1988, 658 patients have been registered and of these, 169 patients have been admitted to the study. Of these, information up to 1 year is available for 145 patients (74 2RHZ/4RH, 71 SEH/EH).

**Characteristics on admission:** The mean age was 31 years (range 13-72 years). The sex ratio was 1:1. An initial induration of 10 mm or more to 1 TU of PPD (RT23 with Tween 80) was seen in 75% of the patients. Of the 145 patients, 108 (74%) had intestinal lesions, 66 (46%) had peritoneal tuberculosis, 20 (14%) had hepatic tuberculosis and 11 (8%) patients had mesenteric tuberculosis; 52 of these patients had combined lesions.

In all, 108 patients (74%) had presented with pain in the abdomen, in association with tenderness of abdomen or alteration in bowel habits or increased borborygmi or distension of abdomen or anorexia.

**Confirmation of diagnosis:** Of 108 patients with intestinal tuberculosis, 91 (84%) had radiographic evidence of intestinal tuberculosis by barium contrast studies, of whom 52% had either direct histopathological or bacteriological confirmation or indirect evidence of tuberculosis elsewhere in the body. Of the remaining, 15 patients had histopathological confirmation and two patients were admitted on the basis of laparotomy findings of multiple strictures of the ileum and peritoneal tubercles.

There were 66 cases of peritoneal tuberculosis, of whom 56 had histopathological confirmation. Ascitic fluid was positive for *M. tuberculosis*, by smear or culture in 6. One patient had exudative ascites with sputum-positive pulmonary tuberculosis and 3 patients had peritoneal tubercles with exudative ascites.

Considering the 20 patients with hepatic tuberculosis, all except 1 had histopathological confirmation while all the 11 patients with mesenteric tuberculosis had the diagnosis confirmed by histopathology.

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

| Status at the end of Rx | 2RHZ/4RH | | SEH/EH | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Symptom free | 63 | *97* | 51 | *89* |
| Clinically improved but still symptomatic | 1 | *2* | 2 | *4* |
| Change of Rx for clinical deterioration | 1 | *2* | 3 | *5* |
| TB death | 0 | *0* | 1 | *2* |
| Total patients with assessable response | 65 | *101* | 57 | *100* |
| **Response not assessable** | | | | |
| Non-tuberculous death | 3 | | 4 | |
| Received less than 75% of Rx | 1 | | 6 | |
| Interruption of 25% or more of Rx | 3 | | 0 | |
| Rx modification | 2 | | 4 | |
| Total patients | 74 | | 71 | |

Out of 65 patients in the rifampicin regimen and 57 in the non-rifampicin regimen, 63 (97%) and 51 (89%) respectively were symptom free at the end of treatment. One patient (2%) in the rifampicin regimen and two patients (4%) in the non-rifampicin regimen had clinically improved but were symptomatic at the end of treatment. One (2%) in the rifampicin regimen and 3 (5%) in the non-rifampicin regimen had a change of chemotherapy for clinical deterioration and 1 (2%) in the non-rifampicin regimen had died of tuberculosis.

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

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

**Collaborative study of brain tuberculoma**

Brain tuberculoma is now being suspected much more often than in the past, probably due to increased awareness of the disease among physicians and the greater

availability of CT scan. There are reports which suggest that chemotherapy alone may be effective even for large brain tuberculomas with increased intracranial tension; however no reports are available on the use of short-course treatment for brain tuberculoma, and also for multiple lesions. A controlled study was initiated in collaboration with the Institute of Neurology (Prof. S. Kalyanaraman), Govt. General Hospital Madras, to evaluate the efficacy of short-course chemotherapy in the management of brain tuberculoma and determine indications for surgery (see 1987 annual report). During the year, the study was extended to the Railway Hospital, Perambur (Dr. Zaheer Ahmed Sujeer).

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

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

Regimen I: $3RHZ_7/6RH_2$

Regimen II: $3RHZ_3/6RH_2$

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

The following investigations are done on admission: CT scan, X-ray (chest and skull), CSF culture, culture of sputum and urine, Mantoux, liver function tests and haematological examinations.

CT scan is repeated at 1 month, 2 months and every 2 months thereafter till 2 consecutive scans are normal. If the size of the mass at the second monthly scan is more than 80% of the mass on admission, a biopsy of the mass is done for histopathology and culture examinations.

So far, 90 patients have been admitted to the study; 50 cases have completed treatment and the results are encouraging.

The intake is continuing.

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

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

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

The chemotherapeutic regimens are as follows:

**(a) Patients with bacilli sensitive to isoniazid and rifampicin at the last examination:**

By random allocation to *either* 3EmbHRZ$_2$/6HR$_2$ *or* 3EmbHRZ$_2$/9HR$_2$, namely ethambutol 1200 mg plus isoniazid 600 mg (without pyridoxine) plus rifampicin 450 mg plus pyrazinamide 2.0 g twice a week for the first 3 months, followed by isoniazid plus rifampicin in the same dosages twice a week for either the next 6 months (total 9 months) or the next 9 months (total 12 months). Every dose is given under supervision. So far, 41 patients have been admitted.

**(b) Patients with bacilli resistant to isoniazid but sensitive to streptomycin:**

6SEmbRZ$_2$/6EmbRZ$_2$: Streptomycin 0.75 g plus ethambutol 1200 mg plus rifampicin 450 mg plus pyrazinamide 2.0 g twice a week for the first 6 months, followed by ethambutol plus rifampicin plus pyrazinamide in the same dosages twice a week for the next 6 months (total 12 months). Every dose is given under supervision; 14 patients have been admitted so far.

**(c) Patients with bacilli resistant to streptomycin and isoniazid.**

6KEmbRZ$_2$/6EmbRZ$_2$: Kanamycin 1.0 g plus ethambutol 1200 mg plus rifampicin 450 mg plus pyrazinamide 2.0 g twice a week for 6 months, followed by ethambutol plus rifampicin plus pyrazinamide in the same dosages twice a week for the next 6 months (total 12 months). Every dose is given under supervision. So far, 8 patients have been admitted.

**(d) Patients with bacilli resistant to isoniazid and rifampicin but sensitive to streptomycin:**

3S$_3$EmbEthZ$_7$/9EmbEthZ$_7$: Streptomycin 0.75 g thrice a week plus daily ethambutol 600 mg plus ethionamide 500 mg plus pyrazinamide 1.5 g for the first 3 months, followed by daily ethambutol plus ethionamide plus pyrazinamide in the same dosages for the next 9 months (total 12 months). Throughout the 12 months, the patient attends thrice a week, when he receives that day's dose under supervision and is supplied with drugs for self-administration for the remaining days. Two patients have been admitted.

**(e) Patients with bacilli resistant to streptomycin, isoniazid and rifampicin:**

3K$_3$EmbEthZ$_7$/9EmbEthZ$_7$: Kanamycin 1.0 g thrice a week plus daily ethambutol 600 mg plus ethionamide 500 mg plus pyrazinamide 1.5 g for the first 3 months, followed by daily ethambutol plus ethionamide plus pyrazinamide in the same dosages for the next 9 months (total 12 months). Throughout the 12 months, the patient attends thrice a week when he receives that day's dose under supervision and is supplied with drugs for self-administration for the remaining days. So far, 12 patients have been admitted.

The intake to all the regimens is continuing.

(started: 1987).

## Screening for renal involvement in sputum positive pulmonary tuberculosis patients

This study was started to estimate the frequency of mycobacteriuria among sputum positive pulmonary tuberculosis patients and to assess the renal function among those with a urine culture positive for *M. tuberculosis* (see 1987 annual report).

When the sputum smear is reported as positive for acid fast bacilli, patients are asked to bring the entire quantity of early morning urine on 3 consecutive days in sterile bottles and the specimens are examined by culture for *M. tuberculosis.* One specimen is examined by culture for non-tuberculous organisms also.

So far, a total of 158 patients have been screened. Of these, culture results are available for 126 patients. Urine culture was positive for *M. tuberculosis* in 2 patients. Two other patients produced organisms that were identified as non-tuberculous mycobacteria.

(started: 1987; expected year of completion of intake: 1989).

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

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

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

Intake to the study was started in September, 1974 and stopped in April, 1985 and a total of 611 children have been admitted to the study. The 5-year follow-up is in progress.

(started: 1974; expected year of completion: 1990).

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

The detailed findings of the first 3 chemotherapy studies on tuberculous meningitis have already been published (Tubercle, 1986, *67*, 17-29). To summarise, 180 patients were treated for 12 months with one of 3 regimens containing rifampicin, with or without pyrazinamide. In all, 27% of the patients died, 39% had neurological sequelae and 34% recovered completely. There was a strong association between the stage of the disease on admission and the mortality. In the first study, when isoniazid was prescribed daily in a dosage of 20 mg/kg, 39% of the patients developed jaundice; however when the dosage was reduced to 12 mg/kg, the incidence was 16%. In the third study, where rifampicin was administered twice a week, the incidence was much lower (5%).

Even though the high mortality could have been due to patients reporting late (87% had stage II or stage III disease on admission), a high prevalence of initial drug resistance (25% to S or H) contributing towards failure of chemotherapy could not be ruled out. A study is therefore being conducted, in collaboration with the Institute of Child Health and Hospital for Children, Egmore, to investigate more intensive regimens, with 5 drugs in the initial phase followed by 2 drugs twice weekly (see 1987 annual report).

Patients aged between 1 and 12 years, who had not received more than 4 weeks of previous anti-tuberculosis treatment and had no evidence of renal or liver disease were eligible; they were randomly allocated, after stratification according to clinical severity, in equal proportions to the following 2 regimens:

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

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

In all, 215 patients were admitted to the 2 regimens (107 to regimen I, 108 to regimen II). On admission, 45 (21%) patients were classified as having stage I disease, 160 (74%) as stage II and 10 (5%) as stage III.

Some other pretreatment characteristics are given below:

| | | |
|---|---|---|
| Age less than 5 years | 75% | |
| Positive tuberculin test with 1 TU | 34% | |
| Contact with a known case of TB | 61% | |
| Abnormal chest radiograph | 51% | |
| CSF smear (alone) positive for AFB | 6% | 47% |
| CSF smear & culture positive | 41% | |

**Response to treatment:** Of the 215 patients admitted to the 2 regimens, 1 died of a non-tuberculous cause and 29 patients discharged themselves against medical advice before completing therapy. The response to the allocated regimen could not be assessed in 35 patients, as their treatment had been modified, mainly because of the development of hepatitis or ocular changes. The analysis of response to treatment is therefore based on 150 patients, after excluding the above 65.

The table below gives the response to treatment in the 2 regimens. It can be seen that 40 patients (27%) had died of tuberculous meningitis, 52 (35%) had neurological sequelae and 58 (39%) had a complete recovery. The response was similar in the two regimens.

| Treatment regimen | No. in analysis | Tb death | | Residual damage | | | | | Complete recovery | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | Total No. | % | severe | moderate | mild | No | % |
| 2 $S_7E_7H_7R_3Z_3/7R_2H_2$ | 66 | 17 | *26* | 20 | *30* | 5 | 14 | 1 | 29 | *44* |
| 2 $S_7E_7H_7R_2Z_2/7R_2H_2$ | 84 | 23 | *27* | 32 | *38* | 0 | 16 | 16 | 29 | *35* |
| Total | 150 | 40 | *27* | 52 | *35* | 5 | 30 | 17 | 58 | *39* |

The table below gives the response according to the stage of the disease on admission. There was a clear association between the stage on admission and tuberculous death, the proportion being 8% for stage I patients and 33% for stage II and stage III combined. Conversely, 76% of stage I patients had a complete recovery against 27% of stage II and stage III.

| Stage of disease | No. in analysis | Tb. death | | Residual damage | | Complete recovery | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| I | 37 | 3 | *8* | 6 | *16* | 28 | *76* |
| II | 107 | 33 | *33* | 45 | *41* | 29 | *27* |
| III | 6 | 4 | | 1 | | 1 | |
| All | 150 | 40 | *27* | 52 | *35* | 58 | *39* |

Of the 40 deaths, 14 (35%) occurred in the first week. In all, 23 (58%) died in the first month, 4 (10%) in the second month, 5 (12%) in the third month, and 8 (20%) between 4 and 6 months.

**Complications while on therapy: (a): Hydrocephalus:** Of the total 215 patients admitted to the 2 regimens, hydrocephalus was suspected in 61 patients. Of these, a CT scan was done and the diagnosis confirmed in 58 patients; in 32 patients, surgery (ventriculo-peritoneal shunt) was performed.

**(b): Blindness and optic disc changes:** Optic atrophy with blindness developed in 5 patients; all of them died (4 had surgery for hydrocephalus) and had the problem till the end. Ten others developed varying degrees of pallor of the optic disc. Of these, in 4, the findings became normal while the remaining 6 patients continued to have the abnormality till the end of treatment. Even though the complication was not considered to be drug related, ethambutol was terminated in all the patients and the other drugs (SHRZ) continued.

**Adverse reactions:** This analysis is also based on all the 215 patients admitted to treatment. Hepatotoxicity was the only problem noticed. Jaundice was present in 19 (9%) patients, while elevated liver function test (LFT) values (more than 100 IU/l) were found in 7 (3%) other patients. In all patients, rifampicin and pyrazinamide were terminated and the treatment modfiied suitably. Repeat LFT values were normal in all except 1 patient, who died due to TB meningitis (serial LFTs in this patient showed considerable lowering of the values).

It may be concluded that despite the administration of intensive regimens which include potent drugs, mortality is likely to be high, indicating once again that the stage of the disease at which treatment is begun is more important than the drug regimen.

To assess the long-term efficacy, the patients are being followed up till 5 years. Patients are seen once a month up to 24 months, once in 3 months thereafter up to 36 months and once in 6 months thereafter up to 60 months.

The follow-up investigations include (a) a complete examination with special reference to the central nervous system; (b) repeat chest radiographs at 3-monthly intervals for patients who have persistent abnormality at the end of treatment, till they become normal; and (c) repeat cerebro-spinal fluid (CSF) examination for cell count. biochemistry and bacteriology every three months for patients with abnormal CSF findings at the end of treatment.

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

The study is in progress.

(started: 1982; year of completion of intake: 1988)

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

Pulmonary function studies are being carried out in patients with tuberculosis of the spine treated with short-course regimens (see 1985-86 annual report). The

treatment had consisted of rifampicin plus isoniazid daily, for either 6 or 9 months; half of those treated for 6 months had radical resection of the spinal lesion with bone grafting. Since these tests had not been undertaken on admission to treatment, comparative pretreatment values for the different groups are not available. However, since allocation of the patients to the three treatment groups was at random, it is highly likely that the three groups were similar in respect of pulmonary function on admission. The aims of the study are to find out (a) whether the correction of deformity by radical surgery makes a significant contribution to improved respiratory function, and (b) whether the presence of a lesion in the thoracic or thoraco-lumbar region of the spine compromises the respiratory function to a greater extent than a lesion in the lumbar region. The following pulmonary function tests are being carried out at yearly intervals using P.K. Morgan Transfer Test Model C.

1. Forced Vital Capacity (FVC)
2. Forced Expiratory Volume in 1 Sec ($FEV_1$)
3. $\frac{FEV_1}{FVC}\%$
4. Maximum Voluntary Ventilation (MVV)

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

(started: 1982; expected year of completion: 1985)

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

The technique of bronchoalveolar lavage (BAL) was utilised to characterise the inflammatory and immune effector cells in the lung parenchyma of patients with sputum smear negative for AFB, but with radiographic appearances suggestive of pulmonary tuberculosis. Patients whose sputa or lavage fluid had shown growth of *M. tuberculosis* in culture were classified as having active pulmonary tuberculosis. All patients had symptoms for less than 3 months and none had received any anti-TB treatment in the past. The lavages in each patient were carried out first from a radiologically normal subsegment and then from radiologically abnormal subsegments. The total cells and differential cells were determined, separately from each site. Of 46 suspected patients in whom BAL was done, 19 patients had bacteriological confirmation, while in 14, the lavage fluid culture was also positive for *M. tuberculosis*.

Eleven patients investigated showed predominance of macrophages (range: 82-96%; mean $\pm$ sd:91.1 % $\pm$ 5%) with a lymphocyte count of 2-14% (mean $\pm$ sd : 6.7% $\pm$ 4%). Another group of 6 patients showed predominance of lymphocytes (range 23-69%, mean $\pm$ sd: 43.8% $\pm$ 18.5%) with a macrophage count of 26-74% (mean $\pm$ sd: 52.4% $\pm$18.3%). In 2 patients, an increased eosinophil count was observed

(65% in one patient and 44% in the other). Thus, two distinct cell profiles, one group with an increase in alveolar macrophages and the other with an increase in lymphocytes, were observed.

It is proposed to study 50 patients.

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

**Alveolitis in miliary tuberculosis**

Miliary disease of the lung due to *M. tuberculosis* is essentially an interstitial lung disease. A detailed study of functional derangement of the gas exchange units in miliary tuberculosis and the role of alveolitis in producing these changes have not yet been undertaken. A study was therefore planned to evaluate in detail the pulmonary function in these patients and also to characterise the alveolitis using the technique of bronchoalveolar lavage (BAL).

It is proposed to undertake BAL studies before and after short course chemotherapy and also do pulmonary function tests such as spirometry, lung volumes and diffusing capacity measurements.

So far, 3 patients have been investigated. It is proposed to admit 20 patients to the study.

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

**Pulmonary function in healthy young adults (15-40 years) in South India**

Since wide changes in pulmonary functions in normal subjects are known to occur due to ethnic variation, physical activity, environmental conditions, altitude of dwelling, tobacco smoking, age, height, sex and socio-economic status, a comprehensive study of pulmonary function was carried out in South Indian subjects residing at Madras. Ethnic South Indians are of Dravidian stock and live in tropical climate at sea level and rice is their staple diet. Two hundred and forty-seven subjects aged between 15 and 40 years were studied. Although the study population is not random, attempts were made to obtain a representative cross-section of normal subjects of Madras city. To achieve this, the subjects included relatives of patients attending the Centre, staff members, manual workers, students and executives. The subjects were eligible for the study if they were ethnic South Indians, with no structural deformity of the thoracic cage, who had been free from respiratory infection for at least three months. None of the subjects had any cardio-respiratory disease, as assessed by detailed history, physical examination, full-plate chest X-rays and 12-lead electrocardiogram.

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

(1) Forced Vital Capacity (FVC)

(2) Forced Expiratory volume in 1 sec ($FEV_1$)

(3) $\frac{FEV_1}{FVC}\%$

(4) Total Lung Capacity (TLC)

(5) Functional Residual Capacity (FRC)

(6) Residual Volume (RV)

(7) $\frac{RV}{TLC}\%$

(8) Effective alveolar volume (VA)

(9) Single Breath Carbon Monoxide diffusing capacity (TLCO)

(10) Transfer co-efficient (KCO)

Correlation coefficients between pulmonary function and various physical parameters were obtained, separately for males and females. Normative prediction equations were developed using the data which are used both in a clinical setting and to adjust comparisons among risk groups for known demographic and treatment differences. Comparisons are also done in a variety of cross-sectional and longitudinal prediction equations to describe "normal lung function."

The study is being continued, and children (7-14 years) and the elderly (more than 40 years) are also being investigated.

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

**Follow up studies in Tropical Eosinophilia (TE)**

Earlier bronchoalveolar lavage and pulmonary function studies had shown that there was intense eosinophilic alveolitis with diffusion defect in Tropical Eosinophilia (J. Clin. Invest, 1987, 80, 216-225). As it had been shown that untreated TE patients presenting with symptoms of long duration could develop intestinal fibrosis, this study was planned to observe the natural history of TE in patients presenting with symptoms of shorter duration (less than 6 months) and who had been treated for 3 weeks with Diethyl Carbamazine Citrate (6mg/kg body weight). The follow up of these patients at 1,3,6,12,24,36,48 and 60 months utilising the technique of bronchoalveolar lavage and pulmonary function is highly satisfactory.

So far, 152 patients have been admitted to the study and it is proposed to investigate a total of 200 patients.

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

**Controlled clinical study of multi-drug therapy in multibacillary leprosy**

As mentioned in the previous (1987) annual report, the Centre is undertaking a controlled clinical trial to assess the relative efficacies of pyrazinamide and rifampicin

in combination with clofazimine and DDS in the treatment of multibacillary leprosy, at the Govt. Royapettah Hospital, Madras.

The following 4 regimens are being investigated:

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

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

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

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

So far, 20 patients have been admitted to the study—6 patients to the first regimen, 5 to the second, 4 to the third and 5 to the fourth regimen.

It is proposed to admit 60 patients to each regimen.

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

# LABORATORY STUDIES

## STUDIES COMPLETED

### Storage of sputum samples with Cetyl Pyridinium Chloride (CPC)

Earlier studies conducted at this Centre and elsewhere had shown that sputum samples which are to be examined by culture cannot be stored at room temperature beyond 3 days as the tubercle bacilli tend to lose their viability. Since the Centre is now involved in the District Tuberculosis Programme to a larger extent and sputum samples are being routinely transported to the central laboratory for culture, several attempts are being made to minimise the loss in viability due to delay in transport of sputum specimens. As one such study, the role of CPC as a preservative agent was explored.

Overnight collection specimens of sputum were homogenised with glass beads, divided into 5 aliquots, and randomly allocated to no storage, or storage at room temperature for 7 or 14 days, with or without CPC. The aliquots stored with CPC, in equal volume, were centrifuged and the deposit inoculated onto Lowenstein-Jensen medium slopes, while the other aliquots were processed by Petroff's method.

In all, 98 specimens were included for this study. The findings are set out in the table below:

| Culture result | Number of specimens | | | | |
|---|---|---|---|---|---|
| | 0 | 7 days | | 14 days | |
| | P* | P | C | P | C |
| Positive | 95 | 75 | 65 | 44 | 65 |
| Negative | 1 | 6 | 4 | 21 | 8 |
| Contaminated | 2 | 17 | 29 | 33 | 25 |

*P = Petroff's method; C = CPC method

It can be seen that after 7 days, the number of cultures positive with CPC was only 65, even less than the 75 obtained without preservative (Petroff's method). However, the culture results remained static up to 14 days of storage with the CPC method. Hence, CPC was not effective as a preservative.

When the quantity of sputum was inadequate, the aliquots were stored only for 7 days. The findings in these specimens were similar to the above, as can be seen from the table on page 42.

| Culture result | No. of specimens | | |
|---|---|---|---|
| | 0 | 7 days | |
| | P | P | C |
| Positive | 176 | 134 | 128 |
| Negative | 4 | 26 | 8 |
| Contaminated | 7 | 27 | 51 |

Thus the study conclusively proved the ineffective role of CPC as a preservative agent in storing sputum samples at room temperature up to 7 days. Hence this study has been stopped and other avenues are being explored.

(started: 1985; completed: 1988)

**A comparison of the Ziel-Neelsen technique for acid fast bacilli by the classical heat-treated and a cold staining method**

The classical Ziel-Neelsen (ZN) staining method for acid-fast organisms involves heating the carbol fuchsin stain over the smear to enable penetration of the dye. In order to avoid the heating procedure, several workers have modified the stain to improve its penetrability. A cold method without any such modification has been reported to give results identical to the standard Z-N method in a comparison carried out with sputum smears by Vasanthakumari et al. (Bull. WHO, 1986, *64*, 742-743). However, they had not graded the positive smears and therefore the sensitivity of the two methods could not be accurately assessed.

A controlled comparison of the same two methods was therefore done with 100 pairs of sputum smears. The positivity was graded by two independent readers; the results by the first reader are tabulated on page 43.

The results show that there was no statistically significant difference between the two methods in the detection or grading of positives. The variation between readers was also not significant (not tabulated). A comparison on a larger number of specimens is being planned. However, the indications are that the cold staining method could provide a feasible alternative under field conditions, as heating sputum smears has often been a problem due to non-availability of basic requirements like spirit lamp and methylated spirit.

(started: 1988; completed: 1988)

| | | Cold staining method | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Neg | 1-10 | 11-50 | 51-100 | + | ++ or more | Total |
| Z-N method | Neg | 45 | 6 | 3 | 2 | 0 | 0 | 56 |
| | 1-10 | 13 | 0 | 0 | 0 | 1 | 0 | 14 |
| | 11-50 | 2 | 2 | 1 | 0 | 0 | 0 | 5 |
| | 51-100 | 0 | 0 | 1 | 0 | 1 | 0 | 2 |
| | + | 1 | 0 | 1 | 1 | 6 | 1 | 10 |
| | ++ or more | 0 | 0 | 1 | 0 | 4 | 8 | 13 |
| | Total | 61 | 8 | 7 | 3 | 12 | 9 | 100 |

**Development of rifampicin resistance in *M. tuberculosis***

In one of the Short Course Chemotherapy studies being undertaken at Madurai (see page 26), 9 patients (out of 425 with drug sensitivity results available) were found to have pretreatment resistance to rifampicin. All 9 patients were also resistant to isoniazid, including 7 resistant to streptomycin as well.

A further 12 patients developed resistance to rifampicin during treatment, and all of them had isoniazid-resistant organisms at the time of emergence of rifampicin resistance. Two of these were isoniazid-sensitive initially but became resistant after the first month of treatment.

Further experiments are being planned in order to get precise information on the pattern of emergence of rifampicin resistance.

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

***In vitro* activity of Rifapentine (MDL 473) against *M. tuberculosis***

The *in vitro* activity of rifapentine ((3-4 cyclopentyl piperazinylaminomethyl)-rifamycin, MDL 473) has been shown to be from two to ten times more than rifampicin (RMP) in Kirchner's liquid medium (Arioli et al; Journal of Antibiotics, *34*, 1026, 1981) and about 4 times more in 7H10 agar medium (Dickinson and Mitchison, Tubercle, *68*, 177, 1987). However, Yates and Collins (Journal of Antimicrobial Chemotherapy, *10*, 147, 1982) have shown the activities of MDL 473 and RMP to be similar on LJ medium. Grosset and colleagues (Revue Francais Maladies

Respiratoire, *11*, 875, 1983) have also reported the two compounds to have similar *in vitro* activity. It was therefore proposed to test the activities of MDL 473 and RMP on LJ as well as 7H11 agar slopes, using South Indian isolates of *M. tuberculosis*, sensitive as well as resistant to RMP.

Rifampicin and MDL 473 were dissolved in dimethyl formamide and incorporated in LJ as well as 7H11 medium to give final concentrations of 0, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 mg/l. The distribution of MICs of 87 cultures tested on LJ medium are presented in the table below. A total of 30 strains were classified as resistant to both compounds while the remaining 57 were sensitive.

| | | MIC with MDL 473 (mg/l) on LJ medium | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 8 | 16 | 32 | 64 | 128 | Total |
| MIC with RMP (mg/l) | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 4 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| | 8 | 0 | 7 | 4 | 1 | 0 | 0 | 0 | 12 |
| | 16 | 1 | 2 | 5 | 3 | 0 | 0 | 0 | 11 |
| | 32 | 0 | 1 | 8 | 8 | 1 | 0 | 0 | 18 |
| | 64 | 0 | 0 | 4 | 6 | 3 | 0 | 0 | 13 |
| | 128 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 30 |
| | Total | 1 | 12 | 22 | 18 | 4 | 0 | 30 | 87 |

Considering the MICs of the 57 strains sensitive to the two compounds, 9 had identical MICs and 3 strains had one dilution higher MIC for MDL 473 than for RMP. The remaining 45 strains had a lower MIC for MDL 473 (23 by one dilution, 16 by two dilutions and 6 by 3 dilutions). Thus, in these 45 strains, MDL 473 appeared to have a 2-8 fold higher activity. However, all strains resistant to RMP were resistant to MDL 473 and vice versa.

On 7H11 agar medium, of the 85 strains tested (results on 2 were lost due to contamination), 29 were resistant to RMP while 28 were resistant to MDL 473, the odd culture having an MIC of 64 with MDL 473 (see table on page 45). Of the remaining 56 cultures, which were sensitive to both the compounds, 16 had identical MICs, while for 4 cultures, the MIC was higher for MDL 473 by 1 or 2 dilutions, all in the lower concentrations (1-4 mg/l). The other 36 showed a lower MIC to MDL 473 than for RMP (21 by one dilution, 13 by two dilutions and 2 by 3 or 4 dilutions) a similar pattern to that on LJ medium.

| | | MIC with MDL 473 (mg/l) on 7H11 medium | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | Total |
| MIC with RMP(mg/l) | 0.5 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | 1 | 3 | 4 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 9 |
| | 2 | 4 | 2 | 6 | 2 | 0 | 0 | 0 | 0 | 0 | 14 |
| | 4 | 1 | 3 | 13 | 4 | 0 | 0 | 0 | 0 | 0 | 21 |
| | 8 | 1 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 5 |
| | 16 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| | 32 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 3 |
| | 64 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 128 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 28 | 29 |
| | Total | 11 | 9 | 23 | 9 | 3 | 1 | 0 | 1 | 28 | 85 |

It may be concluded that the *in vitro* activity of MDL 473 is superior to that of rifampicin, in both LJ and 7H11 medium. It was also found that the MICs of both compounds were lower on 7H11 medium than on LJ medium.

(started: 1987; completed: 1988)

**Antibody levels to tuberculous and environmental mycobacterial antigens in pre-treatment sera from patients with disease due to *M. kansasii***

Patients with pulmonary tuberculosis have high levels of IgG antibodies against several mycobacterial antigens such as *M. tuberculosis, M.bovis, M. scrofulaceum, M. kansasii* and PPD-S. Information on the level of antibodies to various mycobacterial antigens in patients with non-tuberculous mycobacterial disease in India is still lacking. There is a high prevalence of non-tuberculous mycobacterial (NTM) infection with organisms such as *M. avium-intracellulare, M. terrae* and *M. scrofulaceum* in South India. The estimation of antibody levels to the environmental mycobacterial antigens and tuberculous antigens in patients with NTM disease and healthy individuals will reveal the sensitization pattern to these antigens and its usefulness in discriminating between the two groups of individuals.

For this study, serum samples from 13 individuals from whom *M. kansasii* had been isolated repeatedly were included along with 26 serum samples from healthy adults for comparison. Enzyme linked immunosorbent assay (ELISA) was done to

estimate the antibody levels in these serum samples against various tuberculous and environmental mycobacterial sonicate antigens and the data is presented in the table below:

| Antigen | Mean optical density (and standard deviation) | | | |
|---|---|---|---|---|
| | At 1/40 dilution | | At 1/80 dilution | |
| | M. kansasii patients n=13 | controls n=26 | M. kansasii patients n=13 | controls n=26 |
| 1. *M. tuberculosis* H37Rv | .331(.095) | .321(.084) | .243(.064) | .190(.050) |
| 2. *M. tuberculosis* -strain SI | .554(.114) | .379(.108) | .392(.134) | .231(.050) |
| 3. *M. tuberculosis* -strain 7219 | .365(.076) | .343(.125) | .271(.072) | .210(.093) |
| 4. *M. bovis* | .364(.076) | .369(.129) | .298(.056) | .230(.087) |
| 5. BCG | .436(.097) | .468(.059) | .357(.128) | .364(.062) |
| 6. *M. kansasii* | .400(.079) | .332(.065) | .323(.086) | .256(.071) |
| 7. *M. scrofulaceum* | .680(.156) | .354(.085) | .511(.150) | .253(.071) |
| 8. *M. avium* | .411(.084) | .371(.052) | .321(.078) | .301(.056) |
| 9. *M. intracellulare* | .306(.111) | .304(.091) | .247(.113) | .194(.073) |
| 10. *M. terrae* | .258(.072) | .241(.056) | .194(.083) | .161(.038) |
| 11. *M. flavescens* | .330(.103) | .267(.056) | .281(.093) | .172(.039) |
| 12. *M. gordonae* | .356(.110) | .455(.089) | .308(.126) | .330(.094) |
| 13. *M. fortuitum* | .330(.107) | .406(.085) | .290(.128) | .280(.071) |
| 14. *M. chelonei* | .342(.105) | .399(.097) | .288(.115) | .261(.085) |
| 15. PPD-S | .344(.111) | .262(.077) | .270(.119) | .169(.053) |

At 1/40 dilution, the antibody levels to the antigens of *M. tuberculosis-SI, M. kansasii, M. scrofulaceum, M. flavescens* and PPD-S were significantly higher ($P<.05$) in the patients than in the controls. Of these antigens, *M. scrofulaceum* was the most discriminatory with 10 out of the 13 patients having antibody levels 2 standard deviations above the mean value in the controls. At 1/80 dilution, in

addition to the above five antigens, *M. tuberculosis*-H37Rv and *M. tuberculosis*-7219 also showed significantly higher level of antibodies in the *M. kansasii* patients. Again *M. scrofulaceum* was the most discriminatory, with 12 of 13 patients showing antibody levels greater than 2 standard deviations above the mean value in the controls.

In individuals with disease due to *M. kansasii*, the discrimination between patients and controls was higher with *M. scrofulaceum* antigen than with *M. kansasii* antigen. This could be because the immune system is primed first very early in childhood to *M. scrofulaceum* antigens. Later during infection with a cross-reacting mycobacterium like *M. kansasii*, a higher level of antibodies would be formed against *M. scrofulaceum* than against *M. kansasii* antigens. The sera from the *M. kansasii* patients will be absorbed with *M. scrofulaceum* antigens and ELISA will be repeated using the absorbed sera to examine this.

(started: 1988; completed: 1988)

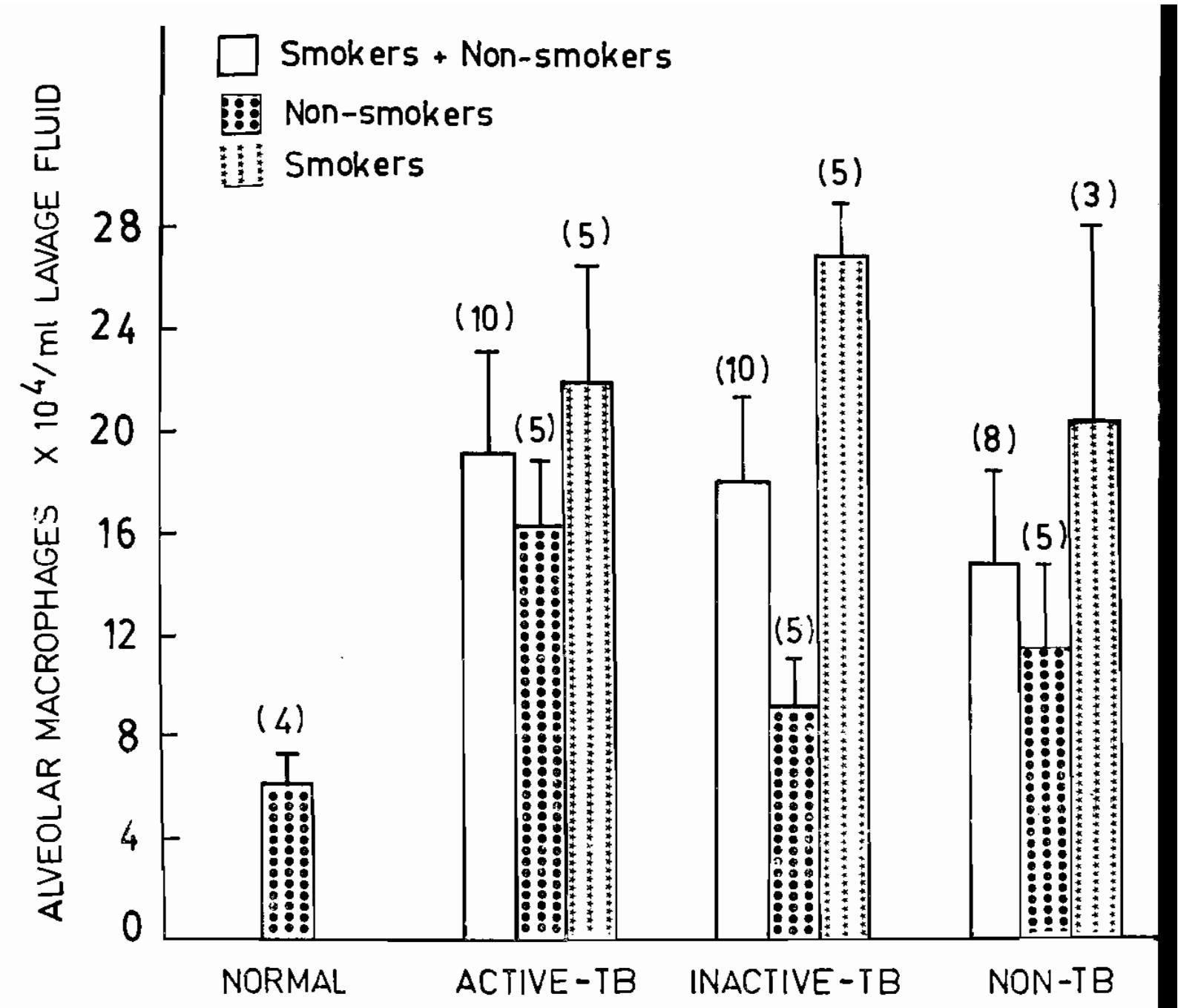
**Hydrogen peroxide release by alveolar macrophages in tuberculosis**

Bronchoalveolar lavage (BAL) is an invaluable means of evaluating immunological responses at the site of inflammation. The cellular composition of lavage in patients with pulmonary tuberculosis has been studied earlier (see 1987 annual report). Further, a series of experiments was designed to study the functions of alveolar macrophages in order to understand the basic immunological mechanisms in tuberculosis.

At first, the total and HLA-DR positive macrophages were enumerated in patients with (a) active pulmonary tuberculosis (8), (b) inactive (treated) tuberculosis (9), (c) non-tuberculous lung disease (6), and (d) normal healthy individuals (5).

The methodology employed for estimating total and HLA-DR positive alveolar macrophages was as follows. Smears of the alveolar cells were stained using modified Wright-Giemsa stain (Diff-Quick, American Scientific Products, U.S.A). Based on the percentage of macrophages and the total number of live cells, the total number of macrophages was calculated and expressed as $10^4$ macrophages per ml of lavage fluid. For estimating HLA-DR positive alveolar macrophages, an assay described by Whistler et al. (J. Immunology 1985, *135*, 172) was employed. In brief, alveolar cells were incubated for 1 hour at 4°C with 1:100 dilution of OKIa1 monoclonal antibody and after washing the cell suspension in RPMI-1640 containing 5% foetal calf serum, further incubation for 1 hour at 37°C with 1:4 dilution of rabbit complement was carried out. The percentage of cells susceptible to OKIa1 and complement treatment was assessed using trypan blue dye exclusion method and expressed as percent cytotoxicity.

The data for total alveolar macrophages are presented in the figure on page 48 The total alveolar macrophage populations in patients with active TB, inactive TB, and non-tuberculous lung diseases were significantly higher than in normal healthy individuals (normal vs. active TB:$p<0.01$, normal vs. inactive TB:$p<0.01$ and normal vs.


Smokers + Non-smokers
Non-smokers
Smokers
ALVEOLAR MACROPHAGES X $10^4$/ml LAVAGE FLUID
0
4
8
12
16
20
24
28
(4)
(10)
(5)
(5)
(10)
(5)
(5)
(8)
(5)
(3)
NORMAL
ACTIVE-TB
INACTIVE-TB
NON-TB

non-TB:$p < 0.05$), but the HLA-DR positive macrophage populations were similar in the groups studied (see table below), taking smokers and non-smokers together. However, an increased number of HLA-DR positive alveolar macrophages was found

| | Mean %** ± SE (M)** | | |
|---|---|---|---|
| | Smokers | Non-smokers | Total |
| (a) Active TB | 54.1±13.0 (4)+ | 38.9±11.3 (4) | 46.5±8.5 (8) |
| (b) Inactive TB | 47.7±2.2 (5) | 44.4±6.0 (4) | 46.2±3.9 (9) |
| (c) Non-TB | 65.0±2.6 (4) | 47.0* (2) | 59.0±5.7 (6) |
| (d) Normal | — | 49.1±5.6 (5) | 49.1±5.6 (5) |

**Percent alveolar macrophages susceptible to OKIal monoclonal and rabbit complement.

+The number of subjects is given in parentheses

*Standard error of the mean (SE) not presented since based on only 2 individuals.

in smokers compared to non-smokers. A slightly decreased level of HLA-DR positive macrophages was observed in the non-smoker group of active tuberculosis patients compared to normal subjects. On the other hand, the HLA-DR positive macrophages in the non-smoker group of inactive TB and non-TB patient groups were similar to that of normal subjects.

As the next step, studies were undertaken on the production of $H_2O_2$ by the alveolar macrophages spontaneously and when stimulated with phorbol myristate acetate (PMA). It was found that there was no difference between the spontaneous and PMA-induced $H_2O_2$ release in any of the groups among the non-smokers. However, $H_2O_2$ release was elevated in the smokers group. Hence, increased production of $H_2O_2$ by alveolar macrophages does not appear to be specific for tuberculosis. If $H_2O_2$ is considered as one of the potential anti-microbial agents that kill intracellular organisms, one would expect a decreased production of $H_2O_2$ by macrophages in patients with tuberculosis. But, since this study has shown no significant differences in the production of $H_2O_2$ between normal individuals and patients with active or inactive TB and with non-tuberculous lung diseases, this, hypothesis does not appear to be substantiated.

(started: 1988; completed: 1988)

## Antibody response to mycobacterial antigen in BCG vaccinated subjects

While a lot of studies have been done in the past on the skin test conversion after BCG vaccination, there are very few reports with regard to the antibody response in vaccinated individuals. Though the role of antibodies in protective immunity in tuberculosis has always been doubted, the immunogenicity of various components of the bacilli in the human host is well established. Hence, an attempt was made to measure the antibodies in circulation after vaccination, both quantitatively (ELISA) as well as qualitatively (IMMUNOBLOT).

Blood samples were collected from subjects from Tiruvallur area immediately before and around 8 weeks after BCG vaccination.

The circulating level of anti-mycobacterial antibodies as measured by ELISA in subjects showed a decrease in their post-vaccinated samples as compared to their prevaccinated samples. This decrease was observed irrespective of the three different crude extracts (PPD, BCG and H37Rv) against which the antibody was measured. This is quite surprising for the following reasons. It is well established that the antibodies that are produced in infection cross react extensively with various mycobacterial antigens *in vitro*, thus demonstrating that among mycobacteria, they share a large number of common antigenic determinants. All the subjects who were included here had prior exposure to atypical mycobacteria as revealed by their skin test positivity (sensitization) to PPD-B and basal level of antibodies as measured by ELISA to various mycobacterial antigens. Hence it is to be expected that the vaccinated dose of BCG should have acted like a booster, thus increasing the antibody levels because of the sharing of common antigenic determinants. Though the skin test to PPD-S became positive after BCG vaccination, the antibody titre decreased. The reason for this is not known. Analysis of antibody profile by immunoblot ( see figures on pages 51 and 52 ) also did not reveal any qualitative variation between pre- and post-vaccinated samples.

(started: 1987; completed: 1988)

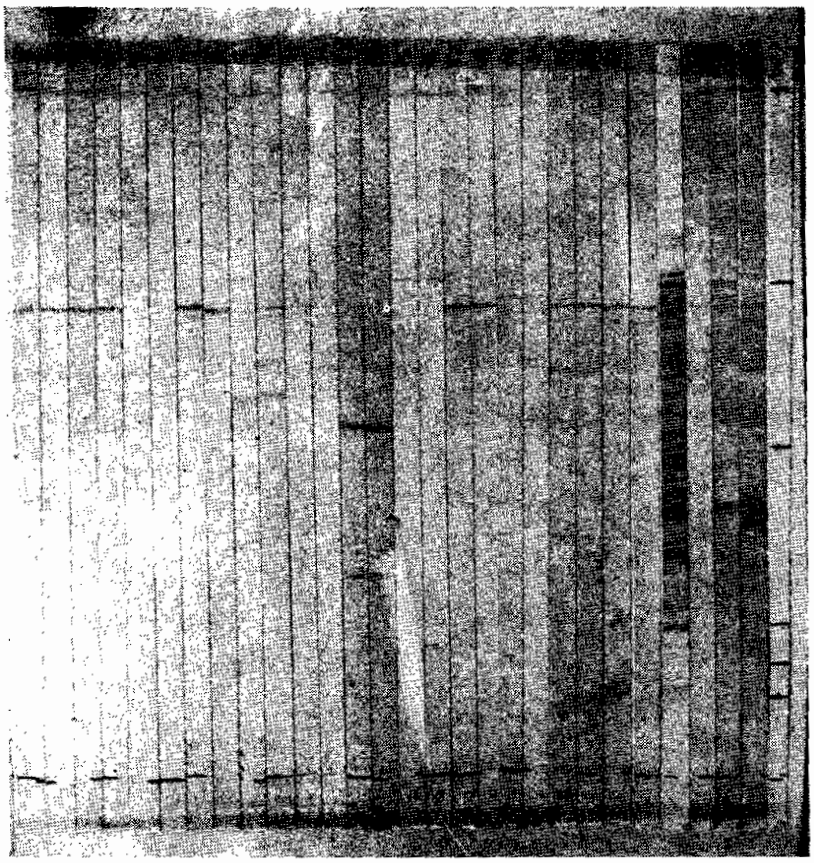
## Antigen-5 in immunodiagnosis of pulmonary and extra pulmonary tuberculosis

Detection of Antigen-5 in broncho alveolar lavage and CSF from patients with tuberculosis, employing competitive inhibition ELISA assay, had not proved as highly sensitive as it was with bactec culture media. The reasons for diminished sensitivity may be: (1) the minute quantities of Antigen-5 available in the specimens; (2) interference due to the presence of antibody.

Preparation of Antigen-5 in sufficient quantity and purity poses many practical problems and hence further work on this antigen has been stopped. Alternative antigens for diagnostic purposes are now being explored.

(started: 1986; completed: 1988)

## ANTIGEN BOUND TO NCP - BCG SONICATE



**Legend**

Odd numbered strips from 1 to 24—Pre vaccinated serum sample
Even numbered strips from 1 to 24—Post vaccinated serum sample

25 — BCG

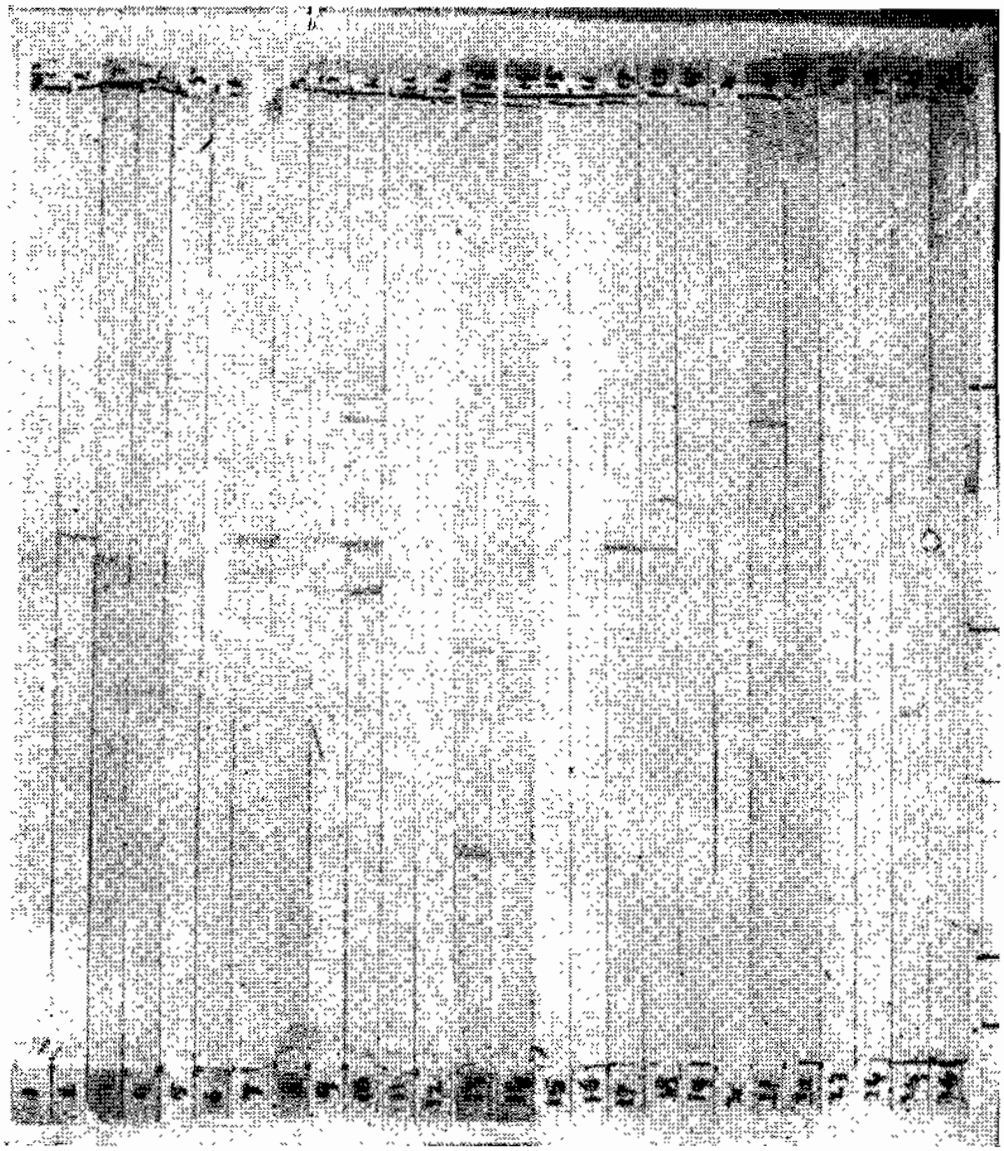
26 — –ve control

27 — +ve control

28 — LL serum

29 — Mor. wt. markers

## ANTIGEN BOUND TO NCP - $H_{37}R_v$ SOL. EXTRACT



**Legend**

Odd numbered strips—Pre vaccinated serum sample

Even numbered strips—Post vaccinated serum sample

**Concanavalin-A-induced suppression of mitogenic responses of lymphocytes in pulmonary tuberculosis**

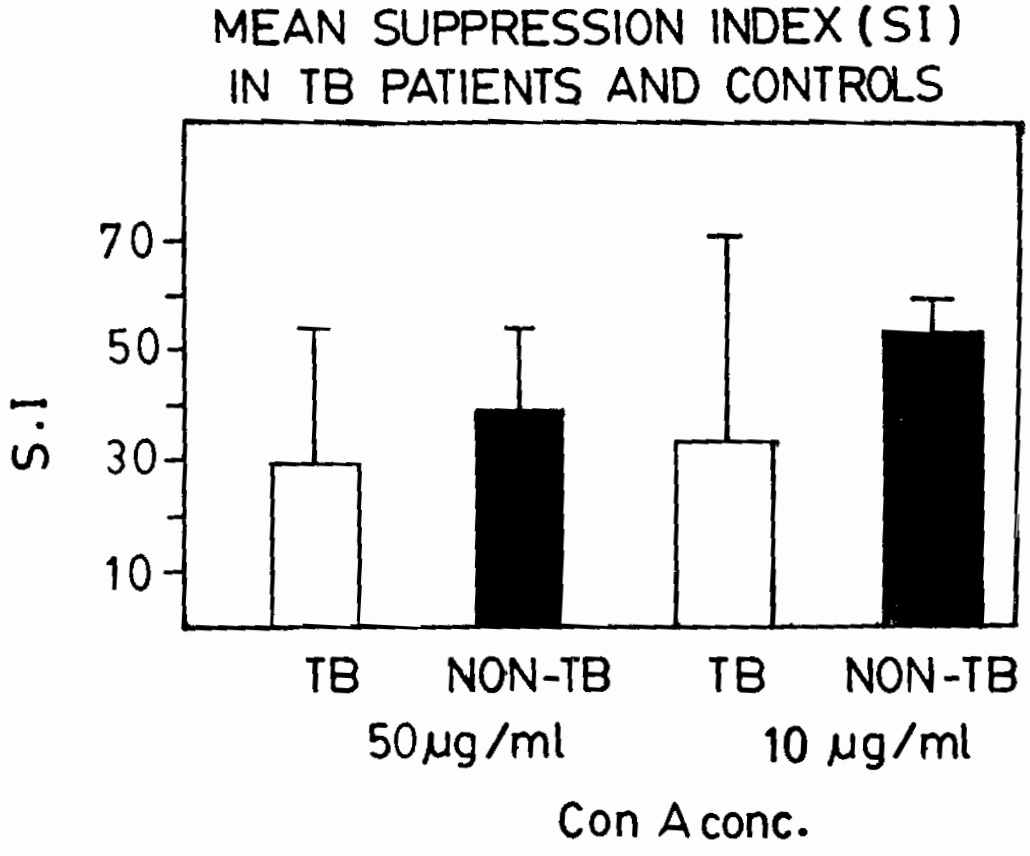
Polyclonal lymphocyte activation with Concanavalin-A (Con-A) induces suppressor activity in normal individuals. In chronic diseases like leprosy and filariasis, Con-A-induced suppression was found to be defective. Con-A-induced suppression was studied in 14 pulmonary tuberculosis patients and 6 normal healthy individuals (controls). Lymphocytes were purified from peripheral blood and stimulated with 10$\mu$g/ml Con-A. After 48-hour incubation at 37°C, lymphocytes were washed and treated with antimitotic agent mitomycin-C. Mitomycin-C-treated cells were co-cultured with non-stimulated lymphocytes of the same individual. The co-cultures were stimulated with 10 or 50$\mu$g/ml of Con-A, and 3H-Thymidine uptake in these cultures was measured. The suppression of response was expressed as suppression index (Sup. I), using the formula:

$$\text{Sup. I.} = \left[1 - \frac{(\text{CPM in cultures with Con-A treated cells})}{(\text{CPM in co-cultures with control cells})}\right] \times 100$$

The table below shows the Sup. I. for all individual subjects. The results are also shown graphically on page 54.

| Suppression Index among | | | |
|---|---|---|---|
| Controls | | PTB patients | |
| 50$\mu$g/ml | 10$\mu$g/ml | 50$\mu$g/ml | 10$\mu$g/ml |
| 64.1 | 56.8 | —09.4 | —16.6 |
| 42.2 | 58.9 | 47.2 | 50.5 |
| 33.8 | 48.9 | 16.7 | 38.2 |
| 35.5 | 42.1 | —22.5 | —53.2 |
| 20.8 | 53.3 | 29.3 | 27.0 |
| 31.6 | 45.8 | 48.0 | 45.3 |
| | | 30.1 | 15.4 |
| | | 58.6 | 57.2 |
| | | 59.6 | 59.5 |
| | | 42.0 | —18.8 |
| | | 25.8 | 62.4 |
| | | 25.1 | 48.9 |
| | | 13.4 | 57.1 |
| | | 41.3 | 72.2 |

From the table and figure, it can be seen that the Sup. I. varied from 20.8 to 64.1 for 50$\mu$g/ml concentration and from 42.1 to 58.9 for 10$\mu$g/ml concentration of Con-A, among the normals. Among the 14 pulmonary tuberculosis patients


MEAN SUPPRESSION INDEX (SI)
IN TB PATIENTS AND CONTROLS
S.I
70
50
30
10
TB
NON-TB
TB
NON-TB
50µg/ml
10 µg/ml
Con A conc.

tested, 10 patients and 5 patients, respectively, showed a similar range of variation with the 50$\mu$g/ml and 10$\mu$g/ml concentration; in 3 patients, the Sup. I. was lower than the lower limit in controls, both for 50$\mu$g/ml and 10$\mu$g/ml. At the 50$\mu$g/ml concentration, the mean Sup.I. among patients was not statistically different from that among controls. But with the 10$\mu$g/ml concentration, using a one-sided test, the Sup. I. in the patients was significantly lower ($p<0.05$) than that in controls. However, based on the results presented here, it is not possible to conclude that there is definite evidence of defective Con-A-induced suppression in tuberculous patients. It is possible that the immunosuppression that is seen in tuberculous patients may be due to other immuno-regulatory mechanisms.

(started: 1988; completed: 1988)

**Comparison of filtration technique and the conventional centrifugation method for the isolation of *M. tuberculosis* from urine of pulmonary tuberculosis patients**

Conventional centrifugation method has been followed for processing urine samples to isolate *M. tuberculosis* by culture. Since tubercle bacilli tend to float on the surface of the sample, there is a chance of failing to recover the tubercle bacilli from urine samples using the conventional centrifugation method. So, in order to recover more number of positive cultures, a filtration technique was applied on urine samples collected from pulmonary tuberculosis patients.

Four hundred and fifty-four early morning urine samples collected from 158 patients were studied. Of the 126 patients for whom results were available, only 2 patients were positive for culture of *M. tuberculosis*. One was culture positive by the centrifugation method and the other by the filtration method, indicating that the filtration method has no additional advantages. Hence, the intake to the study was stoppped.

(started: 1988; completed: 1988).

## STUDIES IN PROGRESS

**Adenosine deaminase (ADA) activity in CSF of patients with tuberculous meningitis (TBM)**

Adenosine deaminase (EC 3.5.4.4), an enzyme which catalyses the deamination of adenosine, forming ionosine and ammonia, has been reported by Ribera et al. (1987) to be of use as a simple diagnostic method for tuberculous meningitis. Hence a study was initiated to estimate the ADA activity in CSF of children with TBM (see 1987 annual report). The method described by Giusti (in Methods in Enzymatic Analysis: 2nd Edition (1974), Verlag Chemic, H.U. Bergmeyer Ed) was standardised. The coefficient of variation of pooled CSF with a mean ADA value of 5.76 was found to be 8% in seven consecutive assays. One hundred and sixty CSF samples collected

from 85 patients with TBM are being assayed in chronological order. Since the lysozyme level is an index of macrophage function, the lysozyme levels also are being estimated. The experiment is in progress.

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

**Evaluation of bioluminescence assay for the estimation of viable tubercle bacilli**

The bioluminescence assay of Adenosine triphosphate (ATP) is a sensitive and rapid technique, which can be used for the estimation of viable bacilli in cultures. It was proposed to evaluate this ATP assay and compare it with the standard calibrated loop method of viable counting.

In cultures of typical strains of *M. tuberculosis* stored at 4°C for less than 6 months, estimation of viable count will be done both by the conventional viable count method and by bioluminescence assay using luciferin-luciferase system and by measuring the ATP level using Biocounter. As 14 cultures (31%) were not viable, it has been decided to screen 100 more cultures and then compare the two methods. After standardization of the test procedures, the method will be applied to study the viability of *M. leprae*.

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

**Fluorescein Diacetate/Ethidium Bromide vital staining of tubercle bacilli**

Determination of viability of tubercle bacilli from smear examination without having to wait for culture is of particular value for patients on treatment, specially those on SCC, who go through a smear-positive culture-negative phase before conversion. It would help decisions regarding change or extension of treatment regimens. Fluorescein Diacetate/Ethidium Bromide (FDA/EB) staining, which is a vital staining technique, has been found useful to differentiate between live and dead *M. leprae* bacilli by a few workers (Kvach J.T., Munguia G & Strand S.H., International Journal of Leprosy, 1984, *52*, 176; Harshon K.V. et al, International Journal of Leprosy, 1987, *55*, 2). The same staining method was found to work well with culture suspension of *M. tuberculosis* and other mycobacterial species. It was therefore proposed to apply the technique for sputum smear examination in order to determine the viability of tubercle bacilli excreted by patients.

However, the method as such, failed to work with sputum smears, perhaps because of the mucoid nature of the samples. Among modifications tried, the treatment of sputum deposit with sputolysin was found to give fairly reliable results. A method has now been standardised to examine the sputum deposit by the FDA/EB method. It is proposed to examine smear positive sputum samples by this method to estimate the proportion of viable bacilli present and compare the same with the culture result.

(started: 1988; expected year of completion: 1989).

## Characterisation and purification of antigenic components of *M. tuberculosis*

Since Antigen-5 was not useful as a diagnostic agent, it was found necessary to develop alternative antigens for diagnostic purposes. As a first step, a mycobacterial antigen, using H37RV strain of *M. tuberculosis*, was developed. The methodology is described below:

*M. tuberculosis*, H37RV strain was grown in Sauton's broth at 37° C. At the end of 8 weeks, the rigid portion of the culture (which corresponds to the culture filtrate antigen) was removed by centrifugation; the bacilli were collected, subjected to ultra-sonication, and separated into various antigenic fractions by differential centrifugation as described below. At first the bacillary suspension was centrifuged at 5000 rpm for 30 minutes to separate the pellet from the supernatant. The pellet was sonicated for 10 minutes and centrifuged at 5000 rpm for a further 20 minutes. The resulting supernatant was then centrifuged at 15,000 rpm for 30 minutes to separate out the cell wall from the supernatant. The resulting supernatant was finally centrifuged at 40,000 rpm for 60 minutes to yield a supernatant containing the soluble extract.

The figure in page 58 shows the distribution of antigens in different preparations (blotted with rabbit antiserum). Of all the preparations, the culture filtrate antigens show the maximum number of bands. Hence the culture filtrate was prepared in large quantities and used for all further studies.

A comparison of the antigen recognition pattern of hyper-immunized rabbit serum and human serum obtained from patients with pulmonary tuberculosis is shown in the figure on page 59. While there are about 25 antigens of the culture filtrate recognised by the rabbit immune serum, only 11 are relevant in the humans with respect to antibody synthesis. Attempts have been made to isolate these relevant antigens by affinity chromatography using the following procedure.

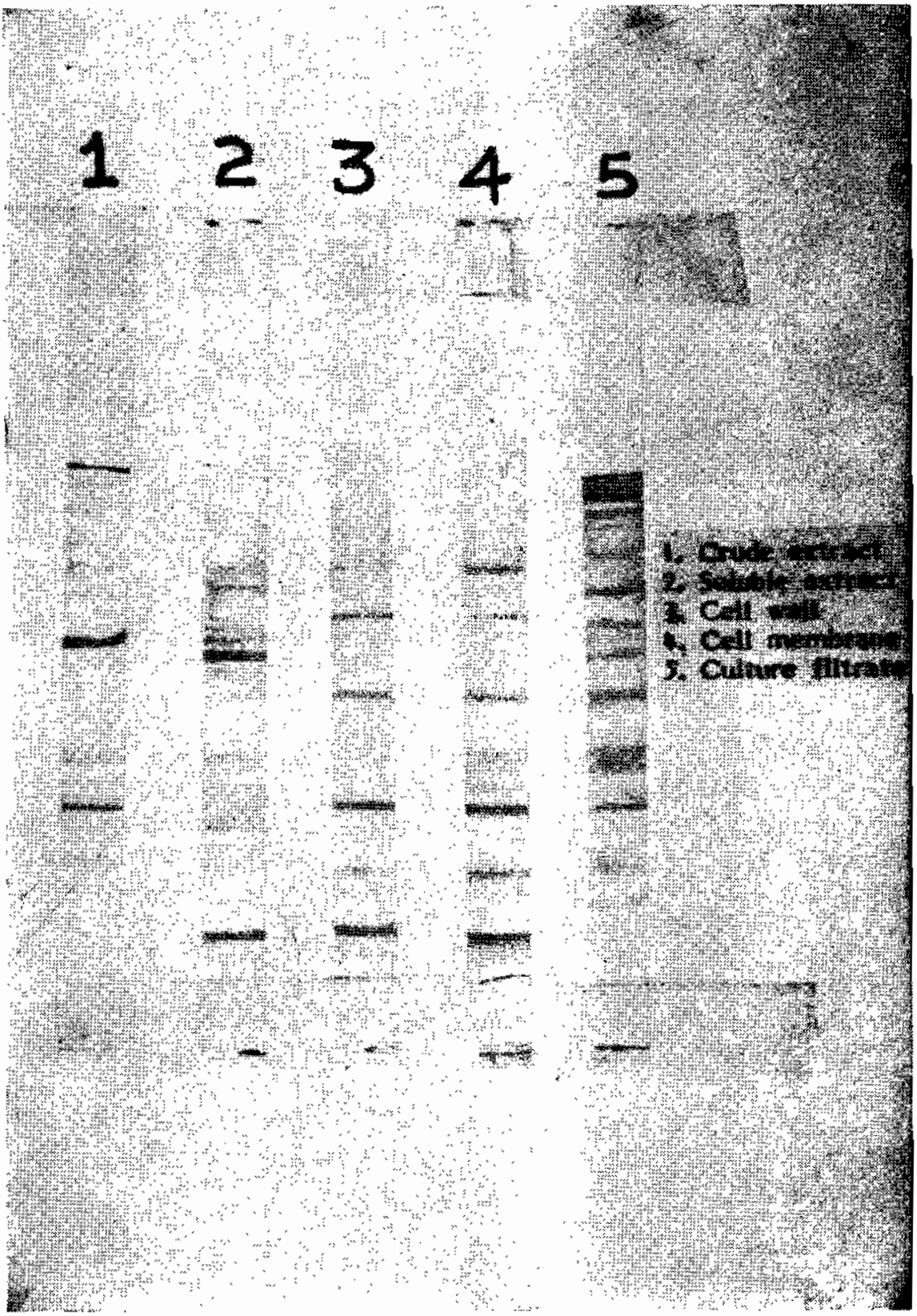
IgG fraction of a large pool of serum from tuberculous patients was prepared by protein-A sepharose affinity chromatography. The IgG thus obtained was again passed through a column of sepharose-4B coupled with culture filtrate antigen. The relevant antibody IgG was eluted with 7M Guanidine Hydrochloride. The affinity purified antibodies thus obtained were hooked up to sepharose-4B beads. The total culture filtrate antigen was absorbed to and eluted from this antibody column.

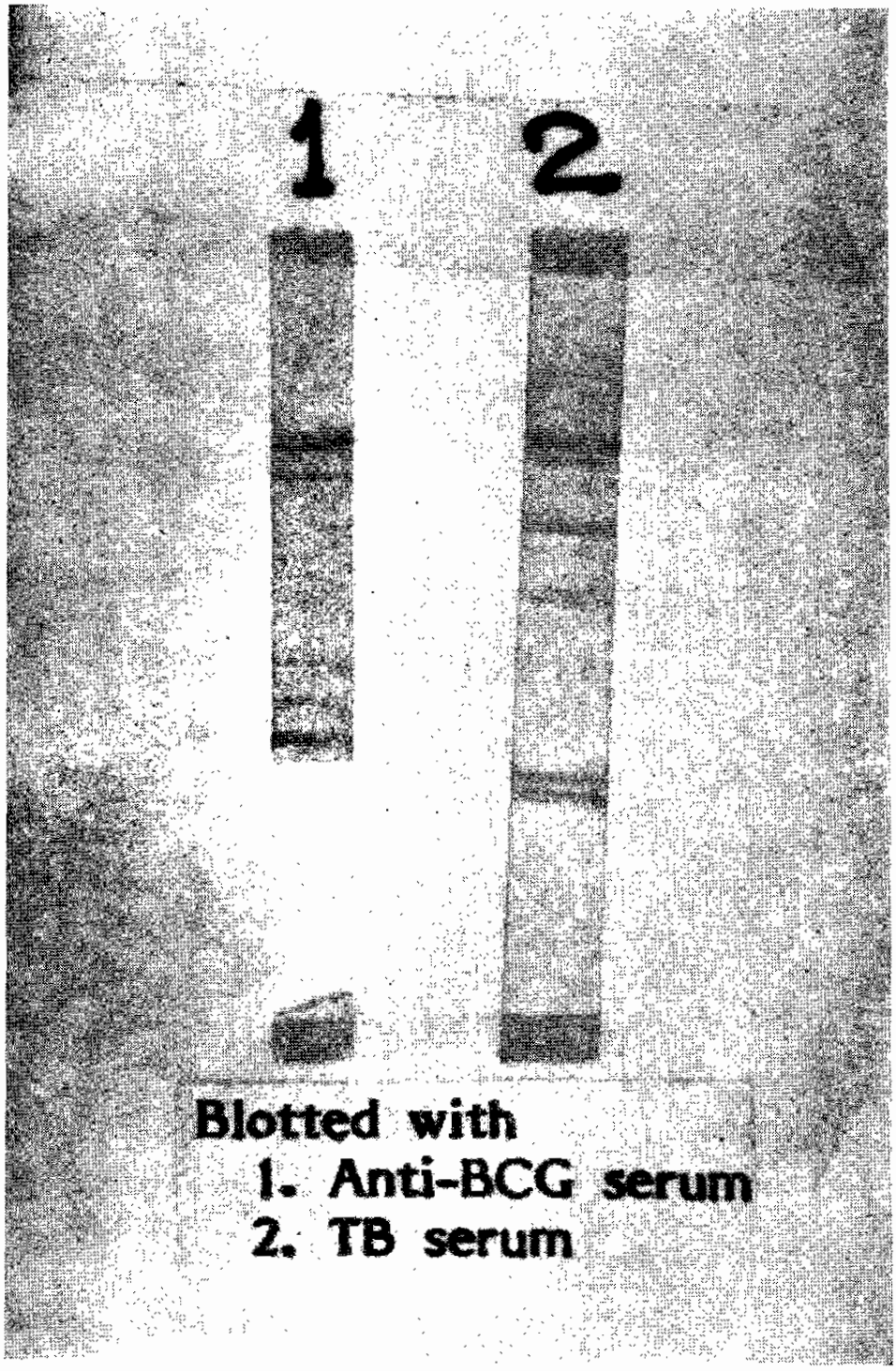
Further work is in progress.

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

## Production of monoclonal antibodies against antigen of *M. tuberculosis*

As a first step towards obtaining relevant antigens for immuno diagnosis of tuberculosis, it was proposed to raise monoclonal antibodies against various antigenic components of *M. tuberculosis* H37Rv. Monoclonal antibodies were raised against


1 2 3 4 5
1. Crude extract
2. Soluble extract
3. Cell wall
4. Cell membrane
5. Culture filtrate

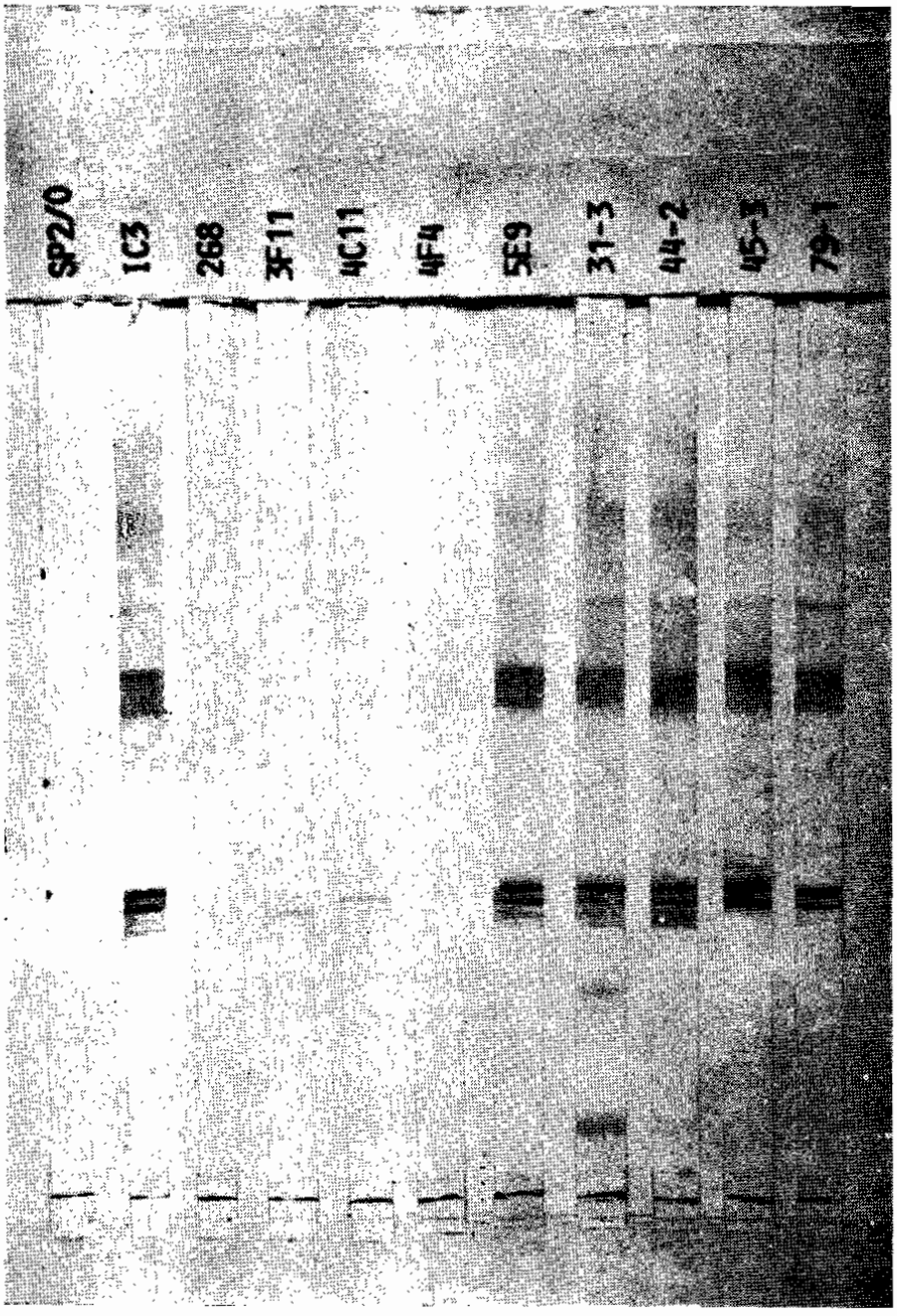

1
2
Blotted with
1. Anti-BCG serum
2. TB serum

culture filtrate antigens of *M. tuberculosis* H37Rv. Balb/C mice were immunized with 70 $\mu$g antigen in incomplete Freund's adjuvant by intraperitoneal route. Sera from immunized mice were tested for the antimycobacterial antibodies using enzyme linked immunosorbent assay (ELISA). Mice showing good antibody response were rested for about a month and boosted by intravenous injection of 100 $\mu$g antigen. On the third day, the mice were sacrificed and spleen cells were fused with mouse myeloma cells (Sp2/0). Fused cells were seeded in 300 wells and maintained at 37°C in 7% $CO_2$ atmosphere. Wells showing colony of hybrid cells were noted and supernatant from these wells was tested for antimycobacterial antibody. The antibody producer colonies were cloned further till purified clones were obtained.

Ten fusions were carried out which resulted in 12 monoclonal antibodies (1C3, 2G8, 3F11, 4C11, 4F4, 5E9, 31-2, 32-1, 44-3, 45-2, 63-2, 79). The table below shows the reactivities of these antibodies with heterologous antigens.

| Monoclonal antibody | M. tuberculosis culture filtrate | Phosphoryl choline-BSA | E. Coli sonicate |
|---|---|---|---|
| 1C3 | + | — | — |
| 2G8 | + | — | — |
| 3F11 | + | — | — |
| 4C11 | + | — | — |
| 4F4 | + | — | — |
| 5E9 | + | — | — |
| 31-2 | + | +/- | — |
| 32-1 | + | — | — |
| 44-3 | + | — | — |
| 45-2 | + | — | — |
| 63-2 | + | — | — |
| 79 | + | +/- | — |

The figure on page 61 shows the reactivity of these monoclonal antibodies against *M. tuberculosis* culture filtrate in Western blots. It was found that all antibodies except 31-2 and 79 showed almost identical reactivity. Also, they bound bands in molecular weight regions 29-33kD and 65kD and a few more minor bands


SP2/0
1C3
268
3F11
4C11
4F4
5E9
31-3
44-2
45-3
79-1

Antibody 31-2 bound two bands, one in 17-18kD and one in 100-111kD region. The cross reaction of this antibody in respect of non-tuberculous mycobacteria is being tested. More fusions will be carried out with alternate immunization strategy for obtaining antibodies that react with antigens that are less immunodominant.

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

### Consistency of bacteriocin typing of *M. tuberculosis* cultures

Screening of 100 rapidly growing non-tuberculous mycobacteria from South Indian patients for bacteriocin production yielded a set of 9 indicator strains (see 1987 annual report). These could classify the typical strains of *M. tuberculosis* cultures into 16 types. More than half of the typical cultures belonged to type 1, which could be divided further into 5 subgroups.

It was proposed to screen pretreatment cultures of some patients to check the reproducibility and consistency of this typing. Twentyfive pretreatment cultures belonging to 10 patients were screened for bacteriocin production. Pretreatment cultures of 5 patients showed consistent results, while those of two patients, although belonging to the same type, varied when subgrouped. Pretreatment cultures of the remaining 3 patients showed inconsistent results, i.e., they were of different types. It is planned to investigate 2 pretreatment cultures from each of 50 patients, in order to assess the usefulness of bacteriocin typing as an additional tool for assessing the epidemiological profile of mycobacteria.

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

### Effect of administration of rifampicin on the adrenocortical functions in patients with pulmonary tuberculosis

Investigations, recently concluded at our Centre, have shown that the diurnal rhythm of cortisol secretion is disturbed in patients with pulmonary tuberculosis; further, a majority of these patients were shown to have adrenocortical insufficiency (or lack of adrenal reserve) on the basis of the tetrecosactrim (synacthen) stimulation test (see 1987 annual report). Rifampicin is a known inducer of the hepatic microsomal enzyme system and has been shown to induce the metabolism of a number of steroid drugs. An investigation was therefore undertaken to study the effect of different rhythms of rifampicin administration (daily and twice-weekly) on the adrenocortical function in patients with pulmonary tuberculosis, and compare the findings with those in patients on treatment with a daily regimen that did not contain rifampicin. The adrenocortical function in these patients is being assessed by studying the diurnal variation of cortisol levels in saliva and by employing the synacthen stimulation test (plasma and saliva).

**Aims:** (1) To study the diurnal variation of cortisol levels in saliva on admission and after 2 months of treatment, and at the end of chemotherapy;

(2) To study the response to synacthen on admission and at 1,2 and 4 weeks after the start of treatment in patients receiving rifampicin daily or twice-weekly and in those receiving a daily non-rifampicin regimen.

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

1. Daily rifampicin regimen ($R_7$)—$2EHRZ_7/6EH_7$
2. Twice-weekly rifampicin regimen ($R_2$)—(a) $2EHRZ_2/4EHR_2$ (b) $2HRZ_2/4HR_2$
3. Daily non-rifampicin regimen ($NR_7$)—1 $EHZ_7/11EH_7$

The diurnal variation of cortisol production, as assessed by salivary levels of cortisol at 2-hourly intervals from 8 a.m. to 8 p.m., is being determined on admission and at 2 months after the start of treatment.

The synacthen stimulation test, based on increase in plasma and salivary levels of cortisol at ½ hr. and 1 hr. after an intramuscular dose of synacthen 0.25 mg, is being performed on the day of admission and at 1,2 and 4 weeks after the start of chemotherapy.

A total of 61 patients have been admitted to the study; of these, 18 have been admitted to the $R_7$ regimen, 22 to the $R_2$ regimen and 21 to the $NR_7$ regimen. The intake has been completed and the assays are in progress.

(started: 1988; expected year of completion: 1989).

**Pharmacokinetics of anti-tuberculosis drugs in patients with renal failure**

Single-dose pharmacokinetics of isoniazid and rifampicin are being studied in patients with different grades of renal failure in an attempt to devise suitable drug dosages for these patients (see 1987 annual report). On the basis of plasma concentrations at 1,2,3,6,8 and 24 hours and the concentrations of drugs and their metabolites excreted in urine over a 24-hour period after drug administration, several pharmacokinetic variables such as the mean peak concentration, exposure (AUC), plasma half-life, renal clearance and the proportions of the drugs excreted in urine are being calculated. Further, several indices of renal failure such as blood urea, nitrogen, plasma creatinine clearance and Fractional Excretion of Sodium (FENa) and the Renal Failure Index (RFI) are being calculated.

The study is in progress. So far, 34 patients with renal failure (15 severe, 15 moderate and 4 mild) comprising of 18 slow acetylators and 16 rapid acetylators of INAH along with 13 control subjects (8 slow and 5 rapid) have been admitted to the study.

(started: 1987; expected year of completion: 1989).

**Development of a reproducible assay for studying the mycobacterial activity of human monocyte derived macrophages**

*M. tuberculosis* is a facultative intra-cellular organism. There is much indirect evidence to show that macrophages are responsible for the destruction of pathogenic mycobacteria. There are a number of reports on macrophage-*M. tuberculosis* interactions in murine and human systems under *in vitro* conditions. However, the results are conflicting and inconclusive. An assay for studying the mycobactericidal activity of human macrophages was designed with an aim to understand the cell-mediated immune responses in tuberculosis.

**Preparation of macrophages:** Peripheral blood monocytes were maintained in culture for seven days; a portion of them were activated with autologous lymphokines generated by stimulating the lymphocytes with 10 mg/l PPD. These monocytes matured into macrophages by day 7.

**Phagocytic challenge:** *M. tuberculosis* was maintained in Dubos broth culture. The peak log phase was achieved between 5 and 7 days of culture. The bacteria were suspended in tissue culture medium and added to the macrophage cultures at a ratio of approximately 2 bacteria per macrophage for phagocytosis. The viability of these organisms was determined immediately after phagocytosis (T0) and at 48h (T48).

Since the assay is vulnerable for large variations and contamination, the reproducibility of the assay was tested by setting up the experiment on each individual's peripheral blood monocytes on 2 or 4 different occasions, at weekly intervals. In all, 3 individuals were investigated; 4 replicates were set up for each assay.

**Viability of *M. tuberculosis* after phagocytosis:** The T0 and T48 cultures were terminated by scraping the macrophages and the lysate was diluted to 1:10, 1:100 and 1:1000 in distilled water; 50 $\mu$l of each dilution was plated on to Dubos agar plates and CFUs were counted after three weeks.

**Results:** The following table gives the break up of the culture results.

| Culture result | No. | % |
|---|---|---|
| Contamination | 66 | *24* |
| No growth | 20 | *7* |
| Less than 300 colonies | 75 | *27* |
| 300 colonies or more | 116 | *42* |
| Total | 277 | *100* |

The degree of contamination and replicate variations were within acceptable limits.

The mean and S.D. of the CFU of *M. tuberculosis* at T0 (immediately after phagocytosis) and T48 (unstimulated as well as stimulated with autologous lymphokine) are represented in the table below.

| | Mean ± S.D. of CFU | | |
|---|---|---|---|
| Subject | T0 | Unstimulated T48 | Stimulated T48 |
| P1 | 66 ± 11 | 145 ± 70 | 195 ± 35 |
| P2 | 48 | 219 ± 27 | 239 ± 25 |
| P3 | 59 | 154 ± 35 | 198 ± 5 |
| P4 | 57 ± 5 | 162 ± 8 | 173 ± 47 |
| S3 | 51 ± 13 | 242 ± 16 | 226 ± 37 |
| S4 | cont. | 167 ± 28 | 264 ± 9 |
| V3 | 52 ± 6 | 145 ± 28 | 262 ± 29 |
| V4 | cont. | 182 ± 18 | 165 ± 41 |

The number of viable bacteria on each occasion as shown by the T0 CFUs appears similar to T48 and all the individuals' macrophages have shown an enhanced growth in the range 2.2 to 4.7 fold. The expected growth is 4-fold (generation time of *M. tuberculosis* is about 20h). However, there was no difference in the CFUs between stimulated and unstimulated macrophages.

Under the present circumstances, it appears that human monocyte derived macrophages are unable to check the multiplication of *M. tuberculosis* even though they have been shown to produce normal amounts of the microbicidal agent hydrogen peroxide. This failure on the part of the macrophage may be due to the fact that human beings are susceptible for tuberculosis, or because peripheral blood monocyte may be a better substitute; more purified lymphokines could be used for stimulation in place of stimulated lymphocyte culture supernatant. These aspects will be investigated in future studies.

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

**Analysis of immune complexes from tuberculous sera**

In our earlier studies (see 1987 annual report), 3 protein bands could be observed in the Polyethylene Glycol (PEG) precipitate obtained from pooled

tuberculosis sera which were found absent in unpooled normal sera. To ascertain the antigenicity of these precipitates, it was necessary to do immuno-blotting.

Immune complexes were precipitated by the addition of PEG from *pooled* sera obtained from 65 patients with tuberculosis and 65 control subjects. When the precipitates were immuno-blotted with rabbit hyper-immune antiserum, it was found necessary that the precipitates should be further purified to detect small quantities of antigen(s).

At present, attempts are being made to further purify the PEG precipitate in the following ways:-

1 Isolation of complexes by adsorption to heat-killed formalized staphylococcus I aureus-A (Staph-A) Cowan-I strain—by their affinity to Protein-A.

2 Isolation by absorption to coated anti-C3d Staph-A—by their bound C3.

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

**Development of DNA probes for *M. tuberculosis***

A study is being undertaken to develop a technique for the detection of mycobacteria in clinical specimens where conventional detection is difficult. One approach that is being attempted is to develop DNA probes that are specific for *M. tuberculosis*. During the first year, experiments directed at obtaining such a probe were the primary focus of our efforts. In brief, lambda gT 11 library consisting of EcoR1 fragments of genomic DNA isolated from *M. tuberculosis* has been obtained from WHO. This library will be screened for clones which hybridized to nick translated total genomic DNA from *M. tuberculosis* Such isolated clones will subsequently be evaluated for their specificity. The procedure for screening and nick translation has been standardized and further work is in progress.

Another approach is to clone BamHi fragments of *M. tuberculosis* in pBR322. Such cloned fragments will be analysed for locating repeat sequences, if any, as well as sequences that are specific to *M. tuberculosis*, by hybridization experiments. In this connection, the mycobacterial DNA has already been isolated. Overnight digestion with BamHi was carried out for complete digestion. The fragments were isolated on 1% agarose gel with ethidium bromide. Each of these fragments ranging from 8 kb to 160 bases were purified by freeze phenol method. The purified fragments were rerun on 1% agarose gel and the presence of DNA was confirmed. As expected, a ladder pattern was obtained from 8 kb to 160 bases. Further work is in progress to clone each of these fragments into pBR322.

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

## HLA studies in tuberculosis

The objective of this project is to use a combination of serological and DNA probes to analyse the genotype of a number of individuals to define whether there exists an association between any serological or DNA marker and the occurrence of tuberculosis. Patients who have been treated for any form of tuberculosis and whose parents or children have also been treated for tuberculosis of any form, are selected for the study. However, these families should have another contact without manifestation of the disease.

Continuous cell lines have been generated from the subjects identified for this study using Epstein Barr Viruses (EBV). This virus infects human B-lymphocytes and transforms them into continuous growing cell lines. One hundred and seven subjects from 22 families have been included in this study. DNA from all the 107 subjects have been prepared and stored under proper conditions. Though EBV transformation per se has been 100% successful, we could recover only 30 cultures and these have been stored in liquid nitrogen. The remaining cultures were lost due to contamination during the long period of *in vitro* cultures.

It is proposed to investigate about 50 such families. When all the families have been included, the DNA samples will be digested with Taq 1 enzyme, electrophoresed and Southern transferred and finally probed with a set of already available HLA class I gene probes.

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

## Histopathological classification of tuberculous lymph nodes

Histopathological studies of tuberculous lymph nodes indicate that it presents a pleomorphic picture like leprosy. Hence a study was undertaken to correlate the histological features with immunological, bacteriological and clinical parameters. It is expected that this approach would (a) help in defining the histological spectrum that exists in TB adenitis, which presents diagnostic difficulties; and (b) provide a basis for elucidating the host response in other forms of tuberculosis.

The first phase of the work will consist of defining the histology of TB adenitis using conventional methods, as well as immunochemical and histochemical techniques. This will be correlated with culture positivity for *M. tuberculosis*, tuberculin reaction and the clinical features of the individual. This part of the study will be based on existing clinical material. It is also proposed to undertake a prospective study, which will include functional studies of the biopsied lymph node in addition to the other criteria

A total of 362 biopsies received as part of the Madras and Madurai studies on tuberculous lymphadenitis during the years 1985-88 were reviewed. Of these, 148 were found to have evidence of tuberculosis and were examined in more detail

tuberculosis sera which were found absent in unpooled normal sera. To ascertain the antigenicity of these precipitates, it was necessary to do immuno-blotting.

Immune complexes were precipitated by the addition of PEG from *pooled* sera obtained from 65 patients with tuberculosis and 65 control subjects. When the precipitates were immuno-blotted with rabbit hyper-immune antiserum, it was found necessary that the precipitates should be further purified to detect small quantities of antigen(s).

At present, attempts are being made to further purify the PEG precipitate in the following ways:-

1. Isolation of complexes by adsorption to heat-killed formalized staphylococcus I aureus-A (Staph-A) Cowan-I strain—by their affinity to Protein-A.

2 Isolation by absorption to coated anti-C3d Staph-A—by their bound C3.

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

**Development of DNA probes for *M. tuberculosis***

A study is being undertaken to develop a technique for the detection of mycobacteria in clinical specimens where conventional detection is difficult. One approach that is being attempted is to develop DNA probes that are specific for *M. tuberculosis*. During the first year, experiments directed at obtaining such a probe were the primary focus of our efforts. In brief, lambda gT 11 library consisting of EcoR1 fragments of genomic DNA isolated from *M. tuberculosis* has been obtained from WHO. This library will be screened for clones which hybridized to nick translated total genomic DNA from *M. tuberculosis*. Such isolated clones will subsequently be evaluated for their specificity. The procedure for screening and nick translation has been standardized and further work is in progress.

Another approach is to clone BamHi fragments of *M. tuberculosis* in pBR322. Such cloned fragments will be analysed for locating repeat sequences, if any, as well as sequences that are specific to *M. tuberculosis*, by hybridization experiments. In this connection, the mycobacterial DNA has already been isolated. Overnight digestion with BamHi was carried out for complete digestion. The fragments were isolated on 1% agarose gel with ethidium bromide. Each of these fragments ranging from 8 kb to 160 bases were purified by freeze phenol method. The purified fragments were rerun on 1% agarose gel and the presence of DNA was confirmed. As expected, a ladder pattern was obtained from 8 kb to 160 bases. Further work is in progress to clone each of these fragments into pBR322.

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

## HLA studies in tuberculosis

The objective of this project is to use a combination of serological and DNA probes to analyse the genotype of a number of individuals to define whether there exists an association between any serological or DNA marker and the occurrence of tuberculosis. Patients who have been treated for any form of tuberculosis and whose parents or children have also been treated for tuberculosis of any form, are selected for the study. However, these families should have another contact without manifestation of the disease.

Continuous cell lines have been generated from the subjects identified for this study using Epstein Barr Viruses (EBV). This virus infects human B-lymphocytes and transforms them into continuous growing cell lines. One hundred and seven subjects from 22 families have been included in this study. DNA from all the 107 subjects have been prepared and stored under proper conditions. Though EBV transformation per se has been 100% successful, we could recover only 30 cultures and these have been stored in liquid nitrogen. The remaining cultures were lost due to contamination during the long period of *in vitro* cultures.

It is proposed to investigate about 50 such families. When all the families have been included, the DNA samples will be digested with Taq 1 enzyme, electrophoresed and Southern transferred and finally probed with a set of already available HLA class I gene probes.

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

## Histopathological classification of tuberculous lymph nodes

Histopathological studies of tuberculous lymph nodes indicate that it presents a pleomorphic picture like leprosy. Hence a study was undertaken to correlate the histological features with immunological, bacteriological and clinical parameters. It is expected that this approach would (a) help in defining the histological spectrum that exists in TB adenitis, which presents diagnostic difficulties; and (b) provide a basis for elucidating the host response in other forms of tuberculosis.

The first phase of the work will consist of defining the histology of TB adenitis using conventional methods, as well as immunochemical and histochemical techniques. This will be correlated with culture positivity for *M. tuberculosis*, tuberculin reaction and the clinical features of the individual. This part of the study will be based on existing clinical material. It is also proposed to undertake a prospective study, which will include functional studies of the biopsied lymph node in addition to the other criteria.

A total of 362 biopsies received as part of the Madras and Madurai studies on tuberculous lymphadenitis during the years 1985-88 were reviewed. Of these, 148 were found to have evidence of tuberculosis and were examined in more detail.

Depending on the extent, cell type and caseation, the granulomata were divided into 4 groups–namely reactive, hypo-reactive, non-reactive and hyper-cellular.

It was found that 94 lymph nodes were of the reactive type. Hyporeactive forms were found in 14 cases, non-reactive in 8 and hypercellular forms in 21 cases. Eleven lymph nodes which showed definite evidence of tuberculosis could not be classified as the sections were too small.

During the year, immunochemical techniques using polyclonal antisera on paraffin sections for mycobacterial and host antigens were standardized. Histochemical staining procedures for plasma cells and mast cells were also established.

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

# EPIDEMIOLOGICAL STUDIES

## STUDIES COMPLETED

### Short Course Chemotherapy for pulmonary tuberculosis under programme conditions

A study, in collaboration with the Institute for Research in Medical Statistics (Madras Chapter), was undertaken in 12 Primary Health Institutions in Chengalpattu District in Tamil Nadu, with the objective of obtaining information on two short-course chemotherapy regimens with respect to their feasibility, acceptability, toxicity and efficacy under programme conditions (see 1987 annual report).

Patients with bacteriologically confirmed tuberculosis, previously untreated or treated for at most 3 months, and aged at least 12 years were admitted to the study. If they were willing to attend the health centre twice a week for drug administration, they were offered a fully supervised twice-weekly regimen of 6 months' duration, namely, rifampicin (300 or 450 mg)*, isoniazid (400 or 650 mg)* and pyrazinamide (1.5 or 2 g)* for 2 months, followed by rifampicin and isoniazid in the same dosages for the next 4 months; a supplement of streptomycin (0.75 g) was given, where feasible. Patients who were unwilling to attend the health centre twice a week were offered a daily self-administered regimen of 6 months' duration with weekly attendance at the health centre for collecting drugs, the regimen being rifampicin (300 or 450 mg)*, isoniazid (300 mg) and pyrazinamide (1 or 1.5 g)* for 2 months, followed by rifampicin and isoniazid in the same dosages for the next 4 months. For both the regimens, a grace period of up to 2 months was allowed to compensate for missed doses/collections. Patients who refused both the short course regimens were offered one of the conventional regimens in the National Tuberculosis Programme—usually a daily regimen of thiacetazone (150 mg) plus isoniazid (300 mg).

The intake to the study was spread over a period of 2 years. During this period, of a total of 647 eligible patients, 508 (79%) accepted short course chemotherapy—352 (69%) the daily and 156 (31%) the twice-weekly regimen. Of the 139 patients who could not be put on short-course chemotherapy, 119 refused short-course chemotherapy and for the other 20, the Medical Officers did not prescribe short-course chemotherapy—in 15 as they were residing in far-off villages and in 5 as they had other complications (blindness, diabetes, mental retardation).

The proportions of patients completing the treatment (i.e., receiving at least 80% of the scheduled chemotherapy) are given by age, for the two regimens, in the

*depending on whether the body-weight was less than 35kg, or 35kg or more

| Age group (years) | Daily regimen | | Twice-weekly regimen | |
|---|---|---|---|---|
| | Number | % completing treatment* | Number | % completing treatment |
| 12-24 | 33 | *84.8* | 19 | *78.9* |
| 25-34 | 62 | *75.4* | 35 | *51.4* |
| 35-44 | 88 | *67.0* | 48 | *54.2* |
| 45-54 | 94 | *56.4* | 29 | *41.4* |
| 55 or more | 76 | *47.4* | 24 | *33.3* |
| Total | 352 | *63.1* | 155* | *51.0* |

* Excluding 1 patient who switched over to the daily regimen

table above. The proportion of patients who completed treatment with the daily regimen (63%) was more than that with the twice-weekly regimen (51%). However, it should be noted that the treatment allocation was not at random. In both the regimens, the likelihood of completing the treatment decreased significantly with increase in age ($p < 0.01$).

**Side-effects:** Side-effects were reported by 65 (18%) of the 352 patients on the daily regimen and 21 (14%) of the 155 patients on the twice-weekly regimen. A Medical Officer interrogated 43 of the former and 11 of the latter and the side-effects were attributed to drugs in 15 (all to pyrazinamide) and 8 (7 to streptomycin and 1 to pyrazinamide), respectively.

**Response to treatment:** Of the 352 patients on the daily regimen and the 155 patients on the twice-weekly regimen, drug sensitivity status at the time of admission was available for 337 and 152 patients, respectively. Of these, culture results were available at the end of chemotherapy for 311 and 135, respectively. The table below gives the proportion of patients culture negative at the end of treatment (5-9 months).

| | Daily regimen | | | Twice-weekly regimen | | |
|---|---|---|---|---|---|---|
| | Total patients | Culture negative | | Total patients | Culture negative | |
| | | No. | % | | No | % |
| All patients | 311 | 226 | *73* | 135 | 98 | *73* |
| Drug sensitive | 263 | 200 | *76* | 115 | 83 | *72* |
| Drug resistant | 48 | 26 | *54* | 20 | 15 | *75* |

It is seen that the proportion of patients culture negative at the end of treatment was the same, namely 73%, for the two regimens. In patients who completed the

course of treatment, however, the corresponding proportion was slightly higher with the twice-weekly regimen (94%) than with the daily regimen (89%).

Among patients on the twice-weekly regimen, the response did not appear to be affected by the initial drug sensitivity status. However, among patients on the daily regiman, culture negativity was substantially higher ($p<0.01$) in patients with drug sensitive bacilli at the start of treatment (76%) than in patients with resistance to isoniazid and/or streptomycin (54%).

Of the 226 and 98 patients, on the daily and twice-weekly regimens respectively, who were negative on culture at the end of treatment, sputum specimens were collected at 36 months from 206 and 82 patients respectively; 184 patients (89%) on the daily regimen and 77 patients (94%) on the twice-weekly regimen continued to be culture negative at 36 months.

**Reasons for default:** Attempts were made, by a medical officer, to contact the patients 'lost' from treatment (i.e. not attending for treatment for 2 months or more from the due date), to find out the reasons for default. The visits were made after the prescribed duration of treatment, irrespective of the time of default. Considering both the regimens, of the 166 'lost' cases, 144 could be contacted, and they gave a total of 182 reasons for default. The distribution of cases according to reasons for default is given below:

| | | | | | |
|---|---|---|---|---|---|
| Out of station | ... | 38 | Did not have bus fare | ... | 6 |
| Unable to be absent from work | ... | 35 | Bus stop too far away | ... | 3 |
| Side effects | ... | 19 | Drugs increase appetite but no money for food | .. | 2 |
| Indifferent | ... | 18 | Forgetfulness | ... | 2 |
| Ill health | . | 17 | Lost ID card | ... | 2 |
| Felt better | ... | 13 | Drugs not available at PHI (according to patient) | ... | 1 |
| Dislike of drugs | ... | 9 | Stopped treatment on the advice of private doctor | ... | 1 |
| Receiving treatment elsewhere | ... | 8 | Way to PHI blocked by river water | ... | 1 |
| Dissatisfied with clinic | ... | 7 | | | |

(started: 1983; completed: 1988).

**Survey of tuberculosis in an urban crowded locality**

A comprehensive survey for tuberculosis in all age groups was undertaken in a population of about 18,000 in Choolai, a crowded locality in Madras city. The objectives of the survey were to estimate the prevalence of tuberculous infection in children and the prevalence of tuberculous disease in adults and children. The methodology of the survey has been described in the annual report of 1987. The main observations are described here.

A total of 17927 individuals were registered, including 287 visitors. Of these, 4563 (25%) were children aged 12 years or less; 2526 children (55%) had received

BCG vaccination, a majority of them soon after birth. The coverages obtained for the various examinations were high—around 90% for most examinations.

The distribution of reaction sizes to 1 TU RT23 was very similar in those with and without BCG vaccination. The antimode was seen at 12 mm. The proportion of children with reaction size of 12 mm or more (see table below) was 26.6% in those with a scar, and 21.0% in those without a scar. Thus the BCG vaccination does not seem to influence the size of reaction to a great extent. The proportion of children without a scar with reaction sizes of 12 mm or more rose steeply from 1.1% in those aged less than 2 years to 6.8% in those aged 2-4 years. The prevalence of infection in children below 12 years is 21% and in those below 5 years is 4.6%. Only 5.5% of children aged below 24 months with a BCG scar showed reaction sizes of 12 mm or more.

| Age group (years) | Sex | With BCG Scar | | | Without BCG Scar | | |
|---|---|---|---|---|---|---|---|
| | | No. of tests read | 12 mm or more to 1 TU RT 23 | | No. of tests read | 12 mm or more to 1 TU RT 23 | |
| | | | No. | % | | No | % |
| 0-23 M | Male | 206 | 11 | *5.3* | 91 | 0 | *0.0* |
| | Female | 142 | 8 | *5.6* | 84 | 2 | *2.4* |
| | Both | 348 | 19 | *5.5* | 175 | 2 | *1.1* |
| 2-4 | Male | 298 | 40 | *13.4* | 128 | 6 | *4.7* |
| | Female | 274 | 34 | *12.4* | 150 | 13 | *8.7* |
| | Both | 572 | 74 | *12.9* | 278 | 19 | *6.8* |
| 5-9 | Male | 502 | 131 | *26.1* | 359 | 73 | *20.3* |
| | Female | 490 | 146 | *29.8* | 358 | 78 | *21.8* |
| | Both | 992 | 277 | *27.9* | 717 | 151 | *21.1* |
| 10-12 | Male | 319 | 150 | *47.0* | 229 | 86 | *37.6* |
| | Female | 295 | 153 | *51.9* | 230 | 84 | *36.5* |
| | Both | 614 | 303 | *49.3* | 459 | 170 | *37.0* |
| 0-12 | Male | 1325 | 332 | *25.1* | 807 | 165 | *20.4* |
| | Female | 1201 | 341 | *28.4* | 822 | 177 | *21.5* |
| | Both | 2526 | 673 | *26.6* | 1629 | 342 | *21.0* |

There were 47 bacteriologically proved cases of tuberculosis, giving a positivity rate of 2.7 per 1000 population. There were 2 children below 5 years with bacteriological confirmation, a rate of 1.3 per 1000 in that age group.

It was found that out of 587 gastric lavage specimens examined, 7 were positive for *M. tuberculosis* and 39 for non-tuberculous mycobacteria; 31 (5%) were contaminated. This rate, under field conditions, compares well with that of sputum (3.7%).

Of 77 positive cultures, 13 were resistant to INH, 5 to streptomycin and 6 to both.

(started: 1988; completed: 1988).

**Feasibility of investigations for childhood tuberculosis in a rural community**

The protective efficacy of BCG in children can best be established by a longitudinal prospective study of a defined population. All the different manifestations of childhood tuberculosis will have to be documented. This involves the collection and processing of several specimens like sputum, gastric lavage, gland biopsy, special X-rays to document skeletal tuberculosis and special investigations for rare forms like abdominal and meningeal tuberculosis. All these investigations are traditionally carried out in a hospital. A study was undertaken to examine the feasibility of conducting such investigations under field conditions.

**Methodology:** Twelve villages from Thiruvalangadu Panchayat Union of Tiruttani taluk, Chengalpattu district were selected for the study; 6002 children aged 0-12 years, identified by means of a house-to-house census, formed the study population. One intake round and two follow-up rounds (at 2 months and 4 months after intake) were completed.

The following examinations were undertaken initially:

(1) All children were screened by health workers. Information on whether the child was suffering from any of the following was elicited:
   (a) fever or cough, of more than one month,
   (b) loss of weight or severe malnutrition like marasmus or wasting,
   (c) glandular enlargement, swelling of joints or small bones, or deformity of the spine.

(2) All children aged 3 months to 12 years were tested with 1 TU RT23, by standard testers and the reaction sizes recorded after 72 hours by standard readers.

(3) All children had a chest X-ray (large X-rays for children aged 6 months to 4 years and MMR for children aged 5 years and above).

(4) A child who had one or more abnormalities on screening by health worker, and/or a reaction size of 10 mm or more to tuberculin testing, and/or

an abnormality on X-ray was clinically examined by a medical officer. Ten percent of children who had none of these were also examined as controls.

(5) Investigations were carried out as indicated below:

(a) Sputum examination (wherever feasible) for all children with respiratory abnormality on clinical examination or abnormality on chest X-ray.

(b) Gastric lavage for children below five years with the above indication and for children with cervical gland enlargement.

(c) Biopsies of all glands >2 cm in the cervical region, or >4 cm in the axillary or inguinal region.

(d) Pleural, ascitic, synovial, or cerebrospinal fluid as indicated on clinical examination.

(e) Skeletal X-rays as indicated.

**Follow-up:** The first round of follow-up was at two months and the second round at 4 months after intake. The following examinations were carried out at each follow-up round.

(1) Screening by health workers, as at intake.

(2) Children whose intake X-rays were abnormal were re-X-rayed after 1 month, and if the abnormality persisted, again at two months.

(3) Clinical examination of eligible children, by a medical officer, as at intake.

(4) Other investigations were also carried out as at intake.

The coverages obtained for the different examinations at intake were found to be around 80% (see table on page 75). It has been possible to get gastric lavages done on two occasions on 80% of those referred, but the yield of positives was low.

The majority of patients in whom lymphnode biopsies were indicated agreed for a biopsy, but were not biopsied because the surgeon to whom they were referred felt that a biopsy was not needed. In all, 34 gland biopsies were done and only 3 were positive for tuberculosis. There is need for applying more stringent criteria for selection of patients for investigations.

The distributions of reaction sizes to 1 TU PPD RT-23 were similar for those with and without BCG scar. An antimode was noted at 12 mm. However, the percentage of children showing reaction sizes of over 12 mm was small, both in those with and without BCG scar (10.7% and 10.6%, respectively).

| Type of examination | No. referred | Examined No. | Examined % |
|---|---|---|---|
| Screening | 6002 | 5625 | *94* |
| Tuberculin testing | 5935 | 5503 | *93* |
| X-ray | | | |
| Large (6 months-4 years) | 2719 | 2433 | *89* |
| MMR (5 years or more) | 3097 | 2925 | *94* |
| Clinical examination | 4065 | 3873 | *95* |
| Gastric lavage | 334 | 278 | *83* |
| Sputum examination | 171 | 134 | *78* |
| Gland biopsy | 58 | 13 | *22* |

Out of 2,581 children who were clinically found to be normal, 128 and 25 had x-ray shadows that were read as doubtful and definite TB, respectively. Only 11 of the 128 and 3 of the 25 children showed tuberculin reactions of 10 mm or more. Of 841 children who were clinically abnormal but were labelled as non-tuberculosis, 47 and 19 had x-ray shadows thought to be doubtful and definite tuberculosis, respectively. Of these, 6 and 2 children had reaction sizes of 10 mm or more.

Children found to be positive for tuberculosis by bacteriology, histopathology or on x-ray and clinical grounds were referred to the nearest hospital for treatment.

(started: 1988; completed: 1988).

## STUDIES IN PROGRESS

### Tuberculosis prevalence survey in Raichur district

With the exception of the National Sample Survey of Tuberculosis conducted in 1955-58, no epidemiological data are available at the national level and particularly in districts where SCC is being monitored by the Centre (see page 9). It was therefore, proposed to conduct sample surveys in these districts.

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

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

So far, 17049 persons have been registered, drawn from 19 villages. Of these, 95% were questioned for symptoms and 1265 were found to be eligible for sputum examination. Of those eligible, sputum samples were collected from 1202 (95%).

The work is in progress.

(started: 1988; expected year of completion: 1989)

**Study of bacteriological quiescence and relapse in sputum positive pulmonary tuberculosis**

The Centre has been monitoring Short Course Chemotherapy under programme conditions in North Arcot district since 1983. Though some information regarding the bacteriological response to treatment is available (see page 17), follow-up to assess relapse rates has not been possible. Hence a study was undertaken to estimate the proportion of patients who were sputum negative at the end of chemotherapy and about two years later. All patients for whom treatment was initiated for smear-positive pulmonary tuberculosis between 1-4-86 and 31-3-88 were included, irrespective of the regimen or the amount of treatment received. The list of patients along with addresses was prepared from the available records at the DTC, the field unit at Vellore and the peripheral health institutions. The patients were visited in their homes, and a spot and an overnight specimen of sputum were collected. At this time, information on symptomatic status as well as particulars of treatment taken were also collected.

So far, 542 individuals have been contacted. Of these, 377 were found in the address given in the treatment cards, and sputum was collected from 345; 32 had died. Another 33 had moved to other taluks or districts, and their current address could be obtained. It is planned to visit them, if possible. As many as 132 patients could not be identified as having lived in the address given on the treatment cards.

The study is in progress.

(started: 1988; expected year of completion: 1989)

# STATISTICAL STUDIES

## A model for the analysis of repeated specimens in clinical trials

A diagnosis of pulmonary tuberculosis, especially in adults, is conclusively made by demonstrating the presence of viable tubercle bacilli in sputum. In developing countries with limited laboratory facilities, examination of sputum smears by direct microscopy is the most important means of diagnosis of tuberculosis. One of the criteria for admission to the pulmonary tuberculosis studies conducted at the Centre is that at least two smears are positive. But smear examination is sometimes rendered ineffective by the occurrence of false negatives. The practice of taking 4 to 6 specimens to diagnose positivity before admission helps to reduce the possibility of false negatives. However, no attempt has been made to quantify the risk of obtaining a false negative finding with this procedure.

An attempt has been made to analyse the results of repeated sputum specimens collected from consecutive patients referred to the Centre. A sample of 440 specimens from 110 patients, collected at 2-4 day intervals is considered in this paper, including 45 patients for whom all 4 specimens were negative. The sputum smears were graded bacteriologically on a 4-point scale of positivity, according to the number of bacilli present (0, 1+, 2+, 3+).

Since the sputum specimens are uniform neither in intensity nor distribution due to various causes, there is still some doubt about the presence of the disease even if 4 negative results are obtained for an individual. The aim of this paper is to quantify the residual doubt about the presence of disease after such a set of negative specimens. This analysis will provide the number of negative specimens that are necessary to attain a specified level of confidence. The overall frequency with which each pair of grades occurs within the same individuals are calculated and presented in the table below.

| Smear result on 1 specimen | Smear result on the other specimen | | | |
|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ |
| 0 | 287 | 59 | 13 | 8 |
| 1+ | – | 99 | 102 | 10 |
| 2+ | – | – | 55 | 24 |
| 3+ | – | – | – | 3 |

**Model for analysis:** We denote the true underlying state of positivity as 0*, 1+*, 2+* and 3+*. The aim of this study is to estimate the quantities of the

type p(0*\0,0,0,0), i.e., the probability of true negatives, given 4 negative results from an individual. The standard procedure in such a problem is to use Bayes theorem. Since specimens were collected on different days, it is reasonable to make the assumption that the specimens were statistically independent. Hence P(0,0,0,0\1+*) may be expressed as $P^4$(0\1+*), where P(0\1+*) is the probability that a 1+ specmen gives negative smear and similarly for others. Therefore the model is a function of the unknown true states P(0*), P(1+*), P(2+*) and P(3+*) and the three error rates P(0\1+*), P(0\2+*), P(0\3+*). Since it is impossible to observe a specimen worse than the true state, the probabilities of false positives are taken to be zero. Care is required to distinguish between 'false negative rate' (e.g., P(0\1+*), and a predictive error rate (e.g., P(1+*\0)).

The approach for estimating the probability of the true underlying states and error rates is based on the EM-Algorithm which consists of two steps, the Maximisation Step (M-Step) and the Expectation Step (E-Step). The algorithm works as follows:

1. Give a starting value for the true states (highest of the 4 grades in this case).
2. M-Step: Maximise the likelihood, using the initial values.
3. E-Step: Using these estimates, estimate the true state.
4. Repeat steps 2 and 3 until convergence is achieved.

The estimated proportion of true states of sputum smear positivity along with the observed one are presented in the table below.

| Smear | Observed % | True % |
|---|---|---|
| 0 | 50 | 36 |
| 1+ | 28 | 23 |
| 2+ | 19 | 31 |
| 3+ | 4 | 10 |
| Total | 100 | 100 |

It can be seen that although half of the specimens were reported to be negative, the estimate of the true state is only 36 percent.

The first table on page 80 shows the error rates. We see for example that if the true state is 2+, there is 12 percent chance that the smear will be reported as negative (0), 58 percent chance that it will be reported as 1+ and 30 percent

| Observed grade | Estimated proportion (%) of smear grades according to state | | | |
|---|---|---|---|---|
| | 0* | 1+* | 2+* | 3+* |
| 0 | 100 | 41 | 12 | 13 |
| 1+ | – | 59 | 58 | 22 |
| 2+ | – | – | 30 | 56 |
| 3+ | – | – | – | 9 |

* True state

chance that it will be reported as the true state i.e., 2+, on a single specimen. Thus, there is $(.12)^4$ or much less than 1 percent chance that a specimen gives rise to 4 negative results. The probability of observing negative results based on different numbers of specimens tested are presented in the table below.

| True smear grade | Proportion of smears missed according to no. tested | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 1+* | 0.41 | 0.17 | 0.07 | 0.03 |
| 2+* | 0.12 | 0.01 | <0.01 | <0.01 |
| 3+* | 0.13 | 0.02 | <0.01 | <0.01 |

The methodology used here leads to straightforward analysis of repeated specimens, enabling the residual doubt about observing a series of negative specimens to be quantified. Since the chance of missing a positive smear by testing 4 specimens is low, the Centre's practice is sufficient to classify a patient as non-tuberculous based on sputum smear. These results are interpretable only for the Centre's laboratory, but the method of analysis can be easily extended to additional observations.

## On applications of P-lambda test and its advantages

The importance of statistical tests of significance needs no emphasis. Each test is generally based on certain assumptions, and the reliability of a test result depends on how far the underlying assumptions are fulfilled. It is sometimes possible to apply different tests of significance to the same data. The factors which generally determine the choice of a test are the simplicity of the test, the nature and type of data, the underlying assumptions validating the test and how far those assumptions are fulfilled. The P-lambda test was used by Fisher for combining

independent tests of the same hypothesis; Fisher's method is Asymptotically Bahadur Optimal (ABO) in the class of all "reasonable" combined tests. The present paper illustrates its use in different types of applications.

**Mathematical basis:** If Z is a continuous random variable with F(z) as its distribution function, $X = F(z)$ can be considered as a random variable uniformly distributed in (0,1) with density function

$$f(x) = \begin{cases} 1 & \text{for } 0 \leqslant x \leqslant 1 \\ 0 & \text{otherwise} \end{cases}$$

Further if $x$ is transformed to $Y = -2 \log x$, the distribution function, G(y) of Y can be derived as

$$G(y) = P(Y \leqslant y) = 1 - e^{-y/2}$$

By differentiating with respect to y, the density function of Y is obtained as $1/2\ e^{-y/2}$ for y ranging from 0 to $\infty$ That is, Y is distributed as chi-square with 2 degrees of freedom.

If $X_1, X_2 \ldots\ldots X_k$ are independent observations, it follows that the sum $\sum_{i=1}^{k} -2 \log X_i$ is a chi-square with 2K degrees of freedom.

The statistic $Y = -2 \sum_{i=1}^{k} \log p_i$ where $p_i$ denote p-values of independent tests of significance of the same hypothesis, is calculated as P-lambda statistic and tested as chi-square with 2K degrees of freedom.

**Combining several tests of the same hypothesis:** Antibody levels in serum are assayed by Enzyme Linked Immunosorbent Assay (ELISA) in a plate consisting of 96 micro wells arranged in 8 rows and 12 columns. Antigen is coated to the surface of the wells and the antibodies bound to the antigen are measured as optical density (OD). As some antibodies may get bound to the surface of the well due to inon-specific binding, reproducibility of the antibody levels is affected. An investigation was carried out to assess the intra - and inter-plate variations by assaying IgG antibody levels to PPD. The ELISA plate was divided into four experimental units, each unit consisting of 24 wells in 3 consecutive columns. Four specimens with different antibody levels were assayed in 4 ELISA plates, adopting a Latin Square Design, the four ELISA plates constituting the "rows" and the four experimental units the "columns" of the Latin Square.

The data were analysed by Analysis of Variance (ANOVA). An example of the "within plate" ANOVA is given on page 82. Sixteen such analyses (4 plates $\times$ 4 specimens) were carried out. The experiment is designed to test the null hypothesis that

| Variation due to | D.F. | S.S. | M.S. | F value |
|---|---|---|---|---|
| Rows | 7 | 0.010601 | 0.002288 | 7.71** |
| Columns | 2 | 0.008225 | 0.004113 | 13.87** |
| Error | 14 | 0.004151 | 0.000297 | |
| Total | 23 | 0.028389 | 0.001234 | |

** indicate statistical significance of F-value at 1% level

there are no differences in the effects of either rows or columns within the plate. The table below presents F-values for "Between rows" and "Between columns" variations for each test specimen separately, indicating the statistical significance. While most

| | Degrees of freedom for F | No. | Plate F-values for the specimen | | | | P-Lambda Statistic (X8) | |
|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | Value | P |
| **Between rows** | 7,14 | I | 1.94 | 11.94 | 4.92** | 7.71** | 35.17 | 0.01 |
| | | II | 5.93** | 4.40** | 1.72 | 2.50 | 28.43 | 0.01 |
| | | III | 4.37 | 10.18** | 4.09* | 13.33** | 39.30 | 0.01 |
| | | IV | 2.85* | 0.71 | 1.86 | 1.96 | 14.25 | 0.06 |
| **Between columns** | 2,14 | I | 1.71 | 13.18** | 10.82** | 13.87** | 34.74 | 0.01 |
| | | II | 0.48 | 9.08** | 15.93** | 5.78** | 30.21 | 0.01 |
| | | III | 11.54** | 2.90 | 10.03** | 40.82** | 36.56 | 0.01 |
| | | IV | 0.12 | 2.72 | 9.02** | 19.06** | 27.31 | 0.01 |

* and ** indicate statistical significance of F-value at 5% and 1% levels, respectively

of the values are seen to be significant, there exist a few non-significant values. As the p-values of F-values for the four test specimens within each plate can be treated as independent observations of uniform distribution in (0,1), P-lambda statistic was calculated from the four P-values within each plate. The value of the statistic and its p-value clearly demonstrate the statistical significance.

**Combining the tests after stratifying the data by a related variable:** If the means of a variable y are to be compared in two groups and if y is correlated with another variable $\times$ and if the distributions of the two groups with regard to $\times$ differ, problems arise in the statistical analysis. The matter will be further complicated if $\times$ and y are observed responses to treatment. When problems of this type

are encountered, the data can be stratified on the basis of values of X, and a suitable test of significance can be carried out in the two groups of each stratum. The tests in the different strata can be considered as independent and combined by the use of the P lambda test. This is illustrated in the following example.

The mean falls in Log Colony Forming Units (LCFU) of tubercle bacilli per ml of sputum in the first two weeks of chemotherapy are compared in two groups of pulmonary tuberculosis patients who were randomly allocated to rifampicin and non-rifampicin regimens. LCFU were estimated before chemotherapy and at the end of 7 and 14 days of chemotherapy.

It was found that the fall in LCFU in either week was correlated with the level of LCFU at the beginning of the week in each regimen. Therefore, the method of covariance analysis can be used in the statistical analysis. But a simple one-tail t-test showed that the mean fall in LCFU in the first week was significantly higher in the rifampicin regimen.

With regard to the comparison of mean falls in the second week, the study of the fall is meaningful only for patients with positive cultures of tubercle bacilli at the end of the first week. However, the proportions of patients with negative cultures were found to differ significantly in the two groups by the chi-square test (10% in rifampicin and 3% in non-rifampicin regimen).

| LCFU* at the beginning of the week | Mean Fall ± SD(n) | | t | p |
|---|---|---|---|---|
| | 4-drug regimen | 3-drug regimen | | |
| 1— | 0.52 ± 1.21 (22) | 0.04 ± 1.38 (12) | 1.04 | 0.15 |
| 2— | 0.38 ± 1.31 (70) | 0.51 ± 1.16 (36) | −0.50 | 0.69 |
| 3— | 1.16 ± 1.19 (64) | 1.01 ± 0.99 (43) | 0.71 | 0.24 |
| 4— | 1.49 ± 1.21 (48) | 1.06 ± 1.19 (30) | 1.51 | 0.07 |
| 5— | 1.56 ± 0.92 (17) | 1.23 ± 1.01 (14) | 0.95 | 0.18 |
| 6.00—6.99 | 3.18 ± 1.50 ( 7) | 1.71 ± 0.59 ( 3) | 1.60 | 0.08 |
| Over-all t-test | | | 1.11 | $\geqslant 0.1$ |
| Value of p-statistic (Chi-square, 12 df) = 21.19; $p < 0.05$ | | | | |

* Log. colony forming units

The mean falls in the second week are shown in the table on page 83, stratified on the basis of LCFU at the beginning of the week. The differences between the 2 regimens were not statistically significant in any stratum. An overall t-test to compare the mean falls in LCFU was also found to be not significant. The P-lambda test appears to be suitable. The two mean falls in each stratum were compared by a single-tailed t-test and then the results of the different tests were combined by the P-lambda test, which gave a significant result ($p \leqslant 0.05$).

The P-lambda test can be used in other situations also, such as undertaking a test of significance after combining the results in several strata which are based on an associated characteristic.

# ELECTRONIC DATA PROCESSING

**Main-frame computer:** The main-frame VAX 750 computer system is being used for analysing the voluminous data generated by the Chengalpattu BCG trial. During the year, corrections of data files containing name and address details were carried out for 110 panchayats and the analysis records of about 1.25 lakh individuals were also corrected up. All culture positive cases from 78 panchayats were listed and verified against basic registers. A total of 5.4 lakh records were converted from EBCDIC format to ASCII representation.

A microcomputer has been hooked up to the main-frame computer to enable transfer of data from 5 1/4" floppy diskettes directly on to the VAX system and vice versa, without having to enter data through the VAX terminal itself. During the year, the IBM-1401H computer system was finally decommissioned since all the punched card data had been transferred on to floppies or magnetic-tape, and subsequently all data storage has been done on 5 1/4" floppies.

Programmes have been written up for the analyses of the data generated from the 3 sample surveys carried out in rural, urban and metropolitan areas among chest symptomatics (see annual report 1987) and are being tested.

**Microcomputers:** There are microcomputers (IBM-PC compatible) in different departments of the Centre, which are utilised within the departments for data storage and interim analyses. Generation of random numbers for coding biological specimens is also done by a computer programme. For large masses of data, however, the data fed into microcomputers are to be transferred to the main-frame VAX system, for which adequate provision has now been made (see above).

The microcomputers are being routinely used to store weekly smear and culture results from several studies and from different areas, and lists are being prepared for use in the Clinic division of the Centre, Madurai, Pondicherry, North Arcot and London. The voluminous data collected during the 3 sample surveys mentioned earlier, consisting of some 35 items of information from each of about 4,000 symptomatics, was posing a problem due to non-availability of data entry time; with the installation of 2 more data entry machines, the entire data was entered on to floppy diskettes and after verification, corrections were carried out. Data from the Cardiopulmonary Medicine Unit of the Centre were also analysed by the use of microcomputers.

The microcomputers are used extensively for all detailed statistical analyses for many laboratory experiments, preparation of reminder and appointments lists and for graphics.

# APPENDICES

## TRAINING PROGRAMMES

### WHO fellow

Mr. Nichol Letan Gelanyang, Malaysia, from 5-9-88 to 16-9-88.

### Trainees

The following underwent training in different departments as follows:

#### Bacteriology

Two batches of 9 students each of Diploma in Medical Technology from Voluntary Health Service Centre, Adyar from 2-5-88 to 14-5-88.

Two Laboratory Technicians from Goa Medical College, Goa, from 16-8-88 to 15-9-88.

#### General

Dr. Sanjoy Gupta and Dr. Devapriya from Institute for Respiratory Diseases, Calcutta, from 12-4-88 to 21-4-88.

Dr. J. Akbar Ali, Dr. V. Gangadharan and Tmt. Rajalakshmi from Govt. Tiruvateeswarar TB and Chest Diseases Hospital, Otteri, Madras, from 25-4-88 to 30-4-88.

Dr. G. Kullayappe and Dr. G. Subba Rao from Kurnool Medical College, Kurnool, from 7-6-88 to 18-6-88.

Dr. S. Dattatreya from Kakatiya Medical College, Warangal, from 7-6-88 to 18-6-88.

Dr. Trilochan Sahoo and Mr. J. Valentine from Christian Medical College, Vellore, on 10-10-88.

#### Others

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

#### Medical students

Christian Medical College, Vellore—1 batch.

**Post-graduate students**

A DTCD student from Thanjavur Medical College, Thanjavur.

Twenty students from Jawaharlal Institute for Postgraduate Medical Education and Research, Pondicherry.

MD students from Kurnool Medical College, Kurnool.

M. Phil (Physiology) students from Postgraduate Institute of Basic Medical Sciences, University of Madras, Madras.

**Nursing and para-medical students**

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

Multipurpose Health Workers from Durgabai Deshmukh Hospital, Andhra Mahila Sabha, Madras–1 batch.

One Medical Social Worker from TB and Chest Diseases Department, Goa Medical College, Goa.

## ICMR—WHO SEMINAR

Two 3-day Seminars (18-20 January, 1988 and 24-26 November, 1988) were organised at the Centre under the joint auspices of the WHO and the ICMR, in order to have an interaction with the District TB Officers and their staff in the 18 districts where the Short Course Chemotherapy programme is being monitored by the Centre. The State TB Officers were also invited.

The Seminars were mainly in the nature of panel discussions on the different components of the programme, viz., case-finding, case-holding, chemotherapy, documentation and sociological aspects. Eminent scientists and workers in the field of TB control also took part in the deliberations.

The subjects covered and the participants are given below:

**18-20 January 1988**

| Subject | Speaker |
|---|---|
| SCC under DTP—Current Status in the 18 districts | Dr. T. Santha Devi |
| A Study of 'Lost' Cases in North Arcot | Mrs. Sudha Ganapathy |

**Panel on "Chemotherapy"**

Moderator: Dr. S.P. Tripathy, Additional Director-General, ICMR

Members: Dr. K.V. Krishnaswami, Dr. K. Jagannath, Dr. K. C. Mohanty, Dr. I. Ranga Rao, Dr. H.M. Kansal, Dr. A. Subramaniam, Dr. R. Prabhakar and Dr. S.P. Gupta

**Panel on "Sociological aspects"**

Moderator: Dr. T.S. Natarajan, Sociologist, Tamil University, Uthagamandalam

Members: Dr. P. Chandrasekar, Mrs. Sudha Ganapathy, Mr. S.A. Rajagopalan, Dr. V.B. Udani, Dr. K.B. Verma and Miss. M.A. Seetha

**Panel on "Documentation"**

Moderator: Dr. S. Radhakrishna, Director, Institute for Research in Medical Statistics (Madras Chapter)

Members: Mr. M.S. Krishnamurthy, Dr. N. M. Sudarsanam, Dr. S.J. Mudholkar, Dr. Pattiwar and Mr. P.R. Somasundaram

**Panel on "Case Finding"**

Moderator: Dr. G.D. Gothi, Former Director, New Delhi TB Centre

Members: Dr. P. Chandrasekar, Dr. S.C. Khasgiwala, Dr. M.S. Chaudry, Dr. N. Sreenivasa Rao, Dr. C.N. Paramasivan and Dr. S.P. Tripathy

**Panel on "Case Holding"**

Moderator: Dr. S.P. Gupta, National Consultant and Co-ordinator (TB), WHO

Members: Dr. P. Chandrasekar, Dr. N. M. Sudarsanam, Dr. V.K. Padmanabhan Dr. S.N. Gupta, Dr. V. Vyahalkar, Dr. J.B. Phaye and Dr. A. K. Sur

**Panel on "Operational Aspects"**

Moderator: Dr. P. Chandrasekar, Epidemiologist, National TB Institute, Bangalore

Members: Dr. T. Santha Devi, Dr. N.M. Sudarsanam, Dr. T.M. Nachinarkinian, Dr. P.K. Chhaya, Dr. A.K. Jain and Dr. S. Radhakrishna

**Valedictory Address**

Mr. S.V. Subramanian, I.A.S., Jt. Secretary, Ministry of Health and Family Welfare, Govt. of India

**24-26 November 1988**

| Subject | Speaker |
|---|---|
| Over-view of Short Course Chemotherapy in 18 districts | Dr. R. Prabhakar |
| Status papers | Jt. Directors / State TB Officers / District TB Officers |

**Panel on "Chemotherapy"**

Moderator: Dr. S.P. Tripathy

Members: Dr. J.N. Khadse, Dr. V.B. Udani, Dr. S.N. Gupta, Dr. K. Jagannath, Dr. C.V. Ramakrishnan, Dr. K.V. Krishnaswami and Dr. R. Prabhakar

**Panel on "Case Finding"**

Moderator: Dr. T.M. Nachinarkinian

Members: Dr. K.B. Varma, Dr. S.H.I. Zaidi, Dr. C.N. Paramasivan, Dr. P. Chandrasekar and Dr. B. Brahmanandam

**Panel on "Case Holding"**

Moderator: Dr. I. Ranga Rao

Members: Dr. V.K. Padmanabhan, Dr. A. Subramanian, Dr. J.B. Phaye, Dr. T. Santha Devi, Dr. S.P. Gupta and Dr. P. Chandrasekar

**Panel on "Programme Implementation"**

Moderator: Dr. B.S. Nagaraja Rao

Members: Dr. A. Gyanendra Singh, Dr. N.M. Sudarsanam, Dr. R. Parthasarathy and Dr. K.V. Krishnaswami

**Panel on "Sociological Aspects"**

Moderator: Dr. A. N. Banerjee

Members: Dr. J.N. Khadse, Dr. P.K. Chhaya, Mrs. Sudha Ganapathy and Dr. T.S. Natarajan

**Panel on "Documentation"**

Moderator: Dr. G. P. Saxena

Members: Dr. B. Brahmanandam, Mr. M.S. Krishnamurthy, Mr. P.R. Somasundaram and Dr. P. Chandrasekar

## STAFF DEVELOPMENT PROGRAMME

1. Mr. S. Ramanujam was awarded a six-month fellowship under the Indo-British ODA Project for training in immunology at the National Institute of Medical Research, Mill Hill, London, from January, 1988.

2. Dr. M. Naseema participated in the IXth International Council for Laboratory Animal Science's Symposium on 'Laboratory animal and health for all' at the National Laboratory Animal Centre, Mahidol University, Bangkok, during January 1988.

3. Mr. P. Venkatesan underwent a 3-day training programme in "Computing with Micro-Vax II" in the Department of Computer Science, University of Madras, Madras, during March, 1988.

4. Mr. P. Venkatesan participated in a 3-week institutional session on 'All India short-term Institute on Statistical Models of Life Time Data and Reliability' in the Department of Statistics, University of Rajasthan, Jaipur, during May-June 1988.

5. Dr. C. Kolappan was awarded a one-year fellowship under the Colombo Plan for training in Epidemiology at the London School of Hygiene and Tropical Medicine, London, from September, 1988.

6. Mr. M. Kannapiran was awarded a one-year TCTP Health fellowship under the Colombo Plan for training in T.B. Immunology at the Royal Post Graduate Medical School, London, from October, 1988.

7. Dr. P. Selvaraj was awarded a one-year fellowship under the Indo-British ODA Project for training in immunology at the National Institute of Medical Research, Mill Hill, London, from October, 1988.

8. Mrs. Chandra Immanuel underwent a short-term training course on "Safety Aspects in the Research Applications of Ionising Radiation" conducted by the Bhaba Atomic Research Centre, Bombay, during December, 1988.

## PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

| Name of conference, venue and date | Title of paper | Name of staff member |
|---|---|---|
| 5th Tamil Nadu Conference on T.B. and Chest Diseases, Salem, 9-10 January, 1988 | Presidential address | Dr. R. Prabhakar |
| -do- | Six month Short-Course regimens in pulmonary tuberculosis | Dr. Paulin Joseph |
| -do- | A comparison of serum and salivary levels of isoniazid | Mr. S. Kailasam |
| -do- | Monitoring Short Course Chemotherapy under DTP —a review | Mr. V. Chandrasekaran |
| -do- | A study of 'Lost' cases in North Arcot district | Miss J. M. Vanaja |
| 43rd Annual Conference of Association of Physicians of India, Pune, 21-25 January, 1988 | Single-breath carbon monoxide diffusing capacity in acute tropical eosinophilia | Dr. V. K. Vijayan |
| Joint meeting of International Epidemiological Association with International Clinical Epidemiology Network and Field Epidemiology Training Programs Conference, Pattaya Resort, Thailand, 24-30 January, 1988 | Prognostic factors in the prediction of bacteriological relapse following Short-Course Chemotherapy of previously untreated pulmonary tuberculosis | Mr. P. V. Krishnamurthy |
| International symposium on 'Nature of Genetic variation in Man', Hyderabad, 25-27 February, 1988 | Significance of acetylator phenotype in the chemotherapy of tuberculosis | Dr. G. Raghupati Sarma |
| U.G.C. sponsored symposium on Bio-Chemistry of Lung, V.P. Chest Institute, University of Delhi, Delhi, 7-9 March, 1988 | Characterization of inflammatory and immune effector cells in lung parenchyma of patients with pulmonary tuberculosis | Dr. V. K. Vijayan |

| Name of conference, venue and date | Title of paper | Name of staff member |
|---|---|---|
| Seminar on 'Statistics-theory and methods', Dept. of Statistics, Loyola College, Madras, 26 March, 1988 | Regression methods for analysis of censored survival data with time dependent covariates | Mr. P. Venkatesan |
| 50th meeting of the Madras Chapter of the Indian Association of Pathologists and Microbiologists, Govt. General Hospital, Madras, 11 June, 1988 | Complement in health and disease (key note address) | Dr. V. D. Ramanathan |
| Symposium on 'Recent advances in Tuberculosis Research', Chandigarh, 3-4 September, 1988 | Drug interactions and adverse reactions to anti-tuberculosis drugs | Dr. G. Raghupati Sarma |
| -do- | Recent advances in chemotherapy: rationale of Short Course Chemotherapy of tuberculosis (key note address) | Dr. R. Prabhakar |
| XIII International Congress of Gastro-enterology and VI European Congress of Diagnostic Endoscopy, Rome, 4-10 September, 1988 | Controlled clinical study of the treatment of abdominal tuberculosis (poster presentation) | Dr. Rani Balasubramanian |
| National Academy of Medical Sciences (India) C.M.E. Programme, Railway Hospital, Madras, 8 October, 1988 | Broncho-alveolar lavage | Dr. V. K. Vijayan |
| VI Annual Convention of Indian Society for Medical Statistics & National Seminar on Statistics in Medicine, Health and Nutrition, Hyderabad, 27-29 October, 1988 | A model for the analysis of repeated specimens in clinical trials | Mr. P. Venkatesan |
| -do- | A new method for the assessment of treatment effects in clinical studies | -do- |

| Name of conference, venue and date | Title of paper | Name of staff member |
|---|---|---|
| VI Annual Convention of Indian Society for Medical Statistics & National Seminar on Statistics in Medicine, Health and Nutrition, Hyderabad, 27-29 October, 1988. | Non-parametric prediction interval for censored survival data (poster presentation) | Mr. P. Venkatesan |
| -do- | Logistic regression and Cox's proportional hazards model in the short course treatment of TB (poster presentation) | Mr. P. V. Krishnamurthy |
| -do- | Application of P-lambda distribution and its advantages (poster presentation) | Mr. G. S. Acharyulu |
| Bhopal Gas Disaster Centre (ICMR), J. N. Hospital, Bhopal, 28 October, 1988 | Broncho-alveolar lavage—Its role among gas victims (guest lecture) | Dr. V. K. Vijayan |
| Silver Jubilee Celebrations, Govt. T.B. Sanatorium, Pondicherry, 14 November, 1988 | Rate of inactivation of isoniazid in relation to therapeutic efficacy and toxicity during treatment of tuberculosis (guest lecture) | Dr. G. Raghupati Sarma |
| 43rd National Conference on TB and Chest Diseases, Calcutta, 11-14 December, 1988 | Controlled clinical study of abdominal tuberculosis | Dr. Rani Balasubramanian |
| -do- | Pott's paraplegia | Dr. Rajeswari Ramachandran |
| -do- | Bacteriological investigation for Short-Course Chemotherapy under District T.B. Programme conditions | Dr. C. N. Paramasivan |
| 43rd National Conference on T.B. and Chest Diseases, Calcutta, 11-14, December, 1988; Symposium on "Assessment of SCC under Programme conditions" | Moderator | Dr. R. Prabhakar |

| Name of conference, venue and date | Title of paper | Name of staff member |
|---|---|---|
| 43rd National Conference on T.B. and Chest Disease, Calcutta, 11-14, December 1988; Symposium on "Assessment of SCC under Programme conditions" | Management of the programme including monitoring | Dr. R. Prabhakar |
| -do- | Implementation of SCC in DTP | Dr. T. Santha Devi |
| -do- | Case holding | -do- |
| -do- | Case finding, and the proportion prescribed Short Course Chemotherapy | Dr. N. M. Sudarsanam |
| -do- | Sociological aspects | -do- |
| -do- | Documentation | Mr. P. R. Somasundaram |
| -do- | Management of the programme including monitoring | -do- |

## LIST OF PUBLICATIONS

### Papers published


1. Vijayan, V.K. Role of steroids in "chronic" Tropical Eosinophilia—A bronchoalveolar lavage study (Abstract): *Indian Journal of Chest Diseases and Allied Sciences*, 1987, *29*, vii.

2. Rajiswamy, Acharyulu, G.S., Rani Balasubramanian, Narayanan, P.R. and Prabhakar, R. Immunological investigations in tuberculous ascites. *Indian Journal of Tuberculosis*, 1988, *35*, 3-7.

3. Lunde, M. N., Paranjape, R., Lawley, T.J. and Ottesen, E.A. Filarial antigen in circulating immune complexes from patients with *Wuchereria bancrofti* filariasis. *American Journal of Tropical Medicine and Hygiene*, 1988, *38*, 366-371.

4. Prema Gurumurthy. Salivary levels of anti-tuberculosis drugs (Abstract): *Indian Journal of Tuberculosis*, 1988, *35*, 91.

5. Rema Mathew. Diagnosis of abdominal tuberculosis (Abstract): *Indian Journal of Tuberculosis*, 1988, *35*, 92.

6. Sudha Ganapathy. Community awareness study of pulmonary tuberculosis (Abstract): *Indian Journal of Tuberculosis*, 1988, *35*, 93.

7. Cameron, M.L., Levy, P., Nutman, T.B., Vanamala, C.R., Narayanan, P.R. and Rajan, T.V. Use of restriction fragment length polymorphisms (RFLPs) to distinguish between nematodes of pathogenic significance. *Parasitology*. 1988, *96*, 381-390.

8. Vijayan, V.K., Kuppu Rao, K.V., Sankaran, K., Venkatesan, P. and Prabhakar, R. Diffusing capacity in acute untreated tropical eosinophilia. *Indian Journal of Chest Diseases & Allied Sciences*, 1988, *30*, 71-77.

9. Selvaraj, P., Venkataprasad, N., Vijayan, V.K., Prabhakar, R. and Narayanan, P.R. Alveolar macrophages in patients with pulmonary tuberculosis. *Lung India*. 1988, *6*, 71-74.

10. Sarma, R.V.S.N., Vallishayee, R.S., Nagabhushana Rao, R.S., Prabhakar, R. and Tripathy, S.P. Use of mebendazole in combination with DEC in bancroftian filariasis. *Indian Journal of Medical Research*, 1988, *87*, 579-583.

11. Kumaraswami, V., Ottesen, E. A., Vijayasekaran, V., Uma Devi, S., Swaminathan, M., Aziz, M.A., Sarma, G.R., Prabhakar, R. and Tripathy, S.P. Ivermectin for the treatment of *Wuchereria bancrofti* filariasis: efficacy and adverse reactions. *Journal of the American Medical Association*, 1988, *259*, 3150-3153.

12. Alamelu Raja, Baughman, R. P. and Daniel, T.M. The detection by immunoassay of antibody to mycobacterial antigens and mycobacterial antigens in bronchoalveolar lavage fluid from patients with tuberculosis and control subjects. *Chest*, 1988, *94*, 133-137.

13. Paramasivan, C.N., Jackett, P.S., Coates, A.R.M., Lowrie, D.B. and Mitchison, D.A. Monoclonal antibodies against *Mycobacterium avium/intracellulare*. *Indian Journal of Medical Research*, 1988, *88*, 13-17.

14. Venkataraman, P., Narayana, A.S.L and Prabhakar, R. Simple qualitative tests for rifampicin in urine. *Indian Journal of Tuberculosis*, 1988, *35*, 114-116.

15. Selvaraj, P., Rajiswamy, Vijayan, V.K., Prabhakar, R. and Narayanan, P.R. Hydrogen peroxide producing potential of alveolar macrophages and blood monocytes in pulmonary tuberculosis. *Indian Journal of Medical Research*, 1988, *88*, 124-129.

16. Alamelu Raja, Machicao, A.R., Morrissey, A.B., Jacobs, M.R. and Daniel, T.M. Specific detection of *Mycobacterium tuberculosis* in radiometric cultures by using an immunoassay for antigen 5. *Journal of Infectious Diseases*, 1988, *158*, 468-470

17. Sudarsanam, N.M. and Ramamoorthy, N.G. Tuberculosis camps: retrospects and prospects. *NTI Newsletter*, 1988, *24*, 52-56.

18. Venkataraman, P., Paramasivan, C.N., Illampuranam, K.J. and Prabhakar, R. Intraspecies differentiation of strains of *Mycobacterium tuberculosis* obtained from Czechoslovakian, Mongolian and South Indian patients. *Indian Journal of Medical Research*, 1988, *88*, 211-216.

19. Paranjape, R.S., Ravoof, A., Acharyulu, G.S., Krishnamurthy, P.V., Tripathy S.P., Prabhakar, R. and Narayanan, P.R. Cell mediated immune response in South Indian pulmonary tuberculosis patients. *Indian Journal of Tuberculosis*, 1988, *35*, 163-170.

20. Thomas, A., Nagarajan, M., Chandrasekaran, V., Lalitha Hari, Somasundaram P.R., Prabhakar, R., Ashok Kumar, Bhatia, V.N. and Roy, R.G. A double-blind controlled clinical trial to assess the role of anti-histamines in the treatment of multi-bacillary leprosy. *Indian Journal of Leprosy*, 1988, *60*, 499-505.

21. Vijayan, V.K., Kuppu Rao, K.V., Venkatesan, P. and Prabhakar, R. Arterial hypoxemia in acute tropical pulmonary eosinophilia. *Lung India*, 1988, *6*, 183-185.

22. Venkatesan, P. and Viswanathan, K. A new method for the assessment of treatment effects in clinical studies. *Technical Report* No. 8/88, 1988, Department of Statistics, University of Madras, Madras.

23. Venkatesan, P. and Viswanathan. K. A model for the analysis of repeated specimens in clinical trials. *Technical Report* No. 9/88, 1988, Department of Statistics, University of Madras, Madras.

24 Rajajee, S. and Narayanan, P.R. Contact sensitization to DNCB. *Indian Journal of Paediatrics*, 1988, *55*, 448-450.

25. Selvaraj, P., Rajiswamy, Vijayan, V.K., Prabhakar, R. and Narayanan, P.R. Hydrogen peroxide release by alveolar macrophages in pulmonary tuberculosis. *Proceedings of the University Grants Commission-sponsored National Symposium on Biochemistry and Molecular Pathology of Lung, V.P. Chest Institute, Delhi*, 1988. p.10.

26. Vijayan, V.K. Characterization of inflammatory and immune effector cells in the parenchyma of patients with pulmonary tuberculosis. *Proceedings of the University Grants Commission—sponsored National Symposium on Biochemistry and Molecular Pathology of Lung, V.P. Chest Institute, Delhi*, 1988. p.13.

27. Weir, Jerry P. and Narayanan, P.R. The use of B-galactosidase as a marker gene to define the regulatory sequences of the herpes simplex virus type 1 glycoprotein C gene in recombinant herpes viruses. *Nucleic Acids Research*. 1988, *16*, 10267-10282.

28. Vijayan, V.K. Bronchoalveolar Lavage. In: Health information for toxic gas victims. *BGDRC Bulletin (ICMR)*, 1988, *1*, 3-4.

29. Rani Balasubramanian, Prabhakar, R. and Rajasambandam, P. A controlled clinical study of the treatment of abdominal tuberculosis. (Abstract) *Journal of Gastro Enterology International*, 1988, *1*, 88.

30. Raghupati Sarma, G. Significance of acetylator phenotype in the chemotherapy of tuberculosis. Proceedings of a symposium on "Nature of genetic variation in man", Environmental Mutagen Society of India, 1988, 263-271.

31. Paramasivan, C.N., Sambasivarao, R., Sivadasan, K., Shampa Anupurba, Reba Kanungo and Prabhakar, R. Non-fermenting gram negative bacteria in human infections. *Indian Journal of Medical Microbiology*, 1988, *6*, 73-79.

**Papers accepted for publication**

1. Kannapiran, M., Chandra Immanuel, Krishnamurthy, P.V. and Raghupati Sarma, G. C-reactive protein levels in patients with pulmonary tuberculosis. *Lung India*.

2. Raghupati Sarma, G. Isoniazid acetylator phenotype. *Indian Paediatrics*.

3. Somasundaram, P.R. Collection of accurate and complete data relating to controlled clinical trials in tuberculosis. Proceedings of the 4th Annual Conference of the Indian Society for Medical Statistics, Bangalore, 24-26 November, 1986.

4. Kuppu Rao, K.V. and Vijayan, V.K. Maximal Expiratory Flow volume loop in Southern Indian sportsmen. *Indian Journal of Physiology and Pharmacology.*

5. Vijayan, V. K., Jawahar, M. S., Reetha, A. M. and Prabhakar, R. Persisting alveolitis in miliary tuberculosis despite treatment with short course chemotherapy. *Indian Journal of Chest Diseases and Allied Sciences.*

6. Venkatesan, P. and Somasundaram, P. R. A probabilistic approach for modelling the joint action of drugs. *Biomedicine.*

7. Indian Council of Medical Research/British Medical Research Council Working Party. A controlled clinical trial of short-course regimens of chemotherapy in patients receiving ambulatory treatment or undergoing radical surgery for tuberculosis of the spine. *Indian Journal of Tuberculosis.*

8. Vijayan, V.K. Practical application series: AIDS and Pulmonologists. *Lung India.*

## JOURNAL CLUB

Meetings of the Journal Club were held regularly once a week. At these meetings, scientific articles of interest, comprising a wide range of subjects, were reviewed by different staff members from each department by turn. This was followed by a discussion about the matter presented and its possible relevance to the research activities of the Centre. Altogether, 35 topics were thus presented and discussed during the year.

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

## LECTURES BY VISITING SCIENTISTS

| Subject | Speaker |
|---|---|
| Nerve Involvement in Leprosy | Dr. H. Srinivasan, Director, Central Jalma Institute for Leprosy, Agra. |
| Community Health Education | Dr. K. A. Pisharody, Consultant on Population & Health Management, Madras. |

## DISTINGUISHED VISITORS

1. Mr. Motilal Vora, Minister of Health, Govt. of India, New Delhi.
2. Prof. Stephen Walter, McMaster University, Hamilton, Ontario, Canada.
3. Dr. Pranab Kumar Das, Immunologist, University of Amsterdam, Amsterdam.
4. Mr. Andrew Stevenson, Managing Director of Churchill Livingstone, Edinburgh, U.K.
5. Dr. David L. Madden, Science Attaché-Designate, Embassy of the United States of America, New Delhi, along with Mr. Manmohan Saxena and Mr. Krishnan.
6. Dr. Eric Ottesen, Chief, Section of Clinical Parasitology, National Institutes of Health, Bethesda, Maryland, U.S.A.
7. Dr. Gunnar Gille, SIDA—Stockholm, Mrs. Anna-Kari Bill, Swedish Embassy (SIDA), New Delhi and Mrs. Amla Rama Rao, Voluntary Health Association of India, New Delhi.
8. The Director-General, British Council Division, London and Mrs. Francis, British Council Division, New Delhi.
9. Dr. P. Ranque, Chief, Filariasis Division, Parasitic Diseases Programme and Dr. C. P. Ramachandran, Secretary, Steering Committee of the Filariasis Scientific Working Group, WHO, Geneva.
10. Prof. Debidas Ray, Professor of Chest Diseases, Christian Medical College, Vellore.
11. Dr. C. R. Krishnamurthy, Chairman, Commission on Bhopal Gas Leak Tragedy, Government of India, New Delhi.
12. Prof. S. K. Jain, Professor of Cardio-respiratory Physiology, V.P. Chest Institute, Delhi.
13. Dr. S. K. Jindal, Asst. Professor of Chest Diseases, Postgraduate Institute of Medical Sciences, Chandigarh.
14. Dr. R. Anantha Narayanan, Principal (retired), Medical College, Trivandrum.

## CONSULTANTS

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

1. Prof. Wallace Fox, MRC TB & Chest Diseases Unit, London.
2. Dr. D. B. Lowrie, British Medical Research Council, London.
3. Dr. Barry Walker, Royal Post Graduate Medical School, London.

## PRIZES AND AWARDS RECEIVED BY STAFF MEMBERS

1. Dr. V. K Vijayan was awarded the Fellowship of the Indian College of Allergy and Applied Immunology, V.P. Chest Institute, New Delhi.

2. Dr. Rani Balasubramanian was awarded the "R.C. Garg Memorial Award" for the best article published in 1987 in the Indian Journal of Tuberculosis for the paper entitled "Kanamycin plus rifampicin plus ethambutol in the re-treatment of patients with tubercle bacilli resistant to isoniazid and streptomycin."

3. Dr. Rajeswari Ramachandran was awarded the "Dr. R. Krishna Memorial Award" for the best paper presented at the 43rd National Conference on TB and Chest Diseases held at Calcutta in December 1988 for the paper entitled "Short course chemotherapy in the treatment of Pott's paraplegia".

## STAFF MEMBERS ON ADVISORY COMMITTEES OF OTHER INSTITUTIONS

| Staff member | Name of committee |
|---|---|
| Dr. R. Prabhakar | Standing Technical Committee, Tuberculosis Association of India, New Delhi. |
| -do- | Planning Board, Dr. M.G.R. University of Medical Sciences, Madras. |
| -do- | Planning and Research—Medical Research Committee of the University of Health Sciences, Vijayawada. |
| -do- | Board of Management, Vision Research Foundation, Madras. |
| -do- | Research Sub-Committee, Vision Research Foundation, Madras. |
| -do- | Scientific Advisory Committee of the Regional Medical Research Centre, ICMR, Port Blair, Andamans. |
| -do- | Editorial Advisory Committee, Lung India, Madras. |
| Dr. V. K. Vijayan | Project Advisory Committee on Clinical Studies and Broncho-alveolar Lavage studies on MIC-exposed people at Bhopal, Indian Council of Medical Research, New Delhi. |
| -do- | Central Crisis Group for Chemical Disasters, Ministry of Environment and Forest, Government of India, New Delhi. |
| -do- | Assistant Editor, Lung India, Madras. |
| Dr. V. Kumaraswami | Associate Editor, Lung India, Madras. |
| Dr. G. Raghupati Sarma | Research Committee of the Drug Addiction Research Centre, Madras. |
| -do- | Editorial Board, Indian Journal of Chest Diseases and Allied Sciences, New Delhi. |

**ACKNOWLEDGMENT**

The Director wishes to express his deep appreciation to Mr. P. R. Somasundaram and Mr. M. S. Krishnamurthy, for their help in editing the report, and in addition, to Mr. S. Sivasubramanian and Mr. R. Segaran for their help in getting it scrutinised and published.