

Professor Denis A. Mitchison
A Visionary In TB Research



(1919-2018)



PROLOGUE

It is a pleasure to write a Prologue to this booklet on Prof. D.A. Mitchison, one of the founders of the ICMR-National Institute for Research in Tuberculosis (ICMR-NIRT). This institute was formerly known as the Tuberculosis Chemotherapy Centre (TCC) when it was established in 1956 under the leadership of Dr. Wallace Fox and Prof. Mitchison. It was later renamed as Tuberculosis Research Centre (TRC) in 1978 and renamed as the National Institute for Research in Tuberculosis (NIRT) in 2011. This booklet on Prof. Mitchison has become possible due to the untiring efforts of Dr. S Radhakrishna, who wholeheartedly took up the responsibility of co-ordinating the efforts of fellow co-researchers of Dr. Mitchison for the writing of the various sections in this book.

Along with Dr. Wallace Fox, Prof. Mitchison initiated the research programme of the Tuberculosis Chemotherapy Centre in 1956. He was responsible for establishing the TB laboratory, which supported the TB clinical trials at Chennai, conducted jointly by the Tamil Nadu State Government, WHO, ICMR and the MRC-UK. He was closely associated with the study carried out for the concurrent comparison of home and sanatorium treatment of pulmonary tuberculosis in South India, which showed clearly that it would be appropriate to treat the majority of the tuberculosis patients at their homes. His research revealed that the South Indian cultures of tubercle bacilli had a wider range of virulence than the British cultures but were, on the average, less virulent. He was also associated with the development of a much more sensitive test for the detection of isoniazid metabolites in the urine and which was used for measuring adherence to anti-TB treatment. His research also showed that fluorescent microscopy yielded as good results as ZN stained microscopy for the detection of TB bacilli in sputum smears, and there was no tendency to produce false positive or false negative results.

Prof. Mitchison had a very imaginative mind and a great ability to propound complex hypotheses to explain unexpected findings - e.g. for lowered virulence in the guinea-pig of Indian strains compared with that of the British strains and its non-impact on the outcome of chemotherapy. One of the major leads for the evolution of the concept of intermittent chemotherapy in tuberculosis patients was the TRC finding from serial serum isoniazid concentration studies that the peak concentration of isoniazid attained was more important than the mere maintenance of a minimal inhibitory concentration of isoniazid (0.2 µg/ml). Prof. Mitchison was an inspirational fellow collaborator of Dr. Wallace Fox and influenced the choice of regimens and design of most of the early TRC clinical studies.

This institute is immensely indebted to Prof. Mitchison for the efforts made by him to develop it into a world-renowned TB research institute. This booklet will remain a tribute to and an acknowledgement of his significant contributions to the development of TCC/TRC/ICMR-NIRT, and to his efforts to control tuberculosis in India and other countries of the world.

Srikanth Tripathy
Director-in-charge, ICMR-NIRT

PREFACE

It is a unique honour to be invited to coordinate the preparation of a booklet on Prof. Mitchison's ground-breaking research in tuberculosis from the erstwhile Tuberculosis Chemotherapy Centre in Madras (now renamed as National Institute for Research in Tuberculosis). And for this I am greatly indebted to the current Director-in-charge, Dr. Srikanth Prasad Tripathy.

In the following pages, there are write-ups by three colleagues. The first is by me, a close associate for over five decades, and describes Prof. Mitchison as a visionary who made stupendous contributions in the field of tuberculosis. The second is by Dr. S.P. Tripathy, a former Head of the laboratory, Director of the Centre and Director-General of the ICMR, and meticulously describes significant research milestones attained in the goal of a world free of tuberculosis. The third is by a fellow bacteriologist of the early days (Dr. T.V.Subbaiah), and gives a graphic description of the physical aspects of the laboratory at the time and work-ethic of Prof. Mitchison, and reminisces over the thrill and excitement of the very early days. In all three accounts, the dynamic personality and the aura of Prof. Mitchison come through vividly.

This is followed by a segment presenting speeches and tributes made by colleagues at a condolence meeting held on 16th July 2018, and a few photographs taken at the Silver Jubilee celebrations of this Centre in 1981.

The next segment sets out the titles, references and abstracts of Prof. Mitchison's papers arising from his research studies at Madras. Finally, there is an Appendix providing biographic details of Prof. Mitchison.

I would like to take this opportunity to thank all my colleagues for their unstinted cooperation, Prof. Mitchison's family for making available rare photographs, and Prof. Andrew Nunn for permission to reproduce some of his slides from the 2018 IUATLD Conference. It is my fond hope that this booklet will enthuse many youngsters to a research career in tuberculosis. And if that does happen, it would be the best possible tribute that we at this Centre could have paid to a great man.

S.Radhakrishna

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Former Chief Statistician, Tuberculosis Research Centre,
Former Director, Institute for Research in Medical Statistics, Madras

VISIONARY IN TUBERCULOSIS RESEARCH

*Talent hits a target no one else can hit
Genius hits a target no one else can see*

Arthur Schopenhaur (Philosopher)

*Good leaders have vision and inspire others to help them turn vision into reality.
Great leaders have vision, share vision, and inspire others to create their own.*

Roy Bennett (Politician)

Denis A. Mitchison, a great visionary in the field of tuberculosis research, was a close friend and a shoulder-to-shoulder research collaborator of the legendary Dr. Wallace Fox (Founder Director of TRC - Tuberculosis Research Centre), and together they made waves with the 'Madras Classic' of late 1950s, a randomized controlled trial of Home treatment and Sanatorium treatment in patients with sputum-positive tuberculosis¹. With the backdrop of his stellar experience of pulmonary tuberculosis, through the first randomised trial of streptomycin versus bed rest in the U.K. in 1948², Mitchison came to Madras in 1956 with great vision and a clear mission. He then jump-started to establish a comprehensive diagnostic and research laboratory, to fit into his thought processes that the clinical evaluation of any chemotherapy in pulmonary tuberculosis in humans could be modelled well through carefully designed and randomized *in vitro* and *in vivo* laboratory

tests that could be quantitatively evaluated and correlated. His small but highly talented young team of colleagues in Madras successfully established a laboratory and experimental animal house of his specifications, for providing bacteriological, pathological, biochemical, pharmacological and animal experimental models to these clinical trials. A series of studies then demonstrated that patients could be successfully treated in their homes, a finding that led to the revamping of the National Tuberculosis Treatment programme of India and that of other developing countries, and had major implications on W.H.O.'s Global treatment policies.

Mitchison was a brilliant analytical bacteriologist and pathologist who piloted a number of studies in TRC which established that:

- (1) Indian strains of tubercle bacilli differed from British strains in several characteristics viz., sensitivity to PAS³ and thiocetazone⁴, hydrogen peroxide sensitivity⁵, virulence in the guinea-pig⁶, but these did not affect the outcome of chemotherapy.
- (2) Established through serial serum isoniazid concentration studies that the attainment of higher peaks was more important than the presence of longer durations of coverage with a minimal inhibitory concentration, a finding that led to the novel concept of intermittent chemotherapy (fully supervised) with high-dosage isoniazid (and streptomycin)⁷.
- (3) Evolved a test to measure the virulence of tubercle bacilli in the guinea-pig, standardised and validated it through carefully designed experiments, and then correlated it with initial disease characteristics of the patient and the outcome of treatment⁸.
- (4) Made invaluable contributions to the overall planning of the clinical trial research programme, and provided critical inputs regarding their conduct, analysis and scientific reporting.

Mitchison was a nephew of J.B.S.Haldane, the internationally famous biologist cum statistician. He had no formal training himself in statistics, and was largely a self-taught statistician who invariably relished citing Snedecor's classic text on Statistical Methods⁹. He employed randomisation, replication and 'blinding' techniques with a fervour that would have pleased R.A.Fisher, and was particularly enamoured of the 'Split-plot' and Factorial designs, Analysis of variance techniques, and, estimation of components of variance. In the context of reporting on a virulence test he had developed for measuring consistent differences between patients and investigating their prognostic impact, he published a paper in the Bulletin of the WHO titled 'Homogeneity of the investigation and a critique of the virulence test' that would have done a statistician proud – so thorough was it in checking on the assumptions for validity of Analysis of variance (normality, homogeneity, additivity) and even making a data transformation¹⁰. Other novelties of this

paper were the use of a linear regression equation to adjust for differences in responses of two strains of guinea-pigs. His use of discrimination analysis for evolving definitions of drug resistance¹¹ was another feather in his statistical cap.

Mitchison had a touch of genius about him, was imaginative, and could propound fascinating explanations to account for unexpected study findings. One such example was in the context of reporting that only one-third of Indian strains were as virulent as British strains while one-third were very attenuated. He hypothesized that the attenuation of Indian strains may be a modification imposed on the bacilli by the need to establish the correct balance between the susceptibility of the impoverished Indian host and the virulence of the parasite¹². Another was his elaborate discussion regarding the lack of association of virulence of cultures obtained immediately before the start of treatment and severity of the patient's disease at that time, and final conclusion that it was probably due to the investigation being just a one-point-in-time study⁸.



After placating Goddess Kali before a drive to Ooty (1957)

Mitchison's achievements are particularly noteworthy as neither trained staff nor automated techniques were available in Madras at the time, and can be largely attributed to his diligence, patience and perseverance. After this outstanding stint of one year in India, Mitchison went back to his parent institute in London, the Royal Post-Graduate Medical School at Hammersmith Hospital, as Director of the MRC Institute of Drug Sensitivity Research in Tuberculosis.



Goodbye Madras, 1958 (Clare, Ruth, Terence & Denis Mitchison)

His close involvement with TRC persisted, however for the next three decades through WHO short term consultant visits, long phone calls and elaborate letters specifying minute details to his Madras colleagues.



D.A.Mitchison and C.V.Ramakrishnan (Senior Clinic Medical Officer)



S.Radhakrishna, D.A.Mitchison and C.V.Ramakrishnan

On his return to London, Mitchison worked on the evolution of pragmatic short-course regimens of chemotherapy, undertook studies of the killing mechanisms of different drugs, developed a technique to measure early bactericidal activity of drugs that had immense prognostic value, introduced the idea of an 8-week phase II study with the proportion culture-negative as the outcome measure, developed a new type of phase II study using modelling of counts of TB in sputum during treatment, worked intensively on new anti-tuberculosis drugs and involved himself in high-dosage Rifampicin trials.



Andrew Nunn - IUATLD Conference at The Hague (2018)

Unbelievable as it may seem, Mitchison worked every day in his lab till he reached the age of 95, permitting himself the luxury of taking Mondays off but that too only after attaining the age of 90! The photographs in the next page of Mitchison bear testimony to his passion for working in the laboratory.



Mitchison – continued to work in the lab till 95!



Mitchison – happiness personified in the lab

His 90th birthday was celebrated in London as a major event in 2009, and was highlighted by the publication of a detailed interview of his life style, thinking processes and work-ethic in a national daily¹³, 'The Guardian'. His views on retirement were exceptional (see below).

Interview

Still saving lives at 90

Rachel Williams

Denny Mitchison fully intends to carry on having life-saving ideas.'

"Nobody in my family in the scientific part ever retired. Well I might, if I get very ill. But how do you give up a whole major part of your life? I view it soberly, but it's a lot of achievement, and I continue to have what I think are really quite interesting and important ideas."

Mitchison was very much a family man, and his 95th birthday was an occasion for a family get-together with four generations represented.



**Mitchison with some family members on his 95th Birthday (2015)
(top: Graeme, Terence, Mark, Denny; bottom: Laura, Clare, Zoe)**



**Four generations of Mitchisons in 2016
(Denny, daughter Clare, her son Ben, and grandson Imogen)**

Mitchison was instrumental in setting up tuberculosis laboratories in several parts of the globe – Hong Kong, Kenya, Uganda, Zambia and Tanzania. He published over 250 papers in reputed scientific journals, was a member of innumerable scientific committees, and received several honours, including the British honour of CMG in 1973, the Medal of Honour from the Union in 1987, British Thoracic Society Medal in 2000, the Stop TB Partnership Kochan prize in 2008, and at the grand old age of 96, the Union Medal in 2015 for "an outstanding contribution to the control of tuberculosis" by his scientific work and actions in the field.

It is no wonder then that at the second plenary session of the 49th Union World Conference on Lung Health at The Hague (October 2018), delegates took a moment to remember him as one of the great pioneers of tuberculosis research, and paid a tribute to his achievements and their impact on management of tuberculosis (see page 10).



“Denny was a giant. His, and Wallace Fox's, brilliant work in advancing the chemotherapy of TB saved millions of lives”

JOHN L. JOHNSON



Andrew Nunn - IUATLD Conference at The Hague (2018)



“The legacy of his intellectual contributions still percolates many areas of TB research and clinical practice and will influence our current and future studies on TB. One can only say this about the work of truly great scientists and Professor Mitchison is up there amongst the greats.”

PHILIP BUTCHER



THE 49TH UNION WORLD CONFERENCE ON LUNG HEALTH

Andrew Nunn - IUATLD Conference at The Hague (2018)

To sum up, Mitchison was “at the centre of many of the advances that occurred in the 20th century”, according to a famous Professor of Infection Pharmacology. With his life-long research collaborator Wallace Fox, Mitchison succeeded in putting the TRC and Madras on the world map of tuberculosis, and saved an estimated 60 million lives from the scourge of this dreaded disease.

On the personal plane, Mitchison was a bubbly person with a twinkle in his eyes and often a dormant pipe in his mouth, and indulged in lengthy phone conversations. He was quite adventurous considering that he drove a bubble car side by side with London’s monster Red buses, but didn’t dare to sample spicy Indian food. He was kind-hearted and a caring host, a strong believer in family values, a fair judge of men and matters, and an inspirational leader with far-sighted thinking who was totally committed to tuberculosis control. He had oodles of talent but nevertheless worked tirelessly, reiterating the truth of Russian ballerina Anna Pavlova’s quote that ‘God gives talent. Work transforms talent into genius’.

But "All good things must come to an end" says an old proverb that dates back to 1374 (Chaucer). And very true it is, for Prof. Mitchison breathed his last on 2nd July 2018, after a life of fulfilment in which he had made gigantic contributions in the field of tuberculosis. His death was followed by a plethora of obits in several journals and newspapers of repute.^{14 - 22}

The world in general, and the TRC in particular, should forever be grateful to this outstanding man. I personally regard myself as very fortunate to have come into contact with him when I was just 20 years of age and at the threshold of my scientific career. And doubly blessed that a close association continued between us for over five decades. My last visit to his hospitable home in Marlborough road, Richmond (see page 12) was in 2005, when I went to the U.K. for an expert meeting at my *alma mater*, the London School of Hygiene and Tropical Medicine.



Richmond home of the Mitchison family (1955 onwards)

All research workers in tuberculosis should draw inspiration from the life, achievements, missionary zeal and enthusiasm of Denis Mitchison, and recall Longfellow's words below from his famous 'Psalm of Life'.

“Lives of great men all remind us
We can make our lives sublime
And, in passing, leave behind us
Footprints on the sands of time”

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Former Director-General, Indian Council of Medical Research

MILESTONES TO A WORLD FREE OF TUBERCULOSIS

A world free from tuberculosis is the goal of scientists engaged in combating this disease which is prevalent in all countries and, half a century back, was known as the disease with the highest morbidity and mortality globally. With a third of the world population having latent tuberculosis with a reactivation rate of 5 to 10% during the lifetime of individuals, eradication of TB from the world, as in the case of poliomyelitis eradication, is impracticable. However, a substantial reduction in the incidence of new cases to negligible levels is possible, and is the aim of achievement of elimination of tuberculosis globally. Today we can think of achieving the goal by 2030, and this has been made possible by the concentrated efforts of a large group of researchers from different countries who have planned and executed a series of carefully planned and designed controlled clinical trials in the chemotherapy of tuberculosis. Credit goes to the collaborative and cooperative efforts of a large team, in which Dr. Mitchison played a very large part and indeed can be given substantial part of the credit because of his foresight and approach in organising clinical trials in several countries.

Dr. Mitchison participated in a controlled clinical trial of bed rest versus streptomycin treatment in 1944 when streptomycin was found to have specific activity against *Mycobacterium tuberculosis*. Though streptomycin therapy proved more effective than bed rest (in sanatorium), the benefit was short-lived as many patients relapsed in a few months with antibacterial resistance to streptomycin. This gave the necessary stimulus to Dr. Mitchison, who thereafter took upon a career of TB research, including studies on two-drug regimens such as PAS plus isoniazid and streptomycin plus isoniazid, when isoniazid was found to be an effective drug against TB in 1948. He developed a good laboratory with culture and sensitivity facilities at the Postgraduate Medical School, London.

India had a large population of over 350 million, with millions of cases of tuberculosis and large mortality rates. When in 1955 a team led by the British Medical Research Council (MRC), World Health Organisation (WHO), Indian Council of Medical Research (ICMR) and Government of India went around parts of India to establish a centre to conduct a controlled clinical trial of Home and Sanatorium line of treatment, both groups treated with a standard regimen of PAS plus isoniazid and selected Madras (now Chennai) for conducting the trial, the WHO selected Prof. Wallace Fox as the Chief Medical Officer and Dr. D.A. Mitchison as the WHO bacteriologist. There was a sanatorium and an outdoor clinic at the time but no laboratory facilities for culture and sensitivity tests. It was necessary to establish a bacteriology laboratory in what was only an empty building. Dr. Mitchison undertook the challenge with the cooperation of ICMR, WHO, BMRC and Tamil Nadu Government, and began establishing the laboratory from scratch. Equipment, chemicals and reagents were supplied by Dr. Mitchison's laboratory in London and by WHO grants. He recruited a whole batch of individuals who had no prior experience and trained them methodically, providing the foundation for a world-class tuberculosis laboratory in Chennai in no time. He trained several young scientists, who later evolved into leaders in their own right.

The journey through the territory of TB chemotherapy in early years was rugged. A major factor determining the choice of the drug regimen was its affordability. Most cases of TB globally occurred in economically poor countries such as India. The choice of drugs was therefore limited to relatively inexpensive ones. PAS, a constituent of standard 2-drug therapy, was very expensive and therefore not available to most patients. Since isoniazid is a bactericidal drug (unlike PAS which is bacteriostatic), we explored the possibility of using isoniazid alone. Three regimens of isoniazid alone – 100 mg twice a day, 200 mg twice a day, and 400 mg in a single daily dose – were compared with a standard regimen of isoniazid plus PAS. In the event, even the best of the three isoniazid-alone regimens (400 mg in a single daily dose) was found to be substantially inferior to the standard combined regimen and therefore unacceptable. However, it was distinctly more effective than 400 mg given in 2 divided doses of 200 mg, a significant finding that led to the exploration of even higher doses of isoniazid but given intermittently (twice or thrice a week). These attempts eventually resulted in effective twice-weekly and thrice-weekly regimens of isoniazid with two or three companion drugs, and formed the basis for formulation of regimens for national tuberculosis programmes in many countries.

Individuals inactivate isoniazid at different rates. About 60% of Indian patients inactivate (acetylate) isoniazid at a slower rate than the remaining 40%, and have greater exposure to isoniazid than the rest. In clinical practice though, there was no difference in response between slow and rapid inactivators when isoniazid was given daily, thrice a week or twice a week. However, when given once a week, the response was substantially inferior

in rapid inactivators. Consequent to these findings, twice-weekly and thrice-weekly regimens of multidrug combinations containing isoniazid were formulated for supervised chemotherapy of TB patients in public health programmes.

As mentioned earlier, affordability of drugs was a major limiting factor in the choice of drug regimens for TB control programmes. When the highly bactericidal drug Rifampicin became available, it was very expensive – about Rs. 20 per daily dose – and was beyond the means of most public health programmes. The World Health Organisation which supplied anti-TB drugs for the Madras Centre could not provide Rifampicin for the trials. M/s Ciba-Geigy, a major drug firm manufacturing Rifampicin, was willing to provide the drug at cost price for the trials, but WHO's policy at the time did not permit direct acceptance from the drug manufacturer. Ultimately, M/s Ciba-Geigy gifted the drug to the British Medical Research Council which in turn gifted it to the Madras Centre, making it possible for it to conduct clinical trials with Rifampicin-containing regimens.

As the Madras trial and those in other countries showed isoniazid-rifampicin containing regimens to be highly effective, developing countries gradually changed their attitude towards the use of rifampicin-containing regimens. Indeed, when the first Rifampicin trial was initiated at Madras, the then Adviser-in-TB to the Government of India and the National Tuberculosis Institute, Bangalore distanced themselves from it. Several years elapsed before they accepted supervised Rifampicin regimens in the TB control Programme, with substantial assistance from the WHO. Today, isoniazid-rifampicin containing regimens form the basis of first line of treatment in the TB control programmes of all countries in the world.

All these achievements were possible because of a series of controlled clinical trials, carefully designed and executed with meticulous care and analysed from numerous angles. Lessons were learnt from successes as well as failures. Each study had a formal protocol, which was carefully designed and extensively reviewed and discussed and revised several times before finalization. Some protocols took over one year from conceptualization to finalization and start of the study. A well-staffed Statistics department provided the necessary inputs for the design and smooth conduct of the study, and analysis of the results.

Prof. Mitchison was closely involved with the planning of all clinical trials at Chennai during the two decades of the formal association of the ICMR with the British Medical Research Council and the World Health Organisation. His involvement with the laboratory studies was even deeper. In addition to establishing a culture laboratory, he undertook a series of laboratory studies on drug sensitivity tests for several anti-tuberculosis drugs used in the clinical trials at the Madras Centre. The finding that Indian

strains of tubercle bacilli were less virulent in guinea pigs compared with British strains of tubercle bacilli was of considerable scientific interest, but this had no impact on the response to chemotherapy with isoniazid-containing regimens. Series of investigations on the estimation of blood levels in pharmacokinetic studies in patients treated with anti-tubercular drugs provided the scientific basis of the success or failure of different regimens, and provided sound basis for optimal use of these drugs in future.

Prof. Mitchison was involved in practically all the laboratory studies and in large parts of the clinical trials, during the year that he headed and built the bacteriology laboratory and thereafter through frequent correspondence and by annual visits of 2 to 6 weeks to the Madras Centre as a consultant, by courtesy of the WHO. While the Madras Centre benefited extensively by association with Professor Mitchison, I believe Professor Mitchison also gained a lot because the association provided him a springboard which allowed him to reach greater heights, enabling him to face different challenges in the control of tuberculosis and in planning studies in collaboration with other countries. He designed a study in Africa on shortening the duration of chemotherapy to 6 months, thus making the regimens more acceptable to patients. Following this, the Tuberculosis Chemotherapy Centre also undertook a series of trials involving short course chemotherapy regimens including intermittent chemotherapy.

Currently, all countries are using standard short course regimens with guidance from WHO. With the high level of success achieved with four-drug regimens, the prevalence and incidence of TB globally have been steadily declining, and it seems likely that the goal of elimination of TB by 2030 will be achieved. Indeed, this might be achieved in India by 2025 itself, a target set by the present Government of India.

One head wind we face in our efforts to eliminate tuberculosis is the emergence of drug resistance in patients who fail on chemotherapy. However, only a small proportion of patients fail on first-line drugs, thanks to well-organised TB control programmes that achieve a success rate of over 90% sputum conversion on first-line drugs. The patients who fail are put on a second line of treatment which converts about 70% of them, and the rest are offered more complex combinations of drugs depending on the sensitivity pattern of the strains. Despite all of this, a few patients fail to respond and end up having Extensively Drug Resistant (XDR) strains which do not respond to any treatment. Such patients, however, are very few in number, representing a miniscule fraction of those initially started on treatment, and will not pose a threat to our achieving the goal of elimination of tuberculosis globally by 2030 A.D.

As a researcher, I found it very interesting and rewarding to discuss with Professor Mitchison any aspect of research in tuberculosis. He was an expert in tuberculosis research, had a rich knowledge of the various aspects of tuberculosis and was convinced that the disease could be brought under control but with collaboration globally. He was also a good teacher and made good researchers out of scientists who interacted with him, and good technicians out of raw, untrained and scantily educated technical staff to whom he taught different techniques with great patience and persisted till he was sure that they had attained perfection. The large number of technicians trained by him in the Madras laboratory in one year is an outstanding achievement. While discussing any research aspect with scientists, Professor Mitchison with his vast background had an advantage and generally dominated in the discussions. He would argue with endless patience till he could convince others. Sometimes this appeared to be arrogant but, more often than not, he was right. However, there was one occasion, when he had to yield with great reluctance, but it turned out, that his 'giving in' during discussion resulted in findings of great importance! The occasion was a discussion among three scientists, Dr. Mitchison, Dr. Fox and Dr. Johannes Holmes, the then Chief of the International Union against Tuberculosis (Paris). The subject was a proposed clinical trial at Madras exploring the use of regimens of isoniazid alone. At that time, the universal belief was that the administration of isoniazid twice daily was necessary. The proposal was to explore two regimens of isoniazid alone—isoniazid 100 mg twice a day and 200 mg twice a day—in comparison with a standard regimen of 5 g of PAS plus 100mg isoniazid twice a day. While agreeing that it was necessary to find out if isoniazid alone was effective, Dr. Holmes suggested that a fourth regimen of isoniazid 400 mg once daily be included. Dr. Fox and Dr. Mitchison argued that once-daily isoniazid would be ineffective, but Dr. Holmes felt that even if it was marginally inferior, it would be more practical and patient-compliant. He was very adamant and refused to collaborate if the fourth regimen was not included. Reluctantly, Dr. Fox and Dr. Mitchison agreed. The results of the trial surprised everyone. Isoniazid 400mg once a day proved to be more effective than 200mg twice a day, proving that twice daily treatment was not really necessary. Giving isoniazid once a day paved the way for exploring possibilities of giving it in higher dosages but intermittently—twice a week or thrice a week, thus making the regimens more patient-compliant and capable of supervising drug intake by patients. This greatly influenced the development of shortened regimes, paving the way for Global TB Control Programmes.

Prof. Mitchison believed every component of a clinical trial would require careful planning and monitoring. Statistics played an important part in every stage of the trial—design, protocol, procedures for implementation, data analysis and interpretation and finally reporting. It is not surprising that the Statistics department in the Madras Centre was established right at the start and had a crucial role to play all along. The controlled clinical trials conducted in Madras in successive decades have proved to be model trials,

with due regard to principles of medical ethics. Indeed, when the first trial was initiated in 1956, there were no international standards for ethical guidelines. The studies conducted by Prof. Mitchison and Dr. Fox in the UK, Madras, Hong Kong, Singapore and Africa set the groundwork for the formulation of ethical guidelines for tuberculosis research to begin with, and later were adopted for research in other diseases.

Association with Prof. Mitchison gave many scientists scope to learn research methodology and make their careers in clinical, bacteriological, immunological and statistical as well as social science research. Many scientists are indebted to Prof. Mitchison for their career development and achievements.

Can we hope to eradicate TB, as we have done with poliomyelitis? Much depends on whether we can have means of killing the dormant tubercle bacilli in billions of people with latent tuberculosis infection. It is possible that with the large number of scientists engaged in research involving molecular biology, an agent may be discovered which can eliminate the dormant tubercle bacilli in the mononuclear cells, eliminate the source of reactivation of tuberculosis and thereby pave the way for eradication of tuberculosis in the world. The world will be looking forward to this D-Day.



Dr.T.V.Subbaiah Ph.D. (London) Dip Bact. (London)

Former Bacteriologist, Tuberculosis Chemotherapy Centre

EARLY LABORATORY DAYS AND NOSTALGIC RECOLLECTIONS

Prof. Dennis A. Mitchison was a unique 'individual', steadfast to the goal he was given; that became his life-time commitment, the development and evolution of drug regimens for the effective chemotherapy of the dreaded pulmonary tuberculosis in humans, be it in isolated institutional care or at home. His initial success came out of chemotherapy with streptomycin in UK under the British Medical Research Council. This was followed by breakthrough studies at the Tuberculosis Chemotherapy Centre, under the banner of the British Medical Research Council, WHO, the Indian Council of Medical Research and the Madras Government, with randomized chemotherapy trials using the then available drugs, streptomycin, p-aminosalicylic acid (PAS), isoniazid, thiacetazone and ethambutol. The first trial established that domiciliary chemotherapy was as effective as sanatorium treatment with the advantages of logistics and compliance to ambulatory chemotherapy.

Leaving Madras with the successes of domiciliary chemotherapy, Dr. Mitchison spent considerable time on strategies for short term chemotherapy, for early acquiescence to obtain better compliance rates, novel combination therapies, to yield speedy quiescence, and, in more recent elegantly designed studies to segment minor recalcitrant persisters as opposed to the major component sensitive to established chemotherapy regimens. His strategy to challenge the persisters with short term aggressive chemotherapy, has reduced the duration of chemotherapy particularly using rifampicin, pyrazinamide and certain recent novel derivatives and drugs.

Dr. Mitchison always concentrated on *in vitro* laboratory studies as he passionately believed that laboratory studies could be tailored to reflect the *in vivo* "cavity". Maybe the future cellular and molecular probes would provide tools to study the pulmonary cavity as an *in vivo in situ* model to resolve the rapid chemotherapeutic or bioengineered interventions to eliminate the dreaded scourge of tuberculosis. As is typical of the ebullient Dr. Mitchison, he may be watching developments with bated breath from interplanetary or interstellar space.

The TCC laboratory in Madras that I joined in the latter half of 1957, was the fountain-head of Dr. Mitchison's international standards of containment, and safeguards of a contemporary clinical laboratory space, fit for dealing with highly infectious aerosol and very slow growing pathogenic microorganisms. The WHO generously provided needed funds and even arranged for the import of all critical instrumentation and equipment. The four main functional areas were:

(1) a laboratory (with a contiguous series of rectangular shaped rooms) and supporting facilities for microscopy and culture, and drug sensitivity studies, (2) a reception room for the receipt of different clinical samples from the patients drawn in the clinic, with decontamination facilities and an annexe for observations, (3) analytical instrumentation testing facilities for rapid clinical, analytical, and biochemical testing and (4) administrative block of three rooms for data collection, storage and analysis.

The experimental animal house was again designed to house guinea pigs, rabbits, mice, hamsters etc., adhering to national and international standards. It was at a safe distance to the west of the main building with appropriate safe-guards. The main animal house had a double corridor system, the healthy corridor for entry and a dirty corridor for exit with air-showers. There were appropriately designed animal inoculation rooms and post-mortem rooms for the qualitative and quantitative evaluation of the disease and keeping the identity of the cultures blind with random numbers. The incinerator to service the experimental animal house was at about 500 meters distance.

The daily working hours for employees were 7 A.M. to 1 P.M.; but all senior staff voluntarily spent the afternoon hours of 2 P.M. to 5 P.M. on research. None wanted to rest! It was an intellectually exciting and rewarding time as the results were unknown until seen to be experienced! Dr. Mitchison desired that people who worked with him always attain something bigger than themselves, be it in the vision, be it about the work at the Centre or any spark with new challenges. When we were all excited that some of the strains of tubercle bacilli isolated pre-treatment from Indian patients differed in isoniazid sensitivity, catalase activity, hydrogen peroxide sensitivity, virulence in the guinea-pigs and other properties suggesting that we have bigger challenges of chemotherapy, he

was excited with theories for attenuated virulence but cautioned us to wait until it became clear that these phenotypic variations had no bearing on the response to the chemotherapy of the patients to the chemotherapy or dosage received. May be these have a bearing on the evolutionary aspect of the tubercle bacilli of Indian origin.

Dr. Mitchison gave credit in all the publications from TCC lab to every colleague who participated in the relevant investigation. That is reflected in almost all the papers published from the lab carrying multiple authors including those from the Clinic and Statistics departments. The list of publications in this booklet (pages 56 to 84) provides the evidence.

Dr. Mitchison was informal and easily approachable for easy and happy resolution of any issues. Since he used to work late in the evening, it was not uncommon to see Mrs. Ruth Mitchison in the lab, carrying their two young children to meet him, all smiles. She was a cheerful, affectionate and kind lady. These are memories of 60 years ago etched in my mind forever!

The life-time commitment of Prof. D.A.Mitchison, to locate the precise vulnerabilities of human tubercle bacilli to available drugs and later tailor these to appropriate chemotherapeutic regimens in humans, borders on a spiritual journey that only a saint with unflinching internal conviction can undertake. He inspired groups of clinical research workers and health care administrators to accept that tuberculosis is curable.

Challenges still remain to reduce the duration of treatment further, take on the problem of persisters, currently being tackled by double-decker therapeutics. Multiple drug-resistant variants and other morbidities of a chronic disease, seek more rapid and early detection. A potential source of the origin of several recalcitrant variants of *Mycobacterium tuberculosis* could be the modified cells of the host in the diseased pulmonary cavity itself, selectively supporting growth of variant human tubercle bacilli. In other words, the diseased pulmonary cavity may have the potential of acting as *in vivo* culture tube. Histopathology studies have taken a back seat for several years as dramatic cure results were achieved with appropriate chemotherapy. In-depth studies on single cell modifications could now be carried with Fluorescence Life time Imaging Microscopy (FLIM) and other techniques for a deeper understanding of how cells within the same tissue, apparently similar, differ in their behaviour. It is for future researchers to use these and similar leads to answer how clusters of cells in pulmonary cavities seen in tuberculosis patients could also harbour variants of *Mycobacterium tuberculosis* that need newer strategies of effective therapies.

At the Silver Jubilee (1981) of Tuberculosis Research Centre



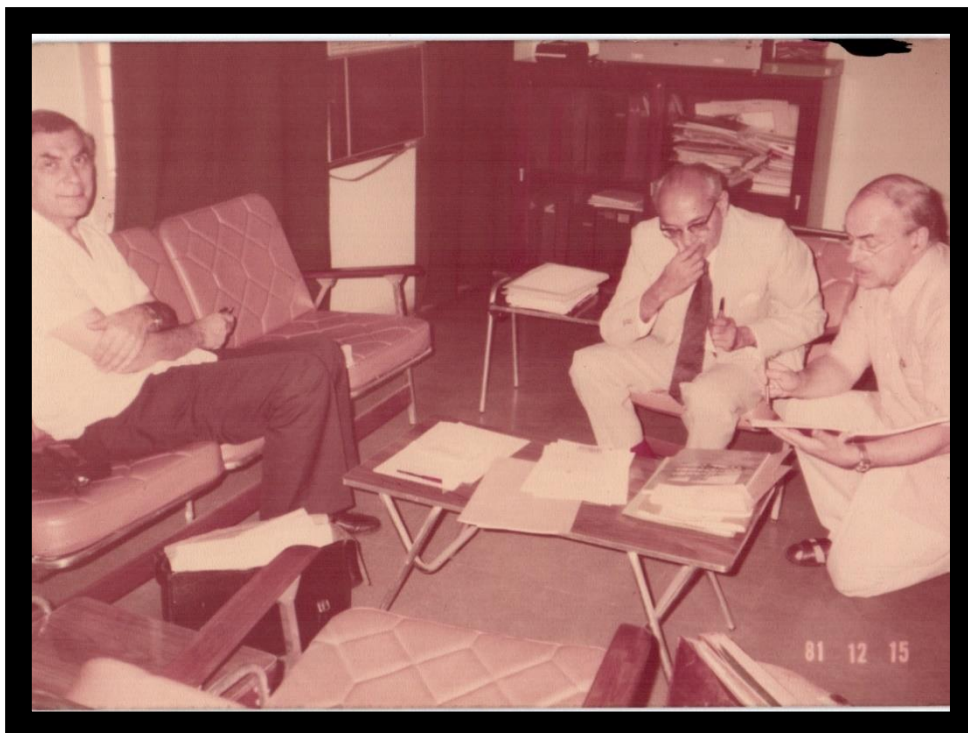
Denis Mitchison and ICMR Director-General Ramalingaswami



Denis Mitchison, Wallace Fox, Ramalingaswami and S.P. Tripathy



Wallace Fox, Ramalingaswami and Denis Mitchison



Wallace Fox, Ramalingaswami and Denis Mitchison

Proceedings of Condolence Meeting on 16th July 2018

Venue: Robert-Koch Auditorium,
National Institute for Research in Tuberculosis (NIRT), Chennai

Chairman: S.P.Tripathy
Former Director-General, ICMR & Former Director, TRC

Dr. Beena E. Thomas, on behalf of the Director-in-charge and staff of the NIRT, organised a touching condolence meeting at the Robert Koch auditorium of the Institute at 5 P.M. on 16th July 2018, to highlight Dr. Mitchison's monumental contributions in the domain of tuberculosis, especially in setting up in 1956 a Tuberculosis Chemotherapy Centre in Madras and nurturing its growth over many decades. A colourful 'lighting-of-the-lamp' ceremony to symbolise the enlightening of the scientific community by Mitchison's research works was followed by tributes to him by many colleagues. The gist of the presentations is set out in the following pages; the complete proceedings are available at <https://you.tube/uGhALIITHPM>.



Overall view of the venue of the Condolence Meeting



Lighted candle on the stage

Opening Remarks by Chairman, S.P.Tripathy



I am deeply grieved by the demise of my teacher and mentor, Prof. D.A. Mitchison. I was a novice in medical research when I joined the Tuberculosis Chemotherapy Centre in March 1962 as a bacteriologist. The Bacteriology laboratory at the Tuberculosis Chemotherapy Centre was established by Prof. D.A. Mitchison in 1956, starting from scratch. He built the laboratory infrastructure by procuring the necessary equipment and other necessities, recruited and trained a whole batch of raw laboratory personnel and thus provided the foundation for a laboratory which in no time achieved the distinction of being one of the best TB laboratories in the world. He established the laboratory during the year of his role as Head of the Laboratory and then handed over a fully functioning laboratory to his successor, Prof. J B Selkon. When I took over the running of the laboratory in 1964, I had little difficulty because the procedures had been well regulated and his advice and guidance from London were always available. He and Prof. Wallace Fox worked as a team and guided the Tuberculosis Chemotherapy Centre (later renamed as Tuberculosis Research Centre and National Institute for Research in Tuberculosis) in the conduct of research. The British Medical Research Council was a part of a collaborative team which formulated and guided the research studies at the NIRT. What NIRT is today because of the strong foundation laid by Prof. Mitchison and Prof. Fox and their continued guidance even beyond the formal collaboration period of 10 years.

Prof. Mitchison and Prof. Fox were deeply involved in the formulation and conduct of the first group of clinical trials, and later provided advice and guidance in the formulation and guidance of studies after the Indian Director, Prof. N.K. Menon took charge of the Centre in 1964.

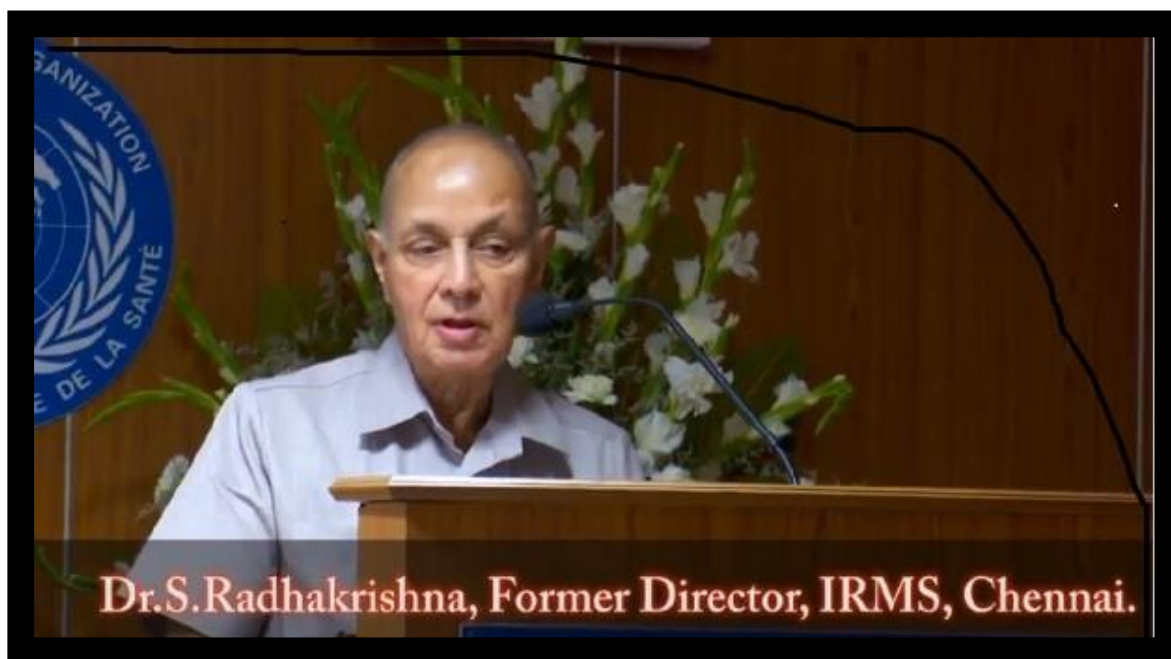
Prof. Mitchison and Dr. Fox were deeply committed to building up the human resource element in the Centre. The staff recruited in the initial years were new to any research. Not only were they provided with initial hands-on research training, as the studies progressed they were also provided with opportunities of research training in Prof. Mitchison's laboratory and in Dr. Fox's Institute in London through international fellowships procured with the help of Prof. Mitchison and Prof. Fox. They also arranged travel facilities for the centre's staff to attend international conferences abroad to present papers based on work done in Madras. Thus, they helped the junior staff to be trained and to evolve into high quality research personnel who could conceive of new avenues of research, plan and conduct quality research projects.

Prof. Fox and Prof. Mitchison not only helped in the establishment of the Madras Centre- they were deeply involved in establishing research centres in other countries- Hong Kong, Singapore, East Africa. These Centres benefitted by interactions with each other and conducted independent studies, avoiding duplication and sometimes collaborating with each other. As a result, Prof. Mitchison and Dr. Fox played a very large role in the conduct of research in tuberculosis globally, in association with WHO and the International Union against Tuberculosis. Because of their contribution, we can visualise the possibility of seeing a tuberculosis-free world in the coming decades.

In retrospect, I realise that much of my career development could be attributed to what I learnt from the Mitchison-Fox team. My original plan of returning to the teaching profession after two years of research at Madras was scrapped. Inspired by the team I continued in medical research which I pursued through my entire career. I have no regrets that I did not go back to a teaching job in the medical profession. The world will miss Prof. Mitchison – his contribution to medical research will always be remembered.

May his soul rest in peace.

‘Down Memory Lane’
by
S.Radhakrishna
(Former Chief Statistician, TRC)



Dr. Mitchison was a close friend and research collaborator of the legendary Dr. Fox who made waves with the Home vs Sanatorium study of the 1960s in Madras. Mitchison was the brilliant bacteriologist in this productive clinico-lab partnership that set the Coom on fire with its path-breaking studies on the characteristics of Indian tubercle bacilli (drug sensitivity, catalase activity, hydrogen peroxide activity, virulence in the guinea-pig) and serial serum isoniazid concentration studies that opened the gateway for intermittent regimens of chemotherapy. His role in the design of TRC’s controlled clinical trials, their conduct and data analysis continued long after he left the shores of Madras, and the postal authorities of the U.K. must have made a tiny fortune with his serial letters to his deputy in the TRC that came with a serial number so that any letter lost in the post could be immediately identified and a copy sought! It would be no exaggeration to say that the brain waves on principles of chemotherapy that were evolved and tested in Madras and several BMRC sites such as East Africa and Hong Kong had their birth in his head. His knowledge of statistical methods and design of experiments was profound and often had qualified statisticians in a spin – not surprising at all if one comes to seriously think of it for he was a nephew to the famous biostatistician J.B.S.Haldane, then permanently settled at the Indian Statistical Institute, Calcutta.



Mitchison in a pensivepose

I joined the TRC in 1956 and therefore saw him in action in the early days – EMA (Early Mitchison Activity) like EBA (Early Bactericidal Activity), had very high potency. After setting up the lab, he left for London after just one year, but his close involvement with the Centre continued for several decades through annual WHO short-term consultant visits, endless phone calls and a barrage of Mahabharat-long letters! I last met him at the memorial meeting for Wallace Fox at the Royal Society of Medicine in London in 2010, when he made a 7-minute Power-point presentation about Fox's contributions that was simply brilliant. I vividly remember that after summarising Fox's stupendous achievements in less than 10 Power Point slides, he ended his talk with a flourish of 'what a great contribution, what a man'!

And now for personal experiences. While in London during 1961-63 in connection with my research training at the MRC and Ph.D at the LSHTM, I used to visit the Mitchison home and interact with him and his family over a cup of tea and mouth-watering Scottish biscuits.



**Ruth and Denis Mitchison at her
Croyden home (1942)**

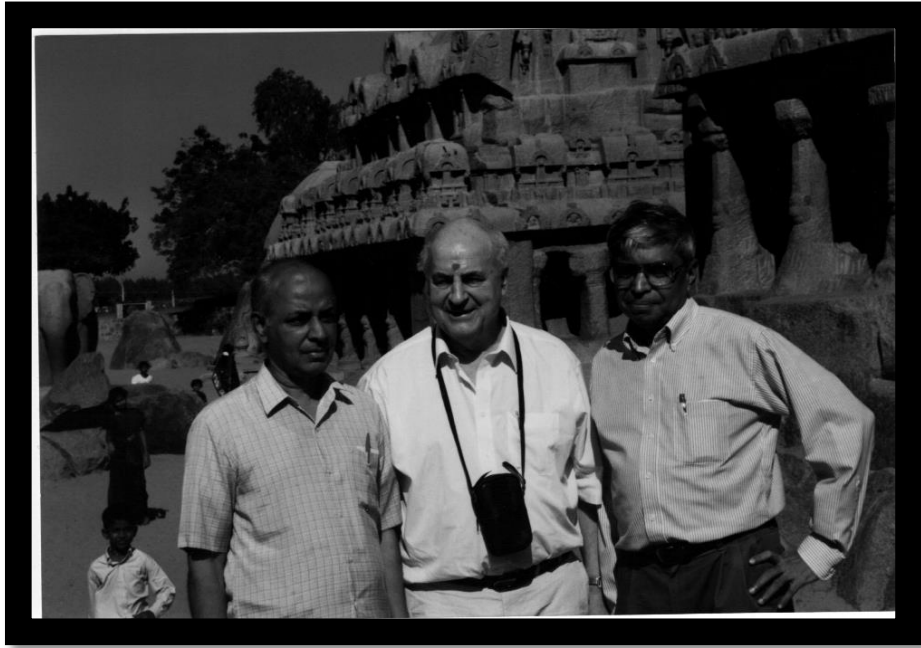


Richmond home of the Mitchisons (from 1955 onwards)

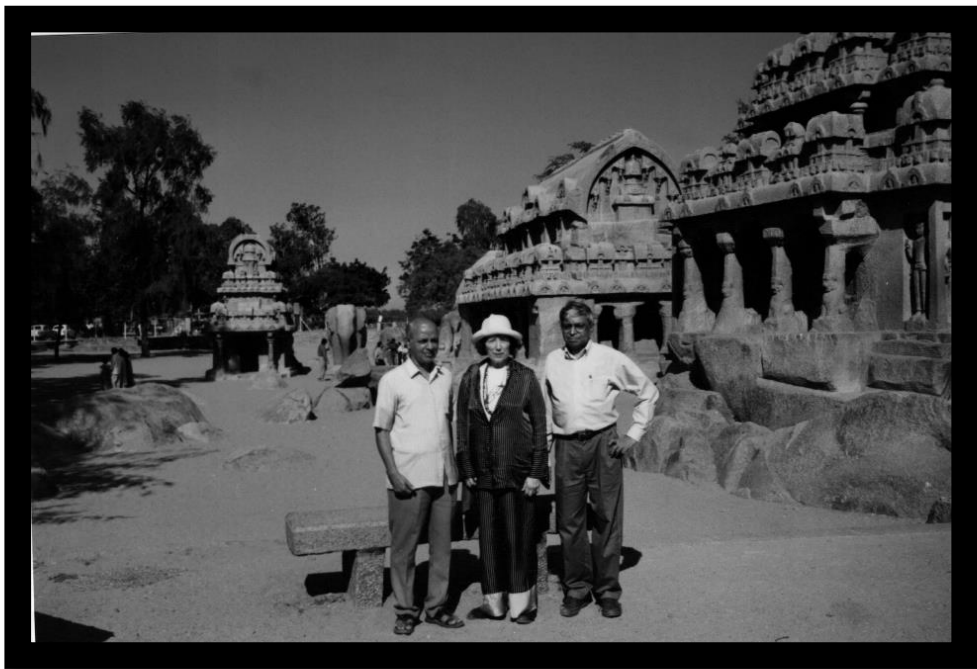
The high tea was usually preceded by a game of shuttle in their backyard, in which his children also participated, especially Graeme and Susan, the older children. We could not have too many dinners at home initially because of my strict vegetarian habits, until his wife Ruth came up with a great solution – to anoint me as chief cook and throw her kitchen open to me for the evening with a variety of vegetables and Indian condiments that she picked up at a local Indian store! The Mitchisons were hospitality personified, and I vividly recall that I was first introduced to the famous London stage by them – a visit to Haymarket theatre to see Terence Rattigan’s play ‘Ross’ which was subsequently made into a film called ‘Lawrence of Arabia’. Mitchison and his wife Ruth, their children and Subbaiah, the TCC bacteriologist on a WHO fellowship at the time, constituted the large group that went after the show for coffee and some eats at a Piccadilly joint at midnight – this was quite an unusual experience for me and a great departure from norm, steeped as I was in the conservative Madrasi culture of gulping thairusadam(Curd Rice) at 8 P.M. and jumping into bed by 9 P.M.

The MRC had an animal station at Porton in Wiltshire at which Mitchison’s group undertook virulence tests (of Indian and British strains of tubercle bacilli) in the guinea-pig. When I expressed a desire to see the activity myself, Mitchison took me there and while driving at 80 m.p.h. on the highway, he used to turn around to me in the back seat and, gesticulating with his arms, expound on some statistical point to me on the design of experiments while I shook with fear and trepidation in the back seat. At that point in time, he didn’t think of anybody’s safety – his, mine or the other two passengers! When we reached Porton, the authorities said I could not enter the lab as a visitor because it was a high security establishment – the wily Mitchison got round this by appointing me as a score recorder of autopsied organs, and I then entered the lab with mask and appropriate head gear – the transformation from Madrasi to chaprasi was then complete!

After Ruth’s demise, Mitchison married Honora, a very bubbly character who infused new enthusiasm into him and the pair came to Madras in 1998 when Subbaiah and I organised a trip to a nearby tourist attraction (coastal town, Mahabalipuram) which they greatly enjoyed.



S.Radhakrishna, D.A.Mitchison and T.V. Subbaiah in Mahabalipuram, a sea-side town near Madras (1998)



S.Radhakrishna, Honora Mitchison and T.V. Subbaiah in Mahabalipuram

When I visited London the next time in 2005, Honora took me out for lunch to an Indian restaurant in Richmond (Mitchison didn't relish Indian food) and we then went home for a cup of tea and a long chat session.



D.A. Mitchison and Honora in their London home (2005)



D.A. Mitchison and S. Radhakrishna in London home (2005)

In recent years, I had little contact with Mitchison who went into a shell after the sudden demise of Honora. But the annual exchange of Christmas cards between us continued without a break for 53 years (1963 – 2016), and it was only last year (2017) that he didn't write himself but got his daughter Clare to acknowledge my greeting and wish me well.

I understand from Gaye Fox that Mitchison died on 2nd July after a painless heart attack. His older son, Graeme, had died of a brain tumour a few months earlier and this had depressed him greatly according to his daughter Clare.

In conclusion, Mitchison was an outstanding scientist with a touch of genius, and the personification of hard work. He was a good leader, and an excellent team man who inspired all who came into contact with him. His contributions in tuberculosis are immense and well acknowledged by national and international bodies. This Institute owes its birth and instant success to him (and Fox), and owes a great debt of gratitude to him. On a lighter note, this Institute may well label him as our Founding Mother since we usually refer to Fox as our Founding Father!

Let us all take this opportunity to thank Mitchison for his labour and gigantic contributions, and pray that his soul may rest in peace.

**Remarks by P.R.Somasundaram
(Former Statistician, TRC)**



Prof. Mitchison came from a distinguished family. His mother Naomi Mitchison was a prolific novelist. Her brother Dr. J.B.S. Haldane was a distinguished biologist and a Visiting Scientist of the Indian Statistical Institute, Calcutta. His brother Dr. N.A. Mitchison was a well-known immunologist.

Prof. Mitchison stayed in Madras for one year, establishing the Bacteriology lab of the Tuberculosis Chemotherapy Centre on a sound basis, so that it was able to support the controlled clinical trials conducted by the Centre. Later he visited the Centre periodically to review the work and facilities.

Prof. Mitchison asked me two statistical questions at my interview for the post of Asst. Statistician. I was able to answer only one but was selected. I had little interaction with him, being mostly involved in routine recording of bacteriological results and analysis of data. But he gave me an excellent testimonial when I applied for the post of Asst. Director.

In Dr. Fox's Tuberculosis and Chest Diseases unit in London, he was referred to as "the Professor". During my stay at Dr. Fox's unit as a WHO Fellow, I visited Prof. Mitchison's unit at Hammersmith Hospital and had discussions with him and his long-term assistant, Ms. Jean Dickinson.

The last time I met him was around 2000 at a hotel in Adyar, when he had come to TRC as a consultant. He came to the car park to meet my wife and daughter.

He continued to work well beyond the age of 80. When most people reach the official retirement age of 60, they are still physically and mentally active, though some opt for Voluntary Retirement earlier. An alternate scheme of Voluntary Extension of service, working 3 or 4 days a week would help utilise the expertise gained.

Prof. Mitchison played a long and valuable innings in the field of tuberculosis, having reached the age of 98 years (his mother was a centurion). May his soul enjoy a well-earned rest.

**Remarks by Manjula Datta
(Former Epidemiologist, TRC)**



In 1978, the TCC was renamed the Tuberculosis Research Centre and the new building was inaugurated. I first saw Dr. Mitchison at that function. He gave a descriptive address, mainly outlining what the TRC can do, which the TCC did not cover. His talk at that time was futuristic. When I saw the number of people who crowded around him, I perked up. What was special about him?

I addressed this question in the only way I knew how. I chased up his publications. The way in which he addressed Virulence was something I don't know how to express this, because not only did I learn about Virulence, I also learned that if you formulate a research question clearly, the design of a study to answer that question would follow.....! I was young and raw, and this was for me a glimpse into a wonderful milieu. In 1979, I moved to the Tuberculosis Prevention Trial, and was never a part of main stream TRC. During that time, Mitchison used to come to TRC as a short term consultant and I went to listen to his lectures. During one such visit, I asked him “how viable count could be used to measure both virulence *and* potency of a drug?” He did not blink. Patiently he explained to me the principle of the viable count and the methodology of how it is done. I could make out that he really, really cared that I understood. This for someone whom he did not know, was not introduced to, and for all purposes a stranger. Figure out his greatness, if you can visualise this episode. I learned that viable count itself was not the outcome but actually the increase in viable count or the decrease in viable count. I saw with astounding clarity, how important it is to define the outcome measure accurately. My second lesson in research methodology. Dr. Mitchison, thank you. I also learned how you

should carry your greatness and the importance of being gracious to greenhorns. What a mentor to have! I really wish I could have worked with him.

What struck me most was the third incident that I remember. Dr. Mitchison has been talking about bacteriology services in the periphery. He did not talk from the podium. He would stay just in front of the audience with the mike in his hand. After the seminar, he was taking questions, and I distinctly remember his words. He said “If you really want to control tuberculosis, you should plan to provide bacteriology services at the district level”.

Today, almost three decades later, our program pundits are saying just that. Did Mitchison have a crystal ball in his pocket? I think it was more mundane. He could put the current knowledge about the mycobacterium, the patient with TB and the population dynamics, and arrive at what was a logical solution. He had a simplistic understanding of the science behind the facts, and an ability to think miles ahead of the times.

A colleague told me that he walked into her room, introduced himself and said “This was where I used to sit. I see that you have the same rickety fan overhead”. He had walked in with his second wife on his arm, like any newly married groom. He was excitedly showing her the room he sat and worked in, with a glow on his face and a sparkle in his eyes! He even took pictures of the room and that old ceiling fan!! As he showed her the clinic, he was as excited as if he was taking her round Buckingham Palace. A bit of nostalgia that you would not have expected from a ‘big’ man. But in that episode I saw the simplicity as a mark of his greatness.

Whenever I saw him he was jolly and pleasant. He always had a twinkle in his eye, which grew deeper if he was pulling your leg. He could pull legs with a very straight face! He was a roly-poly, jolly Santa Claus. And yes, he has left behind a veritable treasure in hearts and minds of those who knew him, and in writing for those who did not know him. I grieve the passing of an intellectual colossus. His passing is a loss, not only for science, but also for deductive reasoning and far-sighted thinking. There are not many like him now-a-days.

For TRC, he has left a set of standards which have survived nearly half a century. Keeping those standards alive, unsullied, unmitigated and undiluted is the best tribute we can pay him. If we did that we would ensure that his soul would indeed rest in peace. May we be found equal to the task!

**Remarks by Jayaraj
(Former Lab. Technician, TRC)**



I would like to illustrate certain milestones in the tremendous research in the field of tuberculosis. Prof D.A. Mitchison, who came from London with a vision and dedication to start a research centre in tuberculosis to help the poor needy people in this tropical country, decided to establish an animal house with rabbits, mice and guinea pigs for research purposes. I at the age of 20, and my team of six members, joined him in this venture, and learnt the procedure of nurturing the animals which was used primarily as models for tuberculosis.

Prof. Mitchison, who stayed in Madras only for one year, brought different strains of animals from London, and taught us how to lineage them, inject them and draw blood from the heart with half puncture without killing the animal. I learnt this technique and am capable of doing it even today without killing the animal. My technique of blood collection from guinea pigs was applauded by Prof. Mitchison, Dr. Selkon and Ms. Elsa Holst.

Prof. Mitchison, was kind enough to teach us many things related to research, and to use the equipment brought from London. In the early days of 1958 when people were afraid of even coming near a TB patient, we youngsters took up the job of Lab assistant, and put our heart and soul to bring the centre to its excellence of today. Our hard work was inspired by

the vision of Prof. Mitchison and the sincere efforts of Dr. Wallace Fox, and resulted in the establishment of an excellent animal house for research purposes.

Many of the researchers and scientists those days were English men and we youngsters from Tamilnadu initially found it difficult to communicate with them. Realising this inadequacy in us, Prof. Mitchison called us every morning at 6.00 am and taught us spoken English. My partner Mari and I showed great interest in learning this foreign language and soon learnt to communicate well in English, which made Prof. Mitchison very happy.

We were able to upgrade the TRC Animal house to become the second largest in the country, next to Haffkine Institute in Bombay. My untiring efforts and dedication took me up the ladder, and started my career as Lab assistant in Bacteriology, in which capacity I did extensive work in the preparation of LJ mediums, culture technique and drug sensitivity tests.

It is always a pleasure for me to enter the campus of TRC (now renamed as NIRT), and recall my happy days here in the company of Dr. Mitchison. I pray that his soul rests in peace.

Tribute by BeenaE. Thomas
(Social Scientist, TRC)



It is amazing that there are some people who make such a lasting impression with their contributions during their life time that even when they no longer exist on planet earth they continue to live.

I have been hearing about one such person – Dr. Mitchison whom I never got to meet but I was instrumental in getting a memorial meeting arranged for him at the NIRT (National Institute for Research in Tuberculosis (the then TCC)) after he passed on a few months ago.

What impressed me the most was that those who worked with him, Dr. S.Radhakrishna, Dr. S.P. Tripathy, both in their eighties, were willing to travel long distances to come to Chennai NIRT to pay such wonderful tributes to him. Barely able to walk, they brightened up as they went on stage taking the audience which brimmed in the auditorium down memory lane on their experiences with Dr. Mitchison. They told us how they had the privilege of working with Dr. Mitchison as he was instrumental in building up the laboratory in NIRT which continues to be the integral part of all the research activities carried out in the centre. They spoke about his attention to detail, his commitment and zeal, his important contributions to EBA (Early Bactericidal Activity) which continues to be relevant even today with drugs used for TB management. They talked about his selfless contribution and his keenness to build up the capacity of the laboratory and the people who worked in this department so that they could carry forward the work even after he left. What impeccable planning, training and implementation to further scientific research in a field that was still nascent till he came and led the path ahead.

My only question is why did we not pay tributes to Dr. Mitchison as a NIRT family when he visited us thereafter on many occasions after he left to the UK? Why did we not give him an opportunity to listen to what we thought of him and tell him that we are deeply indebted and grateful to him. He would have been happy that we continued to cherish his contributions to the then TCC becoming TRC and then a centre of national significance – National Institute for Research in Tuberculosis (NIRT) and that he had a major role to play.

Dr. Mitchison, I just want to say that even though I did not have the fortune of meeting you, I had the fortune of having organised a great memorial gathering for you. If I ever get to meet you in the other world, I would tell you in detail about that memorial gathering which flooded the auditorium.....those who travelled from afar, those who had retired, and those who are currently working, representatives from all departments who helped me understand what a great human being you have been.

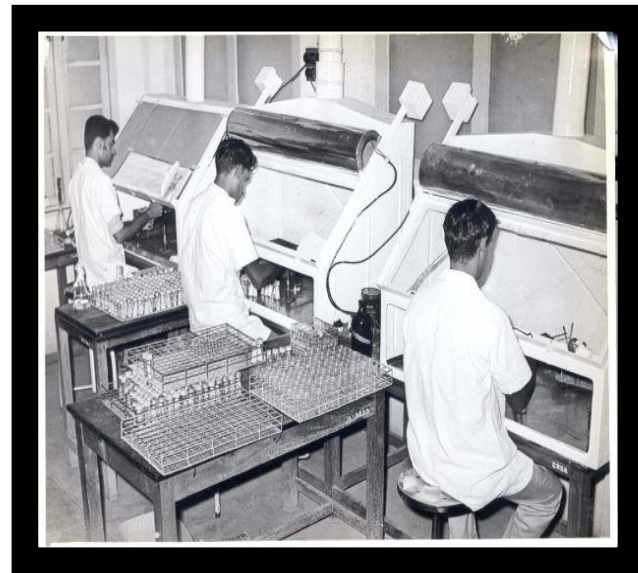
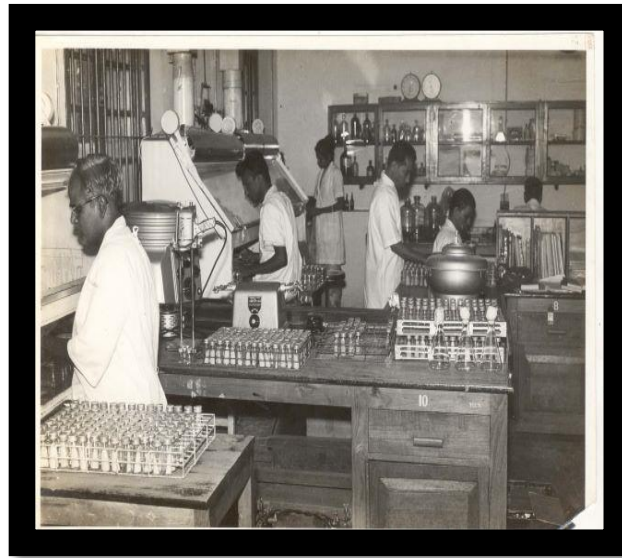
Reminiscences of N.S.Gomathi
(Senior Technical Officer, Bacteriology)



I once had an opportunity to look at some old photographs of the lab set up by Dr. Mitchison. Despite being primitive, the lab looked very neat and compact with all the instruments conveniently and effectively placed. The cabinets used for handling specimens and cultures appeared to be good, providing protection for the workers. Some of the shakers, steel racks that were in use then are still in use in our lab! We still follow the system of discarding contaminated articles into disinfectant baths, and since NIRT trained most of the labs in India, I guess many others in India do so too.

I had a chance to see Dr. Mitchison once in the late 90s when he was on a visit to the Centre as a W.H.O. short-term consultant. I was a lab technician at the time, with little prior knowledge of him or his contributions to NIRT and TB research. But from the way Dr. Paramasivan, our Department Head, was making preparations for the visit, I realised that this was a V.I.P. coming. I was then involved in a study on Early Bactericidal Activity. We had been instructed to count the number of colonies of tubercle bacilli grown over a very small area on 7H11 medium. We had to count even up to 200 in some instances, and that was a laborious task. As we were reporting only grades in routine practice, I wondered why the insistence on counting colonies instead of simply recording a 3+.

Dr. Mitchison entered the lab, looked at our plates and records and expressed satisfaction that two of us were taking independent readings of the colonies, and were cross checking each other's values. Impulsively, I asked Dr. Mitchison why we needed to take the trouble of counting instead of just recording the grade. He looked at me for a second pretty pleased, and explained the concept of EBA beautifully. More importantly, he appreciated technical staff coming forward to understand the procedures instead of doing them mundanely. I realised then how very committed and large-hearted he was that he had taken pains to explain something complex to a young fledgling. This incident made an everlasting impression on me of the greatness of the man.



Message from T.V.Subbaiah (Former Bacteriologist, TCC)

I am sharing some of my thoughts on Prof. Denis Mitchison, and to emphasise how much his absence makes us poorer in our knowledge on strategies for successful chemotherapy of tuberculosis and eventual eradication of this dreaded scourge. He was a Colosuss, hidden as a Saint. Prof. Mitchison, as he preferred to be addressed, was a Giant of a Pioneer in the Architecture and Strategy of Domiciliary Chemotherapy of Human Tuberculosis...a joint enterprise undertaken with all the risks of a new venture beginning in the early 1950s by him and Dr. Wallace Fox, under the joint aegis of MRC of UK, WHO, ICMR and Madras Government. The standardizations needed to quantitate both clinical parameters and correlating laboratory tests needed a superhuman effort at the time, particularly in the complete absence of automated and advanced techniques that are now available. Those in the lab -- me, Dr. Gangadharam, Sreenivasan, Ramamoorthy and Alexander, forming the backbone of the Technical Support--were challenged not only to come up and reach the standards of the MRC of UK, but streamlined to fit into architecture obligated to stand statistically verifiable critique with clinical correlates impacted by short and long-term mono and combination anti-tuberculosis chemotherapy. That these were accomplished by the young and nascent team under the leadership of Dr. Mitchison to the global recognition is an eloquent testimony of the TCC lab.

The foundation Dr. Mitchison laid at TCC stand testimony to the acumen, and his deep understanding of the disease and its causative agents that coexist often hidden and insulated in the host. No job was too small for him. He tirelessly participated in what many may call menial tasks.....repairing a packaged gas plant in the lab at midnight for the lab to function next morning, standardize inoculation loop size to lift exact amount tubercle bacilli for drug sensitivity tests, sterilizing the egg shells before collecting the fluid got inspissation etc. No detail was too small for him, nor was a complex animal experiment, particularly with hamsters, too difficult. He was The Perfect Scientist.

Prof. Mitchison was a trusted colleague and friend, in all its true meaning giving guidance at all times. He shared his family value systems. He brought to the TCC lab his maternal uncle Prof. J.B.S. Haldane. We were all very impressed by the latter's informal eminence. Similarly, Prof. Mitchison brought his mother Mrs. Naomi Mitchison (her husband was a Labour MP), eminent author who had spent her time in Swaziland. I had also the pleasure of meeting at his residence in UK all his brothers who were themselves great scientists in immunology, cell and molecular biology. I was fortunate to catch up with Prof. Mitchison a few years ago in Chennai (Mahabalipuram) when he was there on a brief visit. I had the pleasure of playing host to Prof. Mitchison and his family during their holiday visit to western India's Ajanta and Ellora Caves in Aurangabad where he delivered Guest Lectures and then went on a visit to Kashmir.

Dr. Mitchison was my mentor all the time. I am what I am today because of him. There is never going to be a time for me without Him now or later.

**Message from P.R.Narayanan
(Former Director, NIRT)**

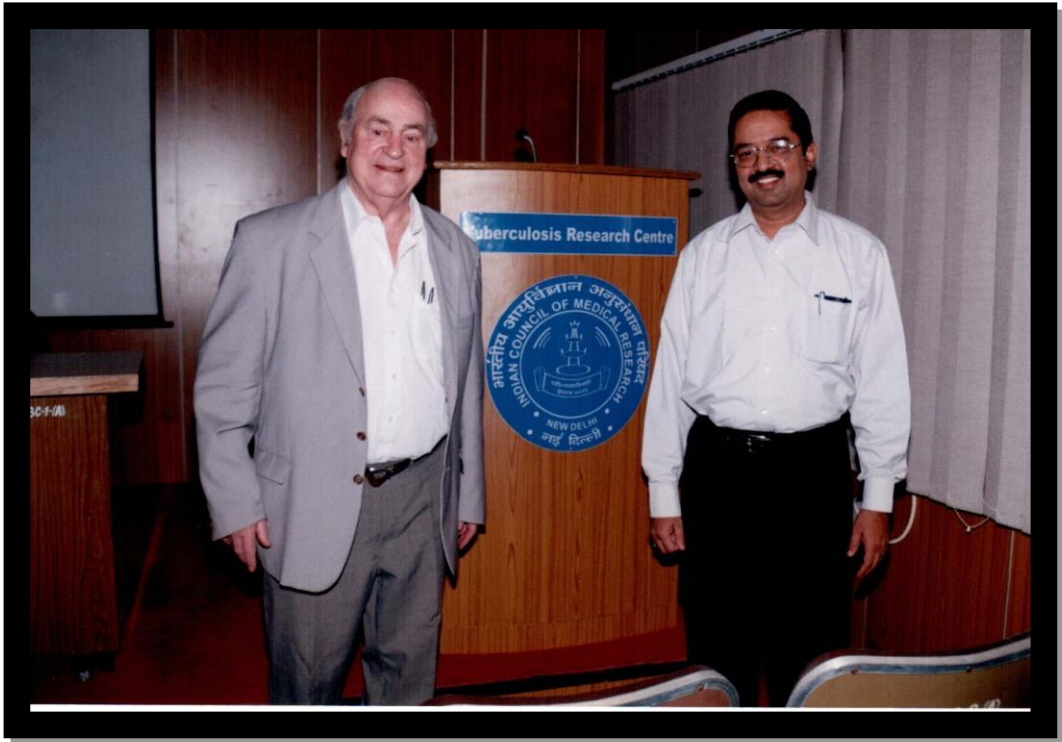
NIRT must be really saddened to hear of the passing of one of the leading TB bacteriologists, Prof. Denny Mitchison, at the age of 98. As a previous employee of NIRT I share their sadness.

With his erstwhile colleague, the late Dr. Wallace Fox, Denny Mitchison designed the most famous Madras Study that changed globally the way TB patients were treated.

He established TB laboratories in many countries. He not only established TB laboratory at TCC, presently known as NIRT, but also guided research for many decades. He authored more than 250 publications and received many prestigious global awards.

His characteristic laugh, his sudden silence during heated discussion of protocols, his humorous discussion on English versus Chennai breakfast over a discussion for a study at TRC, his strong opinion on EBA studies that he wanted to start at TRC that never got started and many more can never be forgotten by me. I can also not forget what a great host he was when on more than one occasion he had invited some of us to his home in London.

Prof. Mitchison was a founding member of TB Alliance's Scientific Advisory Committee at New York for more than 5 years and I had the privilege of being a member of that SAC along with him. His strong opinion during discussions always used to generate lot of heat but the reward of rich learning experience for many of us in the committee kept us cool. We will miss a genuinely true knowledgeable expert. His loss is a loss to TB world. I pray that his soul may rest in peace.



**Prof .D.A.Mitchison and P.R.Narayanan (Director)
in Robert Koch Auditorium, NIRT**



P.R.Narayanan, D.A.Mitchison and C.N.Paramasivan at NIRT

**Message from C.N.Paramasivan
(Former Bacteriologist, NIRT)**

My association with Prof. Mitchison began sometime in 1981 during one of his periodic visits to TRC. I had spent one year (1983 - 1984) with him at RPMS, London. He gave me limitless freedom to work on my own and allowed me to interact with many stalwarts at that time including eminent epidemiologists like Prof. Paul Fine and very many others in UK and elsewhere, although I heard different views about his attitude before going. I had the luxury of establishing a hybridoma lab to raise monoclonal antibodies and he had allowed me to order all my requirements without any hesitation. It was a challenging period and I also worked 10 - 12 hours on any given day and almost on all Saturdays! Truly it was a golden period! I also recall with gratitude the help and support received from his principal Lab Technologist, Brian Allen and other scientists including Doug Lowrie and ARM Coates, presently at St.Georges Hospital Medical School.

I was also associated with him participating in a few multicentric trials thereafter and we had published close to a dozen papers (mostly *in vitro* simulation studies on quinolones) even after my migration to FIND in Geneva. He was always very kind to me and taught me a great deal of science and elementary statistics!

Prof. Mitchison gave a private dinner to 20 to 25 persons on his 90th birthday in London, and this was followed by a special symposium. St. Georges Hospital Medical School conducted this to honour him on this occasion. From FIND, Geneva, my CEO, Giorgio Roscino, and another senior colleague, Rick O'brien were invited along with me. Jacques Grossette was also invited. Amina Zindani, who had published the first EBA paper under the guidance of Prof. Mitchison, took special interest in organizing this event.

During my stay in Geneva till December 2013 he used to occasionally write to me for some details or clarifications on newer diagnostics. My last meeting with him was in October 2016 at his residence and Amina Zindani facilitated my visit through his son. While returning from Liverpool after the UNION Global meeting I had spent a day in London for this purpose. Although I was warned about his periodic temporary memory lapses, I found him to be very lucid and he was talking mostly on the new drug trials and lab support.

I always considered it was my good fortune to have known him.

Photographs taken at the Condolence Meeting

Lighting of the Lamp



Lighting of the lamp by S.Radhakrishna



Lighting of the lamp by P.R.Somasundaram



Lighting of the lamp by Dr. Srikanth Tripathy



View of the audience



Dr. Srikanth Tripathy, Dr. SP Tripathy & Dr.S. Radhakrishna



**Dr. SP Tripathy, Dr.S. Radhakrishna, Mr. P.R. Somasundaram &
Dr. K. Ramachandran**



View of assembled guests



Fox & Mitchison Conference Room at NIRT

**Scientific Publications of
Prof. D.A. Mitchison
from
MADRAS TB CENTRE**

**PUBLICATIONS OF PROF.D.A.MITCHISON FROM MADRAS CENTRE
FOR THE PERIOD 1958 – 2009**

1. Gangadharam PRJ, Mitchison DA, Subbaiah TV, Short EI. The detection of isoniazid in urine. *Tubercle* 1958; 39:191-200.

Three tests for the presence of free isoniazid in urine have been compared in trials on volunteers and patients. Of these, the Short and Case Direct Naphthoquinone-mercuric chloride N-M test was found to be the most sensitive. However, it failed to detect the excretion of isoniazid after a 100 mg. dose by mouth in about 10 per cent of the individuals.

A simple alkaline hydrolysis procedure for conversion of isoniazid metabolites to free isoniazid has been developed and applied to the Direct N-M test. The resulting Combined N-M test detects isoniazid in 98 per cent of urine specimens collected from 0 to twelve hours after taking 100 mg. by mouth and in 80 per cent of specimens collected between twelve and twenty two hours.

Since PAS gives positive results in this test, it cannot be used to detect isoniazid when PAS and isoniazid are being given together to patients. However, when both these drugs are given in the same cachet, the ferric chloride test for PAS can be used to investigate drug taking.

2. Holst E, Mitchison DA, Radhakrishna S. Examination of smears for tubercle bacilli by fluorescence microscopy. *Indian J Med Res* 1959; 47: 495-499.

Equipment for fluorescence microscopy that can be used in normal daylight has been in use at the Tuberculosis Chemotherapy Centre, Madras, for over two years. When it was first introduced, a comparison between this method and the conventional Ziehl-Neelsen method was undertaken to test their relative sensitivities on 1383 routine specimens. This showed that fluorescence microscopy yielded as many positive smears, and had no greater tendency to produce false positive (i.e. smear-positive, culture-negative) results.

3. Tuberculosis Chemotherapy Centre. A concurrent comparison of home and sanatorium treatment of pulmonary tuberculosis in south India. *Bull World Health Organ* 1959; 21:51-131.

In India, as in most under-developed countries, the tuberculosis problem is aggravated by an acute shortage of sanatorium beds. The number of active cases of tuberculosis in the country has been estimated at 2½ million, but only 23,000 tuberculosis beds are available. In these circumstances great importance attaches to the possibility of applying mass domiciliary chemotherapy as a substitute for sanatorium treatment in cases of pulmonary

tuberculosis. The findings of the present study, based on a comparison of the two types of treatment over a period of 12 months, show that despite the manifest advantages of sanatorium care - rest, adequate diet, nursing and supervised medicine-taking - the merits of domiciliary chemotherapy are comparable to those of sanatorium treatment, and that it would therefore be appropriate to treat the majority of patients at home, provided an adequate service were established.

4. Mitchison DA, Wallace JG, Bhatia AL, Selkon JB, Subbaiah TV, Lancaster MC. A comparison of the virulence in guinea pigs of south Indian and British tubercle bacilli. *Tubercle* 1960; XLI:1-22.

Cultures of tubercle bacilli were obtained from 83 South Indian and 29 British patients with radiological evidence of tuberculosis, who were aged 12 years or more and had not had more than two weeks of chemotherapy. All the cultures were sensitive to isoniazid and streptomycin and all were proved to be tubercle bacilli by a variety of identification tests. All the Indian cultures were catalase positive. Of the 63 Indian and 14 British cultures examined, all yielded positive niacin tests, indicating that they were of human type.

The guinea-pig virulence of these cultures was investigated in five series of experiments, two in London, two in Madras and one at Porton. In these experiments three breeds of animals were used, one of which was bred both in London and in Madras. A dose of 1 or 0.1 mg. bacilli was injected intramuscularly, the guinea-pigs were killed six and twelve weeks later and the amount of disease assessed by a score.

As assessed by scores, guinea-pig mortality and culture of guinea-pig spleens, the virulence of Indian cultures was on the average lower than that of British cultures. The Indian cultures had a wide range of virulence, about 30 per cent being as virulent as British cultures, while the remainder were less virulent. The least virulent cultures produced little more than local lesions, and healing occurred between six and twelve weeks, demonstrated both by scores, spleenculture and histological examination. Histological evidence of healing occurred to a greater extent in the spleen and liver than in the lungs. The British cultures were of homogenous high virulence.

The best measurement of virulence was found to be the 'mean index', defined as the score divided by the survival time of the animal. The mean index combined the results of scores and mortality in both the 6-week and 12-week animals, so that additional information was obtained without loss of precision in measuring virulence. As judged by the mean index, Indian cultures were less virulent than British cultures in all the five series of experiments. The scoring procedure was found to be reliable and repeatable in serial experiments. Different observers obtained closely similar scores on the same animals and the results of experiments could be accurately duplicated when carried out in different countries.

The Mantoux reactions of guinea-pigs tested with 100 TU of Old Tuberculin four weeks after infection with Indian cultures were found to be on average the same as those infected with British cultures, but the reactions were more variable in size.

5. Subbaiah TV, Mitchison DA, Selkon JB. The susceptibility to hydrogen peroxide of Indian and British isoniazid-sensitive and isoniazid-resistant tubercle bacilli. *Tubercle* 1960; XLI: 323-333.

A standard, bactericidal, hydrogen peroxide sensitivity test was developed, in which a viable count was done on strains that had been exposed to 0.02 per cent hydrogen peroxide for 90 min. at 37°C. This test has been found to estimate accurately the proportion of catalase-positive, isoniazid-sensitive organisms in a mixture with catalase-negative, isoniazid-resistant organisms.

Among strains from British patients, 8 with full sensitivity to isoniazid were uniformly resistant to peroxide, and 7 which were isoniazid-resistant but still retained some catalase activity, were more susceptible to peroxide, but contained at least 0.11 % peroxide-resistant organisms. In contrast, among strains from Indian patients, 4 of the 7 isoniazid-sensitive, catalase-positive strains were more susceptible to peroxide than any of the British strains and 3 of the 8 isoniazid-resistant, catalase positive strains contained 0.018 % or less peroxide-resistant organisms. All of the 9 catalase-negative, isoniazid-resistant strains from both British and Indian patients were completely susceptible to peroxide.

An association was found between low catalase activity, high susceptibility to peroxide and a high degree of resistance to isoniazid. This association was clearer among British than among Indian strains.

6. Subbaiah TV, Selkon JB, Bhatia AL, Mitchison DA, Radhakrishna S. The addition of para-aminobenzoic acid for catalase to Löwenstein-Jensen medium and the effect of prolonged incubation in the culture of tubercle bacilli. *Tubercle* 1960; XLI: 334-340.

A total of 1792 sputa from tuberculous patients before treatment or during treatment with isoniazid and PAS was as cultured on medium with and without the addition of 10µg/ml. p-aminobenzoic acid. Of these, 528 specimens yielded positive cultures, the amount of growth and the speed of growth being the same on the two media. The failure to achieve improved results with the PABA medium has been attributed to dilution of PAS in the sputum by washing of the centrifuged deposit during culture.

A total of 2814 sputa from patients mainly receiving isoniazid and PAS, isoniazid alone or no treatment, was as cultured on Löwenstein-Jensen medium with and without the addition of 2µg. catalase. No difference was found between the media in the amount and

the speed of growth among the 818 specimens yielding positive cultures, nor was the growth of isoniazid-resistant, catalase-negative organisms improved by the addition of catalase.

In a sample of 1138 sputa, prolonging the period of incubation from 8-9 weeks to 16-17 weeks increased the percentage of positive cultures from 39.1 % by only an additional 0.4%.

The culture method employed in controlled chemotherapeutic trials at the Tuberculosis Chemotherapy Centre, Madras, and in East Africa does not appear to have biased the bacteriological assessments of the results.

7. Tuberculosis Chemotherapy Centre. A concurrent comparison of isoniazid plus PAS with three regimens of isoniazid alone in the domiciliary treatment of pulmonary tuberculosis in south India. *Bull World Health Organ* 1960; 23: 535-585.

Recent studies have shown that treatment of pulmonary tuberculosis with isoniazid plus p-aminosalicylic acid (PAS) at home is, in the majority of cases, as satisfactory as treatment with the same combination of drugs in sanatorium and does not appear to expose the patient's contacts to any special risk. Before mass domiciliary chemotherapy can be introduced, however, a question that has to be decided is what drug or drugs and what dosage and rhythm of administration will be most effective.

This paper presents the results of a controlled comparison of four chemotherapeutic regimens:

- (a) 3.9-5.5 mg/kg body-weight of isoniazid plus 0.2-0.3 g/kg body-weight of PAS (sodium salt) daily in two doses (the standard combined chemotherapy);
- (b) 7.8-9.6 mg/kg body-weight of isoniazid alone daily in one dose;
- (c) 7.8-9.6 mg/kg body-weight of isoniazid alone daily in two doses;
- (d) 3.9-5.5 mg/kg body-weight of isoniazid alone daily in two doses.

Isoniazid plus PAS regimen (a) proved to be the most satisfactory; it was clinically effective and there were very few toxic manifestations. The two isoniazid alone regimens given as two doses a day (c and d) were unsatisfactory in their clinical effectiveness, and peripheral neuritis was a complication with the larger dosage. In contrast, the isoniazid alone regimen consisting of a single daily dose of 7.8-9.6 mg/kg (b) was far more satisfactory, though peripheral neuritis was again a disadvantage.

8. Selkon JB, Subbaiah TV, Bhatia AL, Radhakrishna S, Mitchison DA. A comparison of the sensitivity to p-aminosalicylic acid of tubercle bacilli from south Indian and British patients. *Bull World Health Organ* 1960; 23:599-611.

The sensitivities to PAS of strains of tubercle bacilli obtained before the start of anti-tuberculosis chemotherapy from totals of 147 Indian and 93 British patients have been compared in three investigations, one in Madras, one in London and one simultaneously in both places.

Sensitivity tests were set up on slopes inoculated with about 105 viable units, and the minimal concentrations of PAS inhibiting the growth of 20, 50 or 100 colonies were read. In each investigation, more of the Indian than of the British strains were resistant to PAS with the 20-colony end-point, but no differences were apparent with the 50-colony and 100-colony end-points. Thus, Indian strains contained a small proportion (0.02 or slightly less) of resistant organisms not present in British strains.

Variation from patient to patient in the sensitivity of these strains was of similar magnitude for Indian and British patients. Thus the presence of the resistant organisms was a general characteristic of Indian strains and there was no evidence that a higher proportion of Indian patients had been infected with resistant strains.

A chance factor, such as an increase in inoculum size in the sensitivity test, would allow the resistant organisms characteristic of Indian strains to yield 20 or more colonies on slopes containing the higher PAS concentrations, thus causing the strain to be called resistant in routine tests. Under these circumstances resistant strains would appear sporadically and would not be related to any special tendency for the patient to fail to respond to treatment with PAS. Similarly, a considerable increase in the apparent prevalence of PAS-resistant strains, which occurred between two controlled chemotherapy trials on Indian patients, could be accounted for by a slight increase in the average size of the inoculum used in the tests during these trials.

A tenfold decrease in the inoculum size in PAS sensitivity tests on strains from Indian patients is recommended for future work.

9. Bhatia AL, Devadatta S, Mitchison DA, Radhakrishna S, Selkon JB, Subbaiah TV. The consistency of the virulence in the guinea pig of tubercle bacilli isolated on different occasions from south Indian patients before treatment. *Tubercle* 1961; 42: 317-321.

Isoniazid- and streptomycin-sensitive cultures of tubercle bacilli, isolated pre-treatment from 12 South Indian patients when they first attended the Centre, and at 7 and 42 days thereafter, were tested for their virulence in the guinea-pig.

The variation in virulence between patients was greater than the variation between cultures from the same patient. Thus, consistent differences exist between the virulence of strains of tubercle bacilli isolated from different Indian patients.

No systematic alteration in virulence occurred during the 42-day period, during which no patient was receiving anti-tuberculosis chemotherapy. The variation in virulence between cultures obtained from the same patient was no greater than could be attributed to natural variation in the response of the guinea pigs.

10. Mitchison DA, Bhatia AL, Radhakrishna S, Selkon JB, Subbaiah TV, Wallace JG. The virulence in the guinea pig of tubercle bacilli isolated before treatment from south Indian patients with pulmonary tuberculosis. 1. Homogeneity of the investigation and a critique of the virulence test. *Bull World Health Organ* 1961; 25:285-312.

Virulence tests in the guinea-pig were done on 281 isoniazid-sensitive cultures obtained from the same number of Indian patients on admission to a study of various regimens of domiciliary chemotherapy in the treatment of pulmonary tuberculosis, and on 93 cultures from newly diagnosed, untreated British patients. The tests on 254 of the Indian cultures and on 65 of the British cultures were in DH-breed guinea-pigs at Porton, and the remaining 27 Indian cultures and 28 British cultures were tested in M-breed guinea-pigs at Madras.

In the test, 1 mg of each culture was injected by the intramuscular route into two guinea-pigs for 125 Indian cultures and all 65 British cultures. Half the animals were sacrificed at 6 weeks and the other half at 12 weeks; the extent of tuberculosis in the organs was scored at the post-mortem examination, the maximum score per guinea-pig being 100. Animals dying before the appointed day were similarly scored. The score on each animal was divided by its survival period to give an index. The mean of the square roots of the 6-week index and the 12-week index (the root-index of virulence) was taken as the measure of virulence since it was found to be more acceptable for the analysis of variance technique than the index of virulence used in an earlier publication.

Of the 254 Indian cultures in the Porton series, 143 were stored at -20°C for 44-78 weeks (average, 62 weeks) before being tested. A comparison carried out on pairs of cultures, one stored at -20°C and the other tested fresh, from 20 Indian patients showed no clear evidence of alteration in virulence.

The tests were done in 13 experiments at Porton and in 12 experiments at Madras over a period of two-and-a-half years. The results on the Indian and British cultures in both series, and on strain H37Rv, set up as a control in the majority of the experiments at

Porton, indicated that inter-experimental variation was small in the Porton series and could not be detected in the Madras series.

In the tests on strain H37Rv, variation in the preparation of the infecting suspension did not appear to influence the root-indices of virulence, nor were the viable counts on the suspensions of Indian and British cultures associated with the values of the root-index. However, a known 10-fold decrease in the dose of bacilli lowered the root-index to a small extent.

To obtain comparable results throughout the study, the root-indices of virulence in the Madras series were adjusted to those in the Porton series by allowing for differences in the means and standard deviations of the distributions for the two series. The adjustment appeared to be successful, since the adjusted root-indices in the Madras series were the same, within the limits of error of the test, as the root-indices in the Porton series obtained in tests done in both laboratories on cultures from the same 28 Indian patients.

The results of the tests in the Porton series indicate that to kill all guinea-pigs six weeks after infection would have the advantages of greater efficiency in detecting differences in the virulence of the cultures, of yielding results more acceptable for the analysis of variance technique, and of rapidity.

Cultures of high virulence produced fewer deaths from tuberculosis and lower root-indices in the DH-breed than in the M-breed guinea-pigs, whereas cultures of low virulence produced higher root-indices in the DH-breed guinea-pigs. In consequence, differences in virulence were shown less efficiently with the DH-breed guinea-pigs, about three DH animals being of equal efficiency to one M-breed animal.

11. Bhatia AL, Csillag A, Mitchison DA, Selkon JB, Somasundaram PR, Subbaiah TV. The virulence in the guinea pig of tubercle bacilli isolated before treatment from south Indian patients with pulmonary tuberculosis. 2. Comparison with virulence of tubercle bacilli from British patients. *Bull World Health Organ* 1961; 25:313-322.

A culture of tubercle bacilli was obtained from the sputum of each of 281 South Indian and 93 British patients with pulmonary tuberculosis, of whom all except two had not had more than two weeks of anti-tuberculosis chemotherapy. The virulence in the guinea-pig of these cultures was examined in two series of experiments, one at Porton in which 254 Indian and 65 British cultures were tested, and the other at Madras in which tests were done on 55 Indian and 28 British cultures. Cultures from 28 Indian patients were examined in both series.

The guinea-pigs were each injected with 1 mg of bacilli by the intramuscular route, and were killed 6 or 12 weeks later. The amount of visible disease at post-mortem examination was given a score. The best measure of virulence was considered to be the root-index, defined as the square root of the ratio of the score to the survival period of the guinea-pig.

As assessed by the mortality from tuberculosis in the guinea-pig, by the root-indices of virulence and by the results of culture of the guinea-pig spleens, the Indian cultures were found to be less virulent, on the average, than the British cultures in both the Porton and the Madras series. The Indian cultures also had a wider range of virulence; about one-third were as virulent as the British cultures and about one-third were attenuated to the extent that visible disease was usually confined to the site of inoculation and its draining lymph-nodes. The true variation in the root-indices of virulence was three times larger with the Indian than with the British cultures.

The diameter of the Mantoux reactions of guinea-pigs tested with 100 TU of Old Tuberculin four weeks after infection with the Indian cultures was found to be, on the average, the same as in those infected with the British cultures. The British cultures appeared homogeneous in their ability to cause tuberculin allergy, but the Indian cultures were heterogeneous.

12. Ramakrishnan CV, Bhatia AL, Fox W, Mitchison DA, Radhakrishna S, Selkon JB, Subbaiah TV, Velu S, Wallace JG . The virulence in the guinea pig of tubercle bacilli isolated before treatment from south Indian patients with pulmonary tuberculosis. 3. Virulence related to pretreatment status of disease and to response to chemotherapy. *Bull World Health Organ* 1961; 25:323-338.

This is the last of a series of three reports from the Tuberculosis Chemotherapy Centre, Madras, on a study undertaken with the object of finding out whether differences in the virulence in the guinea-pig of tubercle bacilli isolated from South Indian tuberculous patients before the start of chemotherapy are related to the severity of the patients' disease on admission to treatment and to the subsequent response to chemotherapy. The 281 patients in this study were drawn from the patients admitted to a 1-year comparison of four domiciliary chemotherapeutic regimens: (a) 3.9-5.5 mg/kg isoniazid plus 0.2-0.3 g/kg sodium PAS daily, divided into two doses (PH series); (b) 7.8-9.6 mg/kg isoniazid alone daily in one dose (HI-1 series); (c) 7.8-9.6 mg/kg isoniazid alone daily, divided into two doses (HI-2 series); (d) 3.9-5.5 mg/kg isoniazid alone daily, divided into two doses (H series).

No evidence was found of an association between the virulence of the organisms and any pre-treatment condition of known prognostic importance. There was no association between pre-treatment virulence and progress during treatment in the PH series (the most effective regimen). In the other series, however, the progress was more satisfactory in patients infected with organisms of low virulence than in those infected with organisms of high virulence, the association between virulence and progress attaining statistical significance in the combined HI-2 and H series (the least effective regimens) and only just failing to do so in the smaller HI-1 series.

Possible explanations are put forward both for the absence of an association between virulence and severity of disease on admission and for the presence of an association between virulence and response in the patients treated with isoniazid alone.

13. Subbaiah TV, Bhatia AL, Geraghty E, Mitchison DA, Radhakrishna S, Selkon JB. The virulence in the guinea pig of isoniazid-sensitive tubercle bacilli isolated from south Indian patients before treatment and after three months of chemotherapy. *Bull World Health Organ* 1961; 25: 759-764.

In order to find out whether chemotherapy with isoniazid affects the virulence in the guinea-pig of tubercle bacilli that do not develop resistance to the drug, virulence tests were carried out on isoniazid-sensitive cultures obtained from 20 South Indian tuberculous patients before treatment and after three months of chemotherapy with isoniazid. No significant difference in virulence was observed between the cultures obtained on admission to treatment and those obtained after three months of chemotherapy. This is a finding with important implications for large-scale surveys of the distribution of attenuated strains of tubercle bacilli from untreated patients in India and other countries. Detailed and repeated inquiries as to previous chemotherapy are not important in such surveys, provided that sensitivity tests are done on all the cultures.

14. Devadatta S, Bhatia AL, Andrews RH, Fox W, Mitchison DA, Radhakrishna S, Ramakrishnan CV, Selkon JB, Velu S. Response of patients infected with isoniazid-resistant tubercle bacilli to treatment with isoniazid plus PAS or isoniazid alone. *Bull World Health Organ* 1961;25:807-829.

This study from the Tuberculosis Chemotherapy Centre, Madras, compares patients infected with isoniazid-resistant tubercle bacilli ("R" patients) with those infected with isoniazid-sensitive tubercle bacilli ("S" patients) as regards, first, their pretreatment status and, secondly, their response to a year's chemotherapy, either with isoniazid plus p-aminosalicylic acid (PAS) or with isoniazid alone. With regard to the first comparison, there was, on admission to treatment, little difference between the R and S patients in terms of the extent of the radiographic lesion, the extent of cavitation or the bacterial content of the sputum but there were major differences in the age and sex distributions, the

R patients showing a greater preponderance of young males than the S patients. As to the second comparison, statistically significant differences in the bacteriological response to treatment of the S and the R patients were observed in both the isoniazid-plus-PAS and the isoniazid-alone series, the response of the S patients being much better than that of the R patients. When the response to treatment was assessed in terms of radiographic progress and weight changes, however, hardly any difference was observed between the progress of the S and the R patients. The reasons for the response in the R patients are discussed.

15. Wallace JG, Mitchison DA, Rees RJW, Bhatia AL, Gangadharam PRJ. The virulence of south Indian tubercle bacilli in mice and guinea pigs infected by the intravenous route. *Tubercle* 1961; 42: 212-217.

Cultures of tubercle bacilli from 7 South Indian patients, selected for their low virulence by the intramuscular route in the guinea-pig, and from 6 British patients, were tested for their virulence in the mouse by injecting 0.1 mg. and 0.025 mg. intravenously. With the higher doses no differences were found between the Indian and British cultures in the proportions of mice that died from tuberculosis or in their median survival periods. With the lower dose there was a suggestion that the mice infected with Indian cultures survived longer.

Guinea pigs were infected intravenously with cultures from 2 Indian and 2 British patients, and the number of viable organisms in their spleens were counted at intervals thereafter. Following an initial 2 weeks of growth in the spleens, during which there were only small differences in the growth rates of virulent and attenuated bacilli, the counts on the most attenuated of the Indian cultures decreased, whereas the remaining 3 cultures continued to multiply and killed the guinea-pigs.

The failure to demonstrate the difference in virulence between cultures from Indian and British patients in the mouse is attributed mainly to the route of infection and a consequent lower dosage in the visceral organs. The manifestations in experimental tuberculosis of attenuation shown by isoniazid-sensitive cultures from Indian patients and by isoniazid-resistant cultures from patients of other races are closely similar.

16. Subbaiah TV, Selkon JB, Bhatia AL, Radhakrishna S, Mitchison DA. Addition of catalase to Lowenstein-Jensen medium. *Tubercle* 1961; 42: 537-538.

Sputum specimens expected to contain drug-resistant tubercle bacilli were decontaminated with sodium hydroxide and the washed deposit was inoculated onto 2 slopes of Lowenstein-Jensen medium, one of which contained catalase and one which did not, arranged in a random order prior to the inoculation. The cultures were incubated at 37°C and were read at weekly intervals for 8-9 weeks; their identity was concealed until the readings were completed. All positive cultures were tested for their sensitivity to isoniazid

and for their catalase activity. A total of 124 specimens from the same number of British patients yielded positive cultures on one or both slopes. The amount of growth obtained on medium which contained catalase was similar to that on medium which did not contain catalase, and, in particular, the addition of catalase did not promote the growth of isoniazid-resistant, catalase-negative cultures.

17. Tuberculosis Chemotherapy Centre. The prevention and treatment of isoniazid toxicity in the therapy of pulmonary tuberculosis. 2. An assessment of the prophylactic effect of pyridoxine in low dosage. *Bull World Health Organ* 1963;29:457-481.

A recent report from the Tuberculosis Chemotherapy Centre, Madras, showed that a vitamin-B-complex preparation containing a small amount of pyridoxine (as well as aneurine hydrochloride, riboflavin, nicotinamide, panthenol and cyanocobalamin) was effective in the treatment of peripheral neuropathy caused by daily high-dosage (12.5-15.2 mg/kg body-weight) isoniazid therapy of pulmonary tuberculosis. The present report gives results which show that the B-complex preparation is fully effective in preventing peripheral neuropathy in patients receiving the same high dosage of isoniazid, and that this is due to the small pyridoxine content of only 6 mg daily, and not to any of its other constituents. The low cost of this small dose of pyridoxine makes high-dosage isoniazid therapy, given in combination with other drugs or alone, a possible proposition in developing countries.

Studies in the Centre have produced clear evidence that there is an increase in the frequency of peripheral neuropathy when the dosage of isoniazid is increased from 7.8-9.6 mg/kg body-weight to 12.5-15.6 mg/kg daily, and that its incidence is higher among slow than among rapid inactivators of isoniazid.

The studies also show that increasing the dosage of isoniazid, when given alone, from a moderate daily dosage of 7.8-9.6 mg/kg to the high daily dosage of 12.5-15.6 mg/kg has not materially altered the radiographic or the bacteriological response to treatment.

18. Joseph S, Mitchison DA, Ramachandran K, Selkon JB, Subbaiah TV. Virulence in the guinea-pig and sensitivity to PAS and thioacetazone of tubercle bacilli from south Indian patients with pulmonary tuberculosis. *Tubercle* 1964; 45:354-359.

Isoniazid-sensitive strains of tubercle bacilli, obtained pre-treatment from 280 South Indian patients were tested for their virulence in the guinea-pig and for their sensitivity to PAS; strains from 209 of those patients were also tested for their sensitivity to thioacetazone.

Strains of low virulence more often had a high proportion of PAS-resistant mutants and were more often resistant to thioacetazone than those of high virulence.

There is suggestive evidence that Indian strains can be divided into two groups, the smaller group, comprising about one-quarter of the strains, resembling strains from British patients and the larger group characterized by a lower virulence, a higher susceptibility to hydrogen peroxide, a higher proportion of PAS-resistant mutants and thioacetazone resistance.

19. Mitchison DA, Lloyd J. Comparison of the sensitivity to thioacetazone of tubercle bacilli from patients in Britain, East Africa, south India and Hong Kong. *Tubercle* 1964; 45: 360-369.

Sensitivity tests to thioacetazone were done on cultures of tubercle bacilli obtained from 23 British, 27 East African, 30 South Indian and 48 Hong Kong patients with less than 14 days of previous antituberculosis chemotherapy and with isoniazid-sensitive organisms and from 38 Hong Kong patients with more than 14 days previous chemotherapy and/or isoniazid-resistant organisms. The results were expressed in three ways, one of which was the minimal concentration of thioacetazone inhibiting growth, where growth is defined as a viable count of 0.1 per cent of the count on drug-free medium (the 0.1% MIC).

The 0.1% MIC was 1 µg/ml. thioacetazone or more with 13% of the British cultures, 19% of the African cultures, 73% of the Indian cultures and 70% of the Hong Kong cultures. The other assessments of sensitivity to thioacetazone also indicated that the Indian and the Hong Kong cultures were more resistant to thioacetazone than the British and the African cultures. No associations were found between the sensitivity to thioacetazone and the sensitivity to isoniazid or to streptomycin in the Hong Kong cultures. Thioacetazone-resistant cultures from Indian patients were more often of low virulence in the guinea pig than sensitive cultures, but no such association was found in the 15 Hong Kong cultures tested.

20. Nair CN, Mackay-Scollay EM, Ramachandran K, Selkon JB, Tripathy SP, Mitchison DA, Dickinson JM. Virulence in the guinea pig and susceptibility to hydrogen peroxide of isoniazid-sensitive tubercle bacilli from south Indian patients. *Tubercle* 1964; 45:345-353.

Tests for virulence in the guinea pig and for susceptibility to the bactericidal activity of hydrogen peroxide were done on cultures of isoniazid-sensitive tubercle bacilli obtained from 220 South Indian patients with pulmonary tuberculosis before the start of anti-tuberculosis chemotherapy.

An association was found between the virulence of the cultures and the proportion of their organisms surviving exposure to 0.02% peroxide; 10% or more organisms survived in 11% of 152 cultures of low virulence and in 57% of 68 cultures of high virulence. There was some suggestion that the cultures could be divided into two groups, the larger, containing about 74% of the cultures, having low virulence and high peroxide susceptibility and the smaller having high virulence and low peroxide susceptibility.

21. Tuberculosis Chemotherapy Centre. A concurrent comparison of intermittent (twice-weekly) isoniazid plus streptomycin and daily isoniazid plus PAS in the domiciliary treatment of pulmonary tuberculosis. *Bull World Health Organ* 1964; 31: 247-271.

Previous reports from the Tuberculosis Chemotherapy Centre, Madras, have established that ambulatory treatment of pulmonary tuberculosis with the combination of isoniazid and PAS, administered daily, yields satisfactory results. However, in the usage of any unsupervised regimen, reliance must be placed on the co-operation of patients in self-administering their drugs. Irregularities in drug-taking, which are not uncommon, may lead to unfavourable therapeutic results; this might be avoided by supervised administration of the drugs. Daily supervision is clearly impracticable in developing countries but regimens in which the drug is administered intermittently-say, twice a week or less frequently-are, if effective, more likely to gain general application.

This paper presents the results of a controlled study of a fully supervised intermittent regimen of isoniazid (12.5-16.1 mg/kg body-weight, orally) plus streptomycin (injected in a uniform dose of 1g), given together twice weekly compared with a standard, unsupervised, daily, oral regimen of isoniazid (3.7-6.3 mg/kg body-weight) plus sodium PAS (0.2-0.3 g/kg body-weight), given in two doses. The intermittent regimen was at least as effective as the standard oral regimen, and although the incidence of temporary giddiness in patients receiving this regimen was rather high, this did not appear to have any long-term importance nor did it appear unduly to affect the co-operation of the patients. These encouraging findings suggest a possible change in the orientation of drug administration for tuberculosis in developing countries.

22. Selkon JB, Devadatta S, Kulkarni KG, Mitchison DA, Narayana ASL, Nair CN, Ramachandran K. The emergence of isoniazid-resistant cultures in patients with pulmonary tuberculosis during treatment with isoniazid alone or isoniazid plus PAS. *Bull World Health Organ* 1964; 31:273-294.

Previous reports from the Tuberculosis Chemotherapy Centre, Madras, have described a comparison of four regimens (three of isoniazid alone and one of isoniazid plus PAS) in the treatment of pulmonary tuberculosis and an investigation of the serum isoniazid levels in the patients concerned. The present report studies the emergence of isoniazid resistant

organisms in these patients during treatment. All patients with an unsatisfactory response to treatment yielded resistant cultures, showing that the isoniazid dosage was too low to inhibit sensitive organisms. From the degree of resistance of the first resistant cultures and of the six-month cultures from the patients treated with isoniazid alone it was concluded that resistance emerged in two stages. In the first stage, very early in treatment, highly resistant mutant bacilli grew freely whatever the isoniazid dosage, but mutants of lower resistance were prevented from growing to an extent dependent on the peak isoniazid concentration in the serum. Consequently, when the isoniazid dosage was increased the proportion of patients with resistant organisms decreased, since fewer low-resistance strains were able to develop. In the second stage, organisms with relatively low resistance continued to multiply, though still partially inhibited by isoniazid, and became more resistant, particularly in slow inactivators. The first-stage events determined the results of treatment since, once resistance had emerged, its extent was unrelated to the patient's eventual progress. These findings emphasize the importance of early intensive chemotherapy and adjustment of the isoniazid dosage according to peak serum concentrations rather than concentrations measured three or six hours after the dose. Concomitant administration of PAS prevented emergence of isoniazid resistance in many patients and in others delayed its emergence and reduced its degree, possibly because growth in the second stage was slow.

23. Tuberculosis Chemotherapy Centre. Isoniazid plus thioacetazone compared with two regimens of isoniazid plus PAS in the domiciliary treatment of pulmonary tuberculosis in south Indian patients. *Bull World Health Organ* 1966; 34: 483-515.

Previous report from the Tuberculosis Chemotherapy Centre, Madras, have established that ambulatory treatment of pulmonary tuberculosis with a standard daily regimen of isoniazid plus PAS for one year yields satisfactory results. However, this regimen may be unsuitable for large-scale use in many developing countries, because PAS is expensive, bulky and unpleasant to take, and has poor keeping qualities, especially in tropical countries. It might be possible to overcome these disadvantages by substituting for the PAS a drug, which is equally effective but less expensive and more acceptable, or by reducing the daily dosage of PAS and the period for which it is prescribed.

This paper presents the results over a 12-month period of a controlled comparison of (a) the standard regimen of isoniazid (average 4.5 mg/kg body-weight) plus sodium PAS (average 0.22 g/kg), daily in two divided doses; (b) a regimen of isoniazid (average 6.9 mg/kg) plus thioacetazone (average 3.4 mg/kg), daily in one dose; and (c) a 2-phase regimen of isoniazid (average 5.5 mg/kg) plus sodium PAS (average 0.17 g/kg), daily in one dose for 6 months, followed by isoniazid alone (average 6.8 mg/kg), daily in one dose for the second 6 months. The regimen of isoniazid plus thioacetazone was found to be therapeutically as effective as the standard regimen of isoniazid plus PAS; however, it was

associated with a higher incidence of minor side-effects, and three cases of exfoliative dermatitis. The 2-phase regimen of isoniazid plus PAS followed by isoniazid alone was less effective.

These findings are encouraging for the large-scale use in developing countries of the relatively inexpensive regimen of isoniazid plus thioacetazone; however, any such step should be preceded by carefully planned studies to investigate, under local conditions, the toxicity and the efficacy of the regimen.

24. Devadatta S, Radhakrishna S, Fox W, Mitchison DA, Rajagopalan S, Sivasubramanian S, Stott H. Comparative value of sputum smear examination and culture examination in assessing the progress of tuberculous patients receiving chemotherapy. *Bull World Health Organ* 1966; 34: 573-587.

Bacteriological response is generally considered the best criterion for assessing the efficacy of chemotherapy in patients with pulmonary tuberculosis. The bacteriological methods most commonly used are examination of sputum smears for tubercle bacilli, culture of bacilli from sputum specimens, and drug-sensitivity tests on positive cultures. Culture examination, though more sensitive than smear examination in detecting tubercle bacilli, is time-consuming and economically impracticable as a routine method in most developing countries. A study was therefore undertaken at the Tuberculosis Chemotherapy Centre, Madras, to determine the relative value of smear examination and culture examination in predicting the outcome of treatment and assessing the efficacy of chemotherapeutic regimens in 515 patients (all with bacteriologically confirmed disease and isoniazid-sensitive organisms on admission) receiving isoniazid, alone or with sodium PAS. The results shows that the value of smear examination of overnight sputum specimens at monthly intervals closely approached that of culture examination in assessing the progress of the patients, the percentages of correct predictions by smear and by culture being of the same order. Smear examination was slightly less effective than culture examination in detecting differences in the efficacies of regimens, but it has been estimated that this disadvantage can usually be compensated for by increasing the study population by about 20%.

25. Krishnamurthy DV, Selkon JB, Ramachandran K, Devadatta S, Mitchison DA, Radhakrishna S, Stott H. Effect of pyridoxine on vitamin B6 concentrations and glutamic-oxaloacetic transaminase activity in whole blood of tuberculous patients receiving high-dosage isoniazid. *Bull World Health Organ* 1967; 36:853-870.

An earlier report from the Tuberculosis Chemotherapy Centre, Madras, showed that, in tuberculous patients receiving high-dosage isoniazid (12.5-15.6 mg/kg body-weight), the concomitant administration of 6 mg of pyridoxine prevented peripheral neuropathy. In that study, biochemical determinations of B6 concentrations and GOT activity in whole blood

had been routinely undertaken on all patients on admission to treatment, and at 6, 12, 24, and 52 weeks thereafter; in addition, extra determinations were undertaken for patients who developed peripheral neuropathy. The present paper reports the findings of these investigations, which are: (a) peripheral neuropathy developed predominantly among slow inactivators of isoniazid, and was associated with a substantial reduction in GOT activity but no apparent change in B6 concentrations; (b) the reduction in GOT activity appeared to be due to deficiency of both the coenzyme (pyridoxal phosphate) and the apoenzyme; (c) the concomitant administration of pyridoxine (6 mg or 48 mg) with high-dosage isoniazid to 3 patients with peripheral neuropathy, 1 of whom had convulsions also, resulted in increased B6 concentrations and GOT activity, and no further convulsions; and (d) the concomitant administration of pyridoxine 6 mg daily, as a prophylactic, resulted in a significant increase in B6 concentrations and GOT activity and prevention of the neuropathy.

These findings establish the existence of a definite association between the occurrence of isoniazid-induced toxicity and diminished pyridoxine function.

26. Tripathy SP, Menon NK, Mitchison DA, Narayana ASL, Somasundaram PR, Stott H, Velu S. Response to treatment with isoniazid plus PAS of tuberculous patients with primary isoniazid resistance. *Tubercle* 1969; 50: 257-268.

The response to treatment with isoniazid plus PAS was studied in 30 patients with primary isoniazid resistance and 459 patients with initially isoniazid-sensitive cultures. The patients with primary isoniazid resistance responded substantially less well, as assessed by the extent of radiographic improvement, disappearance of cavitation, negativity on culture and bacteriological status at six and at 12 months; also, cultures isolated from these patients at six months were more often resistant to PAS. Even so, there was suggestive evidence that they had derived some benefit from treatment with isoniazid.

27. Rao KVN, Mitchison DA, Nair NGK, Prema K, Tripathy SP. Sulphadimidine acetylation test for classification of patients as slow or rapid inactivators of isoniazid. *BMJ* 1970; 3: 495-497.

Sulphadimidine acetylation studies were undertaken in 103 patients, 52 of whom had been classified as slow and 51 as rapid inactivators of isoniazid by a standard microbiological assay method. Each patient received sulphadimidine by mouth in a dose of 44 mg./kg. body weight, and free and total sulphadimidine were estimated in blood and urine collected at six hours. The findings suggest that patients may be classified as slow inactivators of isoniazid if the proportion of acetylated sulphadimidine (total minus free) is (a) less than 25% in blood or (b) less than 70% in urine. The sulphadimidine test is easy to perform and the result is available the same day; urine specimens for the test can be stored at room temperature for over a week without any loss of drug.

28. Tripathy SP, Mitchison DA, Nair NGK, Radhakrishna S, Subbammal S. A comparison of various measures of sensitivity of *M. tuberculosis* to pyrazinamide. *Tubercle* 1970; 51: 375-388.

A test, employing Lowenstein-Jensen medium acidified with hydrochloric acid to a pre-inspissation pH of 4.80-4.85, is described for detecting the sensitivity to pyrazinamide of strains of *M. tuberculosis*. The test was performed on cultures from patients with no history of previous chemotherapy with pyrazinamide, and on cultures from patients receiving daily treatment with pyrazinamide.

Sensitivity was measured in terms of the minimal inhibitory concentration of the drug for various sizes of the inoculum, and as proportions of the bacterial population resistant to various concentrations of the drug. For each of these measures, the findings in patients with no history of previous chemotherapy with pyrazinamide were compared with those obtained at 4-12 months after the start of daily treatment with pyrazinamide; the definition of resistance was then chosen such that (a) it discriminated efficiently between the two populations, and (b) it classified only a small proportion of the former population as resistant.

Four definitions of resistance were chosen - a minimal inhibitory concentration of 200 µg/ml. or more employing an inoculum containing approximately 0.4 mg. (moist weight) of bacilli per ml. and a 10-colony end-point, and proportions of 20% or more on 25µg/ml., 5% or more on 50µg/ml., and 1% or more on 100µg/ml. The efficiency of the four definitions was of the same order. Further, highly satisfactory agreement was obtained between pairs of definitions in the classification of individual cultures as sensitive or resistant.

High viable counts on the drug-free acidified medium were associated with high minimal inhibitory concentrations but had little effect on the proportions resistant.

Wild strains with consistent resistance to pyrazinamide were rare. Finally, in patients with an unfavourable response to a daily regimen containing pyrazinamide, resistance had usually emerged four to six months after the start of treatment.

29. Tuberculosis Chemotherapy Centre. A controlled comparison of a twice-weekly and three once-weekly regimens in the initial treatment of pulmonary tuberculosis. *Bull World Health Organ* 1970; 43: 143-206.

A previous report from the Tuberculosis Chemotherapy Centre, Madras, demonstrated the value of a fully supervised twice-weekly regimen of high-dosage isoniazid plus streptomycin in the treatment of newly diagnosed tuberculous patients with drug-sensitive cultures. A logical consequence of this finding was an investigation of regimens with a

longer interval between successive doses. The present report describes the findings of a controlled study of 3 once-weekly regimens and the twice-weekly regimen. The results confirm that the twice-weekly regimen is highly effective and demonstrate that its efficacy is not influenced by the rate of inactivation of isoniazid or by a reduction (by one-fourth) in the dosage of streptomycin. The results also show that once-weekly chemotherapy from the beginning, whether with high-dosage isoniazid plus streptomycin or high-dosage isoniazid plus streptomycin plus high-dosage pyrazinamide, gives unsatisfactory results. However, when an initial daily phase of 4 weeks with a moderate dosage of isoniazid plus streptomycin preceded the once-weekly phase of high-dosage isoniazid plus streptomycin, the response was highly satisfactory in slow inactivators of isoniazid (as good as with the twice-weekly regimen) but was considerably less satisfactory in rapid inactivators. These findings suggest that if a method of compensating for the insufficiency of this regimen in rapid inactivators of isoniazid can be found, the prospects for evolving a highly satisfactory once-weekly regimen are bright.

30. Tuberculosis Chemotherapy Centre. Controlled comparison of oral twice-weekly and oral daily isoniazid plus PAS in newly diagnosed pulmonary tuberculosis. *BMJ* 1973;7-11.

A controlled clinical trial was undertaken in 247 patients with newly diagnosed pulmonary tuberculosis to assess the relative efficacies of a fully supervised twice-weekly oral regimen of isoniazid plus PAS (para-aminosalicylic acid) and a standard self-administered daily regimen of the same drugs following an initial intensive phase of two weeks of daily streptomycin, PAS, and isoniazid. Among patients who had isoniazid-sensitive cultures initially and who attended the clinic regularly the numbers with favourable bacteriological response at the end of the year of chemotherapy were 79 (88%) out of 90 for the twice-weekly regimen and 72 (87%) out of 83 for the daily regimen; the numbers of patients with considerable radiographic improvement were 54 (60%) and 53 (64%) respectively. Complaints of vomiting or diarrhoea that did not require a reduction of the PAS dosage were made on one or two occasions by 23 (21%) out of 109 twice-weekly and 25 (23%) out of 108 daily patients, and on at least three occasions by 4 (4%) and 12 (11%) respectively. Finally, all five patients who had chemotherapy changed on account of hypersensitivity to PAS had been receiving the daily regimen, as also had one patient who died of agranulocytosis.

31. Tuberculosis Chemotherapy Centre. A controlled comparison of two fully supervised once-weekly regimens in the treatment of newly diagnosed pulmonary tuberculosis. *Tubercle* 1973; 54: 23-45.

Four hundred and fifteen patients with pulmonary tuberculosis were admitted to a controlled study of a year's treatment, on an outpatient basis, with one of the following two fully supervised regimens:

SH/SHOW: Streptomycin 1g or 0.75 g plus isoniazid 400 mg administered daily for the first four weeks, followed by streptomycin in the same dosage plus isoniazid 13 mg/kg or 17 mg/kg body-weight, administered once a week for the rest of the year. Pyridoxine 6 mg was incorporated in every dose of isoniazid.

SPH/SPHOW: Streptomycin, isoniazid and pyridoxine in the same dosage as in the SH/SHOW regimen, plus sodium PAS 6 g throughout the year, all the drugs being administered daily for the first four weeks and once a week for the rest of the year.

The regimen, the streptomycin dosage and the once-weekly isoniazid dosage were allocated at random for each patient.

The main analyses in this report concern 359 newly-diagnosed patients (181 SH/SHOW, 178 SPH/SPHOW) with cultures sensitive to isoniazid and streptomycin on admission. About 90 per cent of the patients had cavitated disease and a positive sputum smear on admission, and 40 per cent were rapid inactivators of isoniazid. The condition on admission was similar for the two series.

Two patients (both SH/SHOW) died of tuberculosis and four (all SH/SHOW) had their chemotherapy changed on account of radiographic or clinical deterioration in the presence of a positive sputum. At one year, 85 per cent of the SH/SHOW and 87 per cent of the SPH/SPHOW patients were classified as having a favourable response, mainly on the basis of culture results at 10, 11 and 12 months. Among those who had an unfavourable response, approximately half had responded well initially but had a bacteriological relapse by one year. Considerable or exceptional radiographic improvement was shown by about three-fourths of the patients in each series, and cavitation had disappeared in about half. Resistance to isoniazid was observed at one year in 8 per cent of the SH/SHOW and 5 per cent of the SPH/SPHOW patients, and resistance to streptomycin in 8 per cent and 2 per cent, respectively. It is concluded that the addition of PAS to the SH/SHOW regimen did not confer an appreciable benefit.

Slow inactivators of isoniazid responded considerably better than rapid inactivators, the proportion with a favourable response at 1 year being 93 per cent and 72 per cent respectively in the SH/SHOW and 95 per cent and 76 per cent respectively in the SPH/SPHOW series. The relative insufficiency of isoniazid in the rapid inactivators was manifested by less efficient elimination of isoniazid-sensitive organisms and greater frequency of streptomycin resistance. The duration of coverage with a bacteriostatic concentration of isoniazid (0.2 µg/ml) following a once-weekly dose and the total exposure to isoniazid (expressed as the area under the time-concentration curve) were substantially higher in the slow inactivators (30-34 hours; 85-111 units) than in the rapid

inactivators (14-15 hours; 36-51 units). The peak concentration in the former, however, was only 22 per cent higher than that in the latter, the difference being of the same order as that between SPH/SPHOW and SH/SHOW patients, and isoniazid high-dosage (17 mg/kg) and low-dosage (13 mg/kg) patients. These findings suggest that response to once-weekly chemotherapy with isoniazid is largely determined by the duration of coverage and the total exposure to isoniazid.

Streptomycin 0.75 g (approximately 20 mg/kg body-weight) was therapeutically as effective as 1g and less toxic. The isoniazid dosage of 17 mg/kg in the once-weekly phase appeared to be slightly more effective than the dosage of 13 mg/kg, but only in rapid inactivators.

32. Sarma GR, Kailasam S, Mitchison DA, Nair NGK, Radhakrishna S, Tripathy SP. Studies of serial plasma isoniazid concentrations with different doses of a slow-release preparation of isoniazid. *Tubercle* 1975; 56:314-323.

The suitability of a slow-release matrix preparation of isoniazid for use in once-weekly chemotherapy has been investigated in South Indian patients. Serial plasma isoniazid concentrations were determined up to 6 hours following doses of 15, 30, 45 and 60 mg/kg body-weight in rapid inactivators and up to 10 hours following doses of 15, 30 and 45 mg/kg in slow inactivators. The isoniazid levels were sustained, and the peak concentrations (per unit dose) were considerably lower than with ordinary isoniazid. It was estimated that a matrix isoniazid dose of 35 mg/kg in slow inactivators and 50 mg/kg in rapid inactivators would produce a peak similar to that attained with a non-toxic dose of ordinary isoniazid 15 mg/kg in slow inactivators.

A second investigation showed that matrix isoniazid 40 mg/kg in rapid inactivators produced a coverage (with 0.2 µg/ml) and exposure similar to those attained in slow inactivators with a highly effective dose of ordinary isoniazid 15 mg/kg, while 30 mg/kg gave substantially lower values. In both investigations, disproportionately large increases in plasma isoniazid concentrations were observed in rapid inactivators with an increase in the matrix isoniazid dose. In slow inactivators, both doses of matrix isoniazid, 30 and 40 mg/kg, resulted in coverage and exposure that were substantially higher than those obtained with ordinary isoniazid 15 mg/kg.

33. Hong Kong Chest Services, Tuberculosis Research Centre, British Medical Research Council. Sputum-smear-negative pulmonary tuberculosis. Controlled trial of 3-month and 2-month regimens of chemotherapy. First report. *Lancet* 1979:1361-1363.

Of 1072 Chinese patients with radiographically active pulmonary tuberculosis and no microscopic evidence of acid-fast bacilli in sputum examinations, only 691 (64%) were

sputum-culture negative. All patients were randomly allocated to selective chemotherapy (antituberculosis chemotherapy not being started until the activity of the disease had been confirmed), to daily streptomycin, isoniazid, rifampicin, and pyrazinimide for 2 months or 3 months, or to a standard 12-month control regimen. During the subsequent 12 months, 64% of the patients in the selective chemotherapy series started antituberculosis chemotherapy. Both 2-month and 3-month regimens were inadequate for patients whose pretreatment sputum cultures were positive (relapse rates 14% and 7%, respectively, in patients with drug-sensitive strains) but in the patients whose first cultures were negative the relapse rate was only 1% after both short-term regimens.

34. Hong Kong Chest Service, Tuberculosis Research Centre, British Medical Research Council. A controlled trial of 2-month, 3-month and 12-month regimens of chemotherapy for sputum smear-negative pulmonary tuberculosis: The results at 30 months. *Am Rev Respir Dis* 1981;124:138-142.

Of 1,033 Chinese patients with radiologically active pulmonary tuberculosis but with sputum negative for acid-fast bacilli on 5 initial microscopic examinations, 370 (38%) had 1 or more initial sputum cultures that yielded tubercle bacilli. All patients were randomly allocated to (1) selective chemotherapy, anti-tuberculosis chemotherapy not being started until active disease had been confirmed, or to (2) daily streptomycin, isoniazid, rifampin, and pyrazinamide for 2 months or (3) the same 4 drugs daily for 3 months, or to (4) a 12-month control regimen.

In patients with 1 or more of their initial sputum cultures positive, the short-course regimens were inadequate, being followed by bacteriological relapse rates of 15 and 9%, respectively, during 30 months, compared with 0% in the control series. In patients with all their initial cultures negative, the corresponding relapse rates were 4, 2 and 0%, and in the selective chemotherapy series, 53% of the patients had treatment started during the 30 months because active disease was confirmed (bacteriologically in 40%).

It is important to continue studying short-course chemotherapy for smear negative patients because in many countries they represent a high proportion of those treated.

35. Hong Kong Chest Service/Tuberculosis Research Centre, Madras/British Medical Research Council. A study of the characteristics and course of sputum smear-negative pulmonary tuberculosis. *Tubercle* 1981;62:155-167.

A total of 302 Chinese patients were diagnosed on clinical and radiographic grounds by chest physicians from the Hong Kong Chest Service as having radiographically active pulmonary tuberculosis, but had sputum negative for acid-fast bacilli on 5 recent microscopical examinations. They were not given anti-tuberculosis chemotherapy until active disease had been confirmed by positive bacteriological findings, or by radiographic

or clinical deterioration during close observation. Of the 283 patients assessed up to 30 months, 200 (71%) had active disease confirmed and had chemotherapy started during the 30 months. A further 42 (15%) had evidence of changing lesions on serial chest radiography, and hence of recently active disease.

A number of characteristics of patients and of their bacteriological and radiographic status were tested singly and in combination for association with the presence of active disease confirmed on admission or at any time during the 30 months. Patients with radiographic lesions which were larger and classified as "active" on independent radiological assessment, and with a history of blood-streaked sputum or frank haemoptysis were more likely to have unquestionably active disease on admission or at some time during the 30 months, than patients without these characteristics.

36. Tuberculosis Research Centre. Ethambutol plus isoniazid for the treatment of pulmonary tuberculosis-a controlled trial of four regimens. *Tubercle* 1981; 61:13-29.

A controlled clinical trial of 4 regimens was undertaken in patients with bacteriologically positive, newly-diagnosed pulmonary tuberculosis. The regimens were: ethambutol 15 mg/kg + isoniazid 400 mg daily (E7 H7); ethambutol 45 mg/kg + isoniazid 15 mg/kg, twice a week (E2 H2); ethambutol 90 mg/kg + isoniazid 15 mg/kg, once a week plus isoniazid 15 mg/kg, mid-way between the weekly doses (E1 H2); ethambutol 90 mg/kg + isoniazid 15 mg/kg, once a week (E1 H1). All patients received streptomycin 1g plus ethambutol 25 mg/kg body-weight plus isoniazid 400 mg daily for the first 2 weeks. The total duration of treatment was 12 months for all patients.

There were 484 patients admitted to the study. After excluding 60 (41 with initial drug resistance to isoniazid), there remain 424 patients (107 E7 H7, 101 E2 H2, 107 E1 H2, 109 E1 H1) in the main analyses. The pretreatment characteristics of the 4 groups were broadly similar.

A favourable response at 12 months was observed in 96%, 88%, 93%, and 75% of patients respectively; the differences between the E1 H1 regimen and the other 3 regimens were all significant ($p < 0.03$) as was that between the E7 H7 and E2 H2 regimens ($P = 0.05$).

Among the slow inactivators of isoniazid, the proportions with a favourable response at one year were similar in the 4 groups (range 91-95%). However, among the rapid inactivators, the proportion with a favourable response in the E1 H1 group was only 57%. There was suggestive evidence that the E1 H2 regimen was superior to the E2 H2 regimen.

Of the patients with bacteriologically quiescent disease at 1 year, approximately a half, at random, had no further chemotherapy and were followed up for a 4-year period. Bacteriological relapse requiring retreatment occurred in 15% of 54 E7 H7 patients, 26% of 38 E2 H2, 33% of 43 E1 H2 and 54% of 37 E1 H1 patients, a significant difference ($p < 0.001$). A final evaluation of long-term (5-year) favourable response achieved by the 12-month regimens was 83% for the E7 H7, 63% for the E2 H2, 63% for the E1 H2 and 33% for the E1 H1 regimens.

In general, the regimens were well tolerated and the incidence of adverse reactions to the drugs was low. Of the 424 patients, 6 (1.4%) developed visual disturbance during the year of chemotherapy.

37. Tuberculosis Research Centre. Study of chemotherapy regimens of 5 and 7 months' duration and the role of corticosteroids in the treatment of sputum-positive patients with pulmonary tuberculosis in south India. *Tubercle* 1983; 64:73-91.

A controlled clinical trial of 3 short-course chemotherapy regimens was undertaken in patients with bacteriologically positive, newly diagnosed pulmonary tuberculosis. The patients were allocated at random to receive one of 3 regimens: rifampicin, streptomycin, isoniazid and pyrazinamide daily for 2 months, followed by streptomycin, isoniazid and pyrazinamide twice weekly for 3 months (R/5) or for 5 months (R/7), and the same regimen as (R/7) but without rifampicin (Z/7). Further, half the patients in each series, selected at random, were prescribed daily prednisolone for the first 8 weeks.

Of the 509 patients admitted to this phase of the trial, 390 had pre-treatment sputum cultures sensitive to isoniazid and streptomycin. At 2 months, in 92% of R/5 and R/7 patients (combined) and in 72% of Z/7 patients all cultures were negative ($p < 0.00001$). All 390 patients had a favourable response at the end of chemotherapy. At follow up for 24 months from the time of admission a bacteriological relapse requiring retreatment was observed in 5.4% of R/5, 0.0% of R/7 and 3.9% of Z/7 patients. The differences between R/7 and each of the other 2 series were significant ($p < 0.03$).

Including a second phase of the study to increase the numbers in the Z/7 series, a total of 269 patients with drug-sensitive sputum cultures received the Z/7 regimen. All of them had a favourable response to treatment and only 2.6% had a bacteriological relapse requiring retreatment. These results demonstrate that in patients with drug-sensitive cultures, good results can be achieved with a short-course regimen that does not include rifampicin.

Among patients with sputum cultures resistant to both isoniazid and streptomycin, 6 of 23 in the R/5 and R/7 series had an unfavourable response to treatment, compared with 13 of

16 in the Z/7 series ($p < 0.01$). These results demonstrate that in patients with drug-resistant cultures, short course regimens that do not include rifampicin may be unsatisfactory.

Prednisolone given for the first 8 weeks did not influence the speed of sputum conversion or the response to chemotherapy in patients with drug-sensitive cultures and it had no effect on the rate of bacteriological relapse. However, in patients with culture resistant to streptomycin and isoniazid the response to chemotherapy was less favourable in those who had taken prednisolone than in those who had not. Thus, 4 of 12 receiving prednisolone in the R/5 and R/7 series had an unfavourable response compared with 2 of 11 not receiving it ($p > 0.2$), and all of 10 and 3 of 6, respectively, in the Z/7 series ($p = 0.04$).

The most common adverse drug reaction was arthralgia, which was complained of by 24% of patients in the R/5 and R/7 series and 46% in the Z/7 series ($p < 0.001$); all except 5 continued their normal activities without any interruption or modification of the regimen. Hepatitis occurred in 17 (2.5%) patients.

38. Hong Kong Chest Service, Tuberculosis Research Centre, British Medical Research Council. A controlled trial of 2-month, 3-month, and 12-month regimens of chemotherapy for sputum-smear-negative pulmonary tuberculosis. Results at 60 months. *Am Rev Respir Dis* 1984;130:23-28.

Of 1,019 Chinese patients with radiographically active pulmonary tuberculosis but with sputum negative for acid-fast bacilli on 5 initial microscopic examinations who were studied for 5 years, 364 (36%) had 1 or more initial sputum cultures positive for *Mycobacterium tuberculosis*. All 1,019 patients were randomly allocated to (1) selective chemotherapy (anti-tuberculosis chemotherapy not being started until the disease had been confirmed to be active); or to (2) daily streptomycin, isoniazid, rifampicin, and pyrazinamide for 2 months; or 3) for 3 months; or to (4) a standard 12-month control regimen.

In the 364 patients with 1 or more of their initial sputum cultures positive, the short-course regimens were inadequate, being followed by relapse rates of 32 and 13% respectively, during 60 months, compared with 5% in the control series. In the 655 patients with all their initial cultures negative, the corresponding relapse rates were 11, 7, and 2%. In the selective chemotherapy series, 57% of the patients had treatment started during the 60 months because their disease was confirmed to be active.

39. Tuberculosis Research Centre, National Tuberculosis Institute. A controlled clinical trial of 3- and 5-month regimens in the treatment of sputum-positive pulmonary tuberculosis in south India. *Am Rev Respir Dis* 1986; 134: 27-33.

A controlled comparison of 3 short-course regimens was undertaken in patients with newly diagnosed, sputum-positive, pulmonary tuberculosis in South India. The regimens

were: (1)R3: rifampicin plus streptomycin plus isoniazid plus pyrazinamide daily for 3 months; (2) R5: the same as regimen R3 followed by streptomycin plus isoniazid plus pyrazinamide twice weekly for 2 months; (3) Z5: the same as regimen R5 but without rifampicin. The distributions of various pre-treatment characteristics were similar in the 3 series. At the end of treatment, 6 patients (3 R3, 3 Z5) of 694 (228 R3, 230 R5, 236 Z5) with drug-sensitive organisms initially were classified as having an unfavourable response. By 24 months (21 months of follow-up for the R3 regimen and 19 months for the R5 and Z5 regimens), a bacteriologic relapse requiring treatment occurred in 20% of 200 R3, 4% of 187 R5, and 13% of 199 Z5 patients, the difference between the R3 and R5 series being highly significant ($p = 0.00001$). Considering patients with cultures initially resistant to isoniazid, 4 of 57 in the R3 and R5 series combined had an unfavourable response to treatment compared with 13 of 26 in the Z5 series ($p < 0.0001$). Of the 4 patients with an unfavourable response in the R3 and R5 series combined, resistance to rifampicin emerged in 2. Complaints of arthralgia were made by 45% of the R3 and R5 patients combined and 70% of the Z5 patients ($p < 0.00001$). However, chemotherapy was modified in only 5 and 12%, respectively. Jaundice occurred in 7% of the R3 and R5 patients and 1% of the Z5 patients ($p < 0.00001$).

40. Paramasivan CN, Jackett PS, Coates ARM, Lowrie DB, Mitchison DA. Monoclonal antibodies against *Mycobacterium avium/intracellulare*. *Indian J Med Res* 1988; 88:13-17.

Ten hybridoma cell lines producing monoclonal antibodies (Mabs) against *M. avium/intracellulare*(Mai) serotype 8 were raised by the fusion of BALB/c mouse myeloma cells (SP2) to spleen cells from immunized BALB/c mice. The specificity of the monoclonal antibodies was defined using their differing abilities to bind to sonicates from a range of mycobacterial species and strains. The Mabs showed strain and species specificity. Three Mabs bound only to Mai serotype 8 and 1 Mab bound only to Mai serotypes 8 and 16, the only serotypes tested. The results indicate that Mabs specific for Mai species and serotypes can be produced. These could be useful for serodiagnostic and for epidemiological purposes.

41. Cheng SH, Walker KB, Lowrie DB, Mitchison DA, Swamy R, Datta M, Prabhakar R. Monocyte antimycobacterial activity before and after *Mycobacterium bovis* BCG vaccination in Chingleput, India, and London, United Kingdom. *Infect Immun* 1993; 61:4501-4503.

Monocytes from purified protein derivative S Mantoux-negative children and young adults inhibited intracellular growth of *Mycobacterium microtimum* in Chingleput than in London. *Mycobacterium bovis* BCG vaccination did not enhance bacteriostasis with the Indians but did so with the Londoners. No evidence was found for involvement of

cytokines such as macrophage-activating factor and granulocyte macrophage-colony stimulating factor in the differences.

42. Herbert D, Paramasivan CN, Venkatesan P, Kubendiran G, Prabhakar R, Mitchison DA. Bactericidal action of ofloxacin, sulbactam-ampicillin, rifampin, and isoniazid on logarithmic- and stationary-phase cultures of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 1996; 40:2296-2299.

The bactericidal actions of ofloxacin and sulbactam-ampicillin, alone and in combination with rifampin and isoniazid, on exponential-phase and stationary-phase cultures of a drug-susceptible isolate of *Mycobacterium tuberculosis* were studied *in vitro*. In exponential-phase cultures, all drugs were bactericidal with the higher concentrations of ofloxacin (5 µg/ml) and sulbactam-ampicillin (15 µg of ampicillin per ml) being as bactericidal as 1 µg of isoniazid per ml or 1 µg of rifampin per ml. In two-drug combinations, both drugs increased the levels of activity of isoniazid and rifampin and were almost as bactericidal as isoniazid-rifampin; they also appeared to increase the level of activity of isoniazid-rifampin in three-drug combinations. In contrast, ofloxacin and sulbactam-ampicillin had little bactericidal activity against stationary-phase cultures and were less active than isoniazid or rifampin alone. Furthermore, in two-drug or three-drug combinations, they did not increase the level of activity of isoniazid, rifampin, or isoniazid-rifampin. These findings suggest that ofloxacin and sulbactam-ampicillin are likely to be most useful in the early stages of treatment and in preventing the emergence of resistance to other drugs but are unlikely to be effective as sterilizing drugs helping to kill persisting lesional bacilli.

43. Sulochana Das, Cheng SH, Lowrie DB, Walker KB, Mitchison DA, Vallishayee RS, Narayanan PR. The pattern of mycobacterial antigen recognition in sera from Mantoux-negative individuals is essentially unaffected by *Bacille Calmette-Guerin* (BCG) vaccination in either South India or London. *Clin Exp Imm* 1992; 89; 402-406.

Paired sera were obtained before and 8 weeks after routine BCG vaccination from 20 PPD-S Mantoux-negative individuals who were living adjacent to the Chingleput BCG vaccine trial area in Tamil Nadu and from seven Mantoux-negative school children in London, UK. Most subjects became Mantoux-positive after vaccination. In ELISA tests against soluble extracts of BCG or *Mycobacterium tuberculosis* H 37 Rv or against PPD-S, pre-vaccination antibody titres of South Indian subjects were about twice those of British subjects but there was no increase in titre of antibodies after vaccination of either population. Western blotting showed that even before vaccination, and even in British subjects, antibodies were present that recognized numerous antigenic components in extracts of BCG and *M. tuberculosis*. There was no consistent difference between band patterns with South Indian and British subjects and any effect of vaccination on the patterns was minimal.

44. Sulochana Das, Chan SL, Allen B, Mitchison DA, Lowrie DB. Application of DNA fingerprinting with IS986 to sequential mycobacterial isolates obtained from pulmonary tuberculosis patients in Hong Kong before, during and after short-course chemotherapy. *Tubercle and Lung Dis* 1993; 74; 47-51.

A total of 266 *Mycobacterium tuberculosis* isolates were subjected to DNA RFLP analysis. They were obtained from monthly sputum cultures from patients treated with short-course chemotherapy and then followed up for 2 years. They originated from 42 patients who relapsed after short-course chemotherapy and from a further 42 patients who yielded a single isolated positive culture after chemotherapy. The isolates consisted of one obtained pre-treatment and the last obtained during chemotherapy, together with either two isolates cultured at least 2 months apart during relapse or the single post-chemotherapy isolate. They were coded before DNA RFLP analysis and assigned to groups with identical or near identical band patterns on visual inspection.

After decoding, it was evident that almost every patient was infected with a strain with a different band pattern (fingerprint). In 100 comparisons of either the pre-treatment isolate against the last positive isolate obtained during chemotherapy, or of the first relapse isolate against the second relapse isolate, 15 had been recorded as different; 4 of these were retrospectively found to be due to reading error (error rate 1.5%), leaving 11 (11%) with marked differences. For 5 (12%) of the 42 patients who relapsed, the fingerprint of the relapse isolate was markedly different from that of the pre-treatment isolate. In contrast, the isolated positive culture was markedly different from that initially present in 36 (90%) of 40 comparisons. The relative contributions by clinical mixed infection and laboratory cross-contamination to the remaining 10-12% discrepancy rates could not be assessed. In 7 (8%) of the total 84 patients, the successive isolates showed a one-step gain or loss of either 1 or 2 bands, suggesting evolution of the fingerprint types. This RFLP fingerprinting method was clearly superior to phage-typing as a means of distinguishing between these isolates.

45. Paramasivan CN, Sulochana S, Kubendiran G, Venkatesan P, Mitchison DA. Bactericidal action of Gatifloxacin, Rifampin, and Isoniazid on logarithmic- and stationary-phase cultures of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2005; 49; 627-631.

The bactericidal activity of gatifloxacin, alone and in combination with isoniazid and rifampin, was studied on both exponential- and stationary-phase cultures of *Mycobacterium tuberculosis* strain H37Rv. On log-phase cultures, the bactericidal activity of gatifloxacin at 4 mcg/ml was rapid and was very similar to that of isoniazid. At concentrations of 0.25 and 4 mcg/ml, gatifloxacin enhanced the activity of isoniazid. Killing of the stationary-phase culture was biphasic. During the first 2 days, gatifloxacin at 4 mcg/ml slightly increased the limited bactericidal activities of isoniazid and rifampin.

However, no further additional bactericidal activity was found during further incubation with isoniazid alone or when gatifloxacin was added to either isoniazid or rifampin. This suggested that the stationary-phase culture contained a mixture of occasionally dividing bacilli that were killed during the first 2 days and true static persisters in the residual population that mimicked those in human lesions. In view of the failure of gatifloxacin to add to the sterilizing activity of isoniazid or rifampin during days 2 to 6 of exposure in the stationary-phase culture, it is unlikely to be a sterilizing drug that can be used to shorten the duration of treatment appreciably when it is added to present treatment regimens.

46. Kubendiran G, Paramasivan CN, Sulochana S, Mitchison DA. Moxifloxacin and Gatifloxacin in an acid model of persistent *Mycobacterium tuberculosis*. *J Chemother* 2006; 18:617-623.

Studies in the mouse and in humans suggest that use of moxifloxacin and gatifloxacin may shorten the duration of treatment of pulmonary tuberculosis. We describe here the *in vitro* findings with gatifloxacin and moxifloxacin in regimens similar to those that might be used in the treatment of tuberculosis. The bactericidal activities of moxifloxacin and gatifloxacin were measured alone and in different combinations with isoniazid, rifampicin and pyrazinamide against a 30-day, stationary phase culture, at a pH of 5.9. There was a rapid, irregular fall in colony counts during the first 4 days followed by a slower consistent kill during days 4-21 with a mean kill of -0.36 (SD=2.74) and -0.106 (SD=0.011) log₁₀ CFU/ml/day, respectively. The 4-21-day kill is considered the best assessment of bactericidal activity against persisting bacilli that prolong treatment. The substitution of either of the quinolones for isoniazid in the control regimen of rifampicin, pyrazinamide and isoniazid did not increase bactericidal activity with log CFU of 5.00 and 4.88, but did result in increased bactericidal action with the log CFU of 4.11 and 4.10 for moxifloxacin and gatifloxacin respectively. Moxifloxacin and gatifloxacin had closely similar activities in all drug combinations. Adding moxifloxacin or gatifloxacin to the control regimen resulted in a significant increase in bactericidal action, considered sufficient to reduce the treatment duration.

47. Sulochana S, Mitchison DA, Kubendiran G, Venkatesan P, Paramasivan CN. Bactericidal activity of moxifloxacin on exponential and stationary phase cultures of *Mycobacterium tuberculosis*. *J Chemo* 2009; 21; 127-134.

The bactericidal activity of moxifloxacin, alone and in combination with isoniazid and rifampicin, was studied on exponential and stationary phase cultures of *Mycobacterium tuberculosis* H37Rv strain, the standard strain which is a wild type of *M. tuberculosis* strain, not exposed to any environment, susceptible to all anti-tuberculosis drugs. Moxifloxacin alone was highly bactericidal, being intermediate in activity between isoniazid and rifampicin on both types of culture. The speed of activity was slow with the stationary phase culture, causing a reduction from 6.41 log₁₀ cfu/ml to 2.70 log₁₀ cfu/ml

on day 6 with the higher moxifloxacin concentration of 4 mcg/ml and to 4.08 log 10 cfu/ml with the lower concentration of 0.25 mcg/ml. When added to isoniazid, its activity against both exponential and stationary phase cultures was increased. However, when it was added to rifampicin, no increase in activity was found with either type of culture. Addition of moxifloxacin to isoniazid and rifampicin resulted in a slight increase in activity against the exponential culture but a considerable increase against the stationary phase culture with counts below the limit of detection of 4 and 6 days with both moxifloxacin concentrations. The synergism found with isoniazid, but not with rifampicin, supports the view that isoniazid should be included in combinations with moxifloxacin during the therapy of pulmonary tuberculosis.

APPENDIX

Biographic details of Prof. D.A.MITCHISON

Date and Place of Birth: 6th September 1919, Oxford

Names of parents: Dick Mitchison and Naomi (née Haldane)

Education

Dragon School, Oxford and Abbots Holme School

Trinity College, Cambridge - Natural Sciences

University College, London - Medicine & PG training in Pathology

Employment

Brompton Hospital, London

MRC Unit on Drug Resistance in Tuberculosis

Royal Postgraduate Medical School

Hammersmith Hospital, London

Date of retirement: 31 July 2015

Countries where he established laboratories

India, Kenya, Uganda, Tanzania, Zambia, Hong Kong

Countries collaborating in clinical trial programme

East Africa, India, Hong Kong, Singapore, Czechoslovakia

Important U.K. research collaborators

Wallace Fox, Philip D'Arcy Hart, Ian Sutherland

Awards and honours conferred

CMG from the U.K. Government conferred in 1973

Medal of Honour from the Union in 1987

British Thoracic Society Medal in 2000

Stop TB Partnership Kochan prize in 2008

The Union Medal in 2015 for "an outstanding contribution to the control of tuberculosis".

Date and Place of Death: 2nd July 2018, Kingston

So long, Farewell, mentor to us all



**Photograph taken in June 2013,
at the age of 93**