

## Abstracts

### SESSION I. CLINICAL ASPECTS. *CHAIRMAN:* D L LEIKER (THE NETHERLANDS)

#### **1 The Malta experience—rifampicin and Isoprodian combination treatment for leprosy. G Depasquale**

Since 1972, a total of 247 patients have been treated with a rifampicin–Isoprodian combination for a specific period of time as determined by individual clinical and bacteriological progress, and were subsequently controlled periodically for the possibility of relapse. The age, sex, and disease type distribution is demonstrated and the tolerability to treatment and results are discussed. Practically no patients have relapsed, and to date, 145 patients have been under regular post-treatment control for over 10 years, while a further 57 patients have been controlled for over 5 years.

#### **2 Epidemiology of leprosy in Malta. D L Leiker**

A reconstruction of the distribution and the trend of leprosy in Malta since 1900, based on notifications to the Ministry of Health, records of leprosy hospitals and patient records. A decrease in the incidence since 1900 was found. The most rapid decrease occurred in suburban areas, followed by rural areas. In the last decades most of the new cases occurred in peripheral rural villages, 'at the end of the road'.

Preliminary results of treatment of leprosy in the Netherlands are available with daily rifampicin, and dapson, and clofazimine on alternating days. About 400 patients, one third lepromatous and borderline-lepromatous, mostly pretreated with a single drug, were treated for 1 year with the drug-combination and thereafter released from treatment and kept on observation.

So far, 3–5 years after release from treatment, no relapses were found.

#### **3 First evaluation of the Malta leprosy eradication project. D L Leiker**

Independent assessment of classification, treatment history and the bacteriological and histopathological status of leprosy patients in Malta 4 years after release from treatment with rifampicin and Isoprodian.

Assessment of side-effects of the drug-combination and causes of death.

#### **4 A follow-up investigation of the Malta-project, 1983 and 1986. W H Jopling**

The first follow-up examination, carried out in April 1983, included 116 multibacillary leprosy patients who had completed multidrug therapy (MDT), the majority having commenced MDT in 1972, and the minority subsequently. Length of treatment varied between 5 and 89 months, and side effects were mostly mild. No signs of clinical relapse were found, but 36 patients had positive skin smears; 26 had granular bacilli alone, and 10 had scanty 'solids'. Details of these findings constitute the first part of this report.

The second follow-up examination will take place in the early part of April 1986 in order to discover if any of these 10 patients show clinical or bacteriological evidence of relapse, and these findings constitute the final part of this report.

#### **5 Report of the joint leprosy/tuberculosis project in Paraguay. A E Alvarenga**

On the basis of the agreement between the government of Paraguay and the German Leprosy Relief Association (DAHW), a programme is being developed in this country for the eradication of leprosy and tuberculosis. This joint programme started in 1979.

For programme execution a joint leprosy/tuberculosis organization was created under the centralized management of the directorate of the government leprosy service.

Programme implementation is being effected through the existing health infrastructure. This implies that the programme directorate has to implement the programme through two administratively and organizationally different systems. The leprosy programme has a vertical management whilst the tuberculosis service is of a horizontal nature with several administrative levels responsible for the decision making process. This situation requires a rather elaborate type of coordination in order to achieve a uniform programme implementation.

In both programmes the same combined chemotherapy is being used, namely Isoprodian-RMP. The introduction of this regimen, because of its rapid therapeutic action, has made a significant impact in the fight against both of these endemics.

The anti-leprosy programme was developed in two stages:

1. The start of the project in Asunción and its surrounding smaller towns. About one third of the national population is concentrated in this area.
2. During the second stage, which is now in progress, the programme is being gradually extended to the population of the interior. To this end, the available infrastructure as well as the leprosy prevalence are being taken into account.

Until 31 December 1985 some 1623 cases of leprosy were admitted by the programme. This represents 32% of the total registered cases in the country. Of these patients, 797 (49%) terminated treatment whilst 685 cases (42%) are actually passing through the different phases of the therapy.

In 2 patients only, following 2 years of post-treatment observation, therapy had to be reinstated because of clinical reactivation.

The execution of the tuberculosis programme also underwent a gradual development. The start was in Asunción and its neighbouring populations as well as in settled groups of indigenous populations living in the Paraguayan chaco.

In 1980 the TB-programme had a population coverage of approximately 1.6 million. The principal objective of the programme is to reduce as rapidly as possible the TB morbidity and mortality rates. For this purpose a rapid extension of the programme to obtain the widest possible population coverage is necessary—subject to the availability of resources.

Medical attention to TB-patients is being provided by the government health centres. This type of attention is integrated into the general health service.

For programme implementation the government TB service also can count on the collaboration of several voluntary organizations providing assistance to the indigenous populations. Frequently these organizations employ trained lay workers for the task.

The short-term therapy with Isoprodian-RMP is showing very good results indeed, so much so that in some indigenous populations tuberculosis has been practically eliminated.

To the vast majority of patients anti-TB drugs are issued ambulatory. A few serious cases only are hospitalized in special institutions.

During 6 years of programme activity, within the corresponding areas, 5853 tuberculosis patients were detected, or in other words, some 25% of the total estimated number (22,812) of cases in the country. In detail:

TB cases detected within 6 years	5853
No. cured	2835
Abandoned treatment	1261
Left the area	429
Died	385
Actually on treatment	943

The high number of abandoned cases results from the fact that we have to adapt the old organization to the new requirements.

The introduction of the combination Isoprodian-RMP constitutes a most important advance in the fight against leprosy and tuberculosis, principally this is because of its rapid action and outstanding efficiency in the treatment of these two diseases. The period of treatment is vastly shortened with all its positive consequences derived from this progress.

In the light of these promising results, the ambitious goal of planned disease eradication which has no parallels in other programmes, imposes the need for continuing the ongoing programme until its final realization. To fulfil this attainment it is imperative that the joint TB/leprosy programme can count on the continuation of the invaluable support of the German Leprosy Relief Association (DAHW). This great challenge has found the backing of the Paraguayan Government and its people almost from the onset of the programme.

## **6 Combined treatment schedules for leprosy. A prospective randomized multi-centre study. Clinical results. M Dietrich, Clinical Study Group\***

Confirmed cases of lepromatous or borderline lepromatous patients were randomized to receive one of the three following drug regimens: (a) DDS 100 mg/day, (b) DDS 100 mg/day + Rifa 600 mg/day, (c) Rifa 600 mg/day + Isoprodian, 2 tablets/day. A complete physical check-up, basic laboratory test, skin smears and histology were done before treatment and at regular intervals during the 3 years of treatment. Prior to chemotherapy, a DDS resistance test was performed and in case of DDS resistance the patient was put into group D which is equivalent to group C. 302 patients were randomized in five different centres: Freetown (Sierra Leone), Karachi (Pakistan), Bombay, Madras and Chetput (India). The study design was to treat patients for 3 years and to have a follow-up period of 5 years. Presently there are still 245 patients in the study, 69 in Group A, 90 in Group B, 86 in Groups C and D. There is no statistical difference concerning the variables sex, age or disease classification (BB or LL) in the three groups. We report the results after 3 years of treatment. Of the 102 cases we evaluated, 87 show a regression while 14 patients were clinically classified as stable leprosy. The bacteriological index as well as the acid-fast bacilli in the skin biopsy decreases by

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about the same amount in all treatment groups per year. This preliminary evaluation shows no difference in therapeutic response in combined as well as single-drug therapy. The clinical, bacteriological and histological parameters have clearly improved. The three drug regimens were tolerated well, and there was no difference in side effects as judged by GOT, GPT, B.U.N., and haematology serial examinations. As none of the three drug regimens seemed to be superior, the evidence of relapse and/or the development of DDS resistance in the follow-up period may prove to be the crucial criterium for final judgement.

## **7 The impact of multidrug therapy drug implementation in the Tanzania National TB–Leprosy Programme. H J Chum**

## **8 Effect of clofazimine and dapsone on rifampicin (Lositril) pharmacokinetics in multibacillary and paucibacillary leprosy cases. J M Mehta, I S Gandhi, S B Sane, M N Wamburkar**

A comparative pharmacokinetic study of Lositril (rifampicin) was carried out in 6 multibacillary and 12 paucibacillary leprosy cases. The type of leprosy had no significant effect on rifampicin pharmacokinetics.

The effect of dapsone and clofazimine when given separately and in combination was studied on rifampicin pharmacokinetics in each group of 6 patients. Within group comparison revealed that clofazimine reduced rifampicin absorption significantly ( $P < 0.01$ ) and prolonged the time to reach the peak serum concentration ( $P < 0.01$ ). Since MCR and  $K_e$  were also reduced significantly in RC group, as compared with RDC group ( $P < 0.02$  and  $P < 0.05$  respectively), no significant alteration was seen in overall Auc and  $C_{max}$ , although  $t_{0.05}$  was increased significantly ( $P < 0.02$ ) in RC group.

Dapsone alone did not produce any significant alteration in rifampicin pharmacokinetics parameters, while dapsone with clofazimine reduced rifampicin 1 h serum levels ( $P < 0.05$ ) and Auc ( $P < 0.05$ ) significantly.

Of the three groups, except RC group, both RDC and RD groups were homogenous  $K_a$ ,  $avd$ ,  $C_{max}$  and Auc/ $t_{0.5}$  ratio of RC group were significantly different from those in RD group. While  $K_a$  and  $avd$  were significantly less ( $P < 0.05$  and  $< 0.001$  respectively) and  $C_{max}$  and Auc/ $t_{0.5}$  ratio were significantly more ( $P < 0.01$ ) in RC group. Since clofazimine reduced rifampicin absorption, the difference in  $K_a$  and  $t_p$  became more significant in the post-regimen phase ( $P < 0.01$ ).

## **9 Some clinical impressions of multidrug therapy in leprosy. J M Mehta**

The Poona District Leprosy Committee is running two leprosy control projects, one in the urban area of Poona and the other at Solapur. In these projects, by the end of December 1985, 4532 leprosy patients were given multidrug therapy. Of these, 1828 patients are multibacillary while 2704 are paucibacillary.

The standard regimen recommended by the WHO Technical Report Series 675 is being followed for multi- as well as paucibacillary cases. The patients who have maintained regularity in treatment have shown clinical as well as bacteriological improvement. They did not show major side-effects/toxicity. A detailed analysis of the data is being undertaken. However, a few cases in whom interesting clinical findings were observed are reported here.

In 21 multibacillary cases, the bacteriological index started falling initially and after it dropped to 1·0/1·5, it remained static, even after continuing MDT though they showed definite clinical improvement. The pigmentary changes and the ichthyotic changes seen during clofazimine therapy persist for more than 2 years after stopping clofazimine. In a few patients, pigmentation due to clofazimine therapy is not pronounced, though supervised clofazimine therapy is confirmed. The reason for this is not known.

In 20 paucibacillary patients, those previously treated with dapsone monotherapy and later put on MDT did not show much clinical improvement in respect of the patches, while clinical response in new untreated paucibacillary patients treated with multidrug therapy is excellent, as observed in the disappearance of the patches. It is the observation of some of our colleagues that if MDT is discontinued after 6 months, patients report back within 1 year with relapse in the form of new patches.

## **10 Experience with MDT—clinical, operational and managerial implications.** **M Rangaraj**

## **11 Clinical problems in the initiation and assessment of multidrug therapy.** **M F R Waters, D S Ridley & Marian J Ridley**

The introduction of multidrug therapy is essential to overcome major problems of dapsone resistance, both primary and secondary, and hopefully also of compliance and of microbial persistence. The division into multibacillary and paucibacillary leprosy, as postulated by WHO, depends on both accurate clinical classification and smear taking and reading of a high standard. In this paper, we shall discuss some of the classification problems which we have experienced, as exemplified by smear-negative, neural BB and BL leprosy.

We shall also discuss the assessment of the results of treatment of paucibacillary leprosy, as great difficulty is often experienced in distinguishing between bacterial relapse of treatment from late reversal (upgrading or type 1) reactions. We have observed the latter to occur as late as 3 years after commencing (and continuing on multidrug) therapy in BT leprosy.

## **12 Preliminary evaluation of the effect of WHO/MDT on disabilities in leprosy patients in Malawi (Central Africa).** **J Pönnighaus & G Boerrigter**

In a preliminary analysis of disability rates at registration, at completion of treatment and at 1 year after completion of WHO/MDT, we have shown that the percentage of patients treated with WHO/MDT who developed new or worse disabilities (5·7%) was similar to the percentage of patients treated with dapsone monotherapy who developed new or worse disabilities (2·7–6·1%).

On the other hand, review notes of the field staff appear to indicate that a higher percentage of patients (52%) recovered lost functions during and after WHO/MDT than during dapsone monotherapy (19–28%).

## **13 The use of MDT in three western regions of Nepal.** **P G Kalthoff**

It has been proved that, even under very difficult field conditions like in Nepal, MDT can be introduced in the field if there are detailed instructions available for the PMW, together with

sufficient training provided. In addition, supervision needs to be done, particularly at the beginning to introduce the new habits properly, as well as periodically afterwards to make sure the standard is kept. We are hoping that the new patient recording system will help to improve the standard further, and particularly to allow us precise evaluation of the programme in the future.

#### **14 Operational aspects of the implementation of multidrug therapy at ALERT, Ethiopia. M Becx-Bleumink**

The ALERT Leprosy Control Department is responsible for leprosy control in Shoa Administrative Region. This region is centrally located in Ethiopia; it covers an area of about 85,000 sq. km, with a population of 8.75 million. The region is divided into one urban and eleven rural districts.

Leprosy diagnostic and treatment services are given in 292 centres; 60% of these are attached to general medical services and 40% are leprosy clinics which have been established in those areas where a general medical service does not exist yet. About 50% of the centres are accessible by car during the whole year. Multidrug therapy (MDT), according to the WHO recommendation of 1981, was introduced in January 1983. Paucibacillary patients are treated for a period of at least 2 years and until their skin smears have become negative.

In October 1983 a 'Manual for implementation of Multiple Drug Therapy in Ethiopia' was finalized; a second, revised, edition of this manual became available in February 1985.

During 1983 MDT was introduced in two rural districts (64 clinics); during 1984 in one urban and two rural districts (48 clinics) and in 1985 in two rural districts (61 clinics).

Prior to the introduction of MDT the leprosy control services were reorganized and intensified. This includes clinical and bacteriological examination of the patients under treatment, release from treatment of those patients who were considered of having received sufficient treatment with dapsone monotherapy, introduction of new recording and reporting systems, health education campaigns in the clinics and the communities, redefining of tasks and training of all cadres of staff involved.

During the period 1 January 1983 to 1 July 1985 3401 multibacillary patients and 2759 paucibacillary patients have been put under MDT. By the beginning of July 1985, 740 multibacillary patients and 2285 paucibacillary patients had completed their course of MDT. Until July 1985 one BT relapse has been diagnosed. Evaluation of the results of the treatment is done by way of cohort analysis: Of the 2543 paucibacillary patients who started MDT during the period 1 January 1983 to 31 December 1984, 2297 patients (90.3%) completed their course of MDT within a period of 9 months; 202 patients (7.9%) had their treatment discontinued because of irregularity of attendance; 22 patients (0.5%) had been transferred to a non-MDT area; 12 patients (0.5%) had died and 11 patients (0.4%) continued the treatment after 9 months.

During 1986 the MDT programme will be further expanded to two rural districts. We have planned that by 1990 the whole region will be covered with MDT.

During the period July 1982 to July 1985 the number of patients under chemotherapy in the region has decreased from 20,908 to 10,507. This decrease is mainly due to the release from treatment of over 5500 patients after dapsone monotherapy and the introduction of MDT.

Patients who have been released from treatment are instructed to attend regularly for follow-up examinations. So far 25–30% of the patients came for the appointed follow-up examinations.

About 3500 patients who have been released from chemotherapy since July 1983 continue to need care because of disabilities.

We have experienced that proper planning and organization of the MDT programme, including preparation of a detailed manual, are of extreme importance in order to guarantee proper implementation and evaluation of MDT. Workshops for the staff involved in MDT are conducted

at regular intervals. Priorities for future leprosy control in those areas where the number of patients under treatment has decreased to a large extent have been defined.

We are in the final process of making preparations for the field studies in one of the MDT areas; one study on the incidence of relapses, one on reactions during MDT and during the first year after release from MDT.

Problems we experience in the MDT programme will be discussed. These are especially: Problems related to the severe drought and the acute shortage of public transport, analysis 'by hand' of the many data which are essential for evaluation of MDT and the leprosy situation over the years.

The differentiation between relapse and reaction in paucibacillary patients. This is becoming an increasing problem.

### **15 Combined chemotherapy of multibacillary leprosy of 6 months duration. T Saylan, N Onsun & S R Pattyn**

A treatment regimen of 6 months duration and composed of 2 weeks daily RMP (600 mg), PRO (500 mg) and DDS or CLO (100 mg) followed by 24 weeks RMP (600 mg) once weekly and daily PRO (500 mg) and DDS or CLO (100 mg), was administered to a group of 72 MB patients (45 new cases and 27 cases treated previously). Nineteen patients could be followed for 2 and 3 years after the end of therapy. No relapses were observed. The confidence limit of this result is 17.5.

### **16 The future of leprosy in the Dominican Republic and experiences made with MDT. D Martinez Cruz**

SESSION II. TEST MODELS FOR THE EFFECTIVE CONTROL OF CHEMOTHERAPY FREE COMMUNICATION. *CHAIRMAN: A M DHOPLÉ (USA)*

### **17 The use of rodent models in assessing antimicrobial activity against *M. leprae*. R H Gelber**

The ability of antimicrobial agents to prevent multiplication of *M. leprae* in the mouse foot pad remains the only generally acceptable means of assessing their potential for clinical application. The first screening technique to be utilized, the 'continuous method', employed uninterrupted treatment from the time of footpad infection, initially with the highest concentration of drug tolerated, orally if possible. Unfortunately, this technique did not distinguish between purely bacteriostatic agents and those with bactericidal effects. Thus a method termed the 'kinetic technique' was developed wherein drugs are administered from day 60 to 150 following foot pad infection. Agents that inhibit growth only during administration are considered bacteriostatic and those that appear to limit multiplication even after treatment has been discontinued are considered bactericidal. More recently the 'proportional bactericidal technique' for more direct assessment of *M. leprae* killing was developed. By this technique mouse foot pads are inoculated with 10, 100, 1000 and 10,000 *M.*

*leprae*, mice treated for the first 60 days, and foot pads harvested and *M. leprae* enumerated 1 year later, a time sufficient for any surviving *M. leprae* to multiply. This method allows for a quantitative assessment of bactericidal activity and comparison of the relative killing potential of various agents. The application of these methods will be reviewed and limitations of their utilization detailed.

Because there are no well established means of predicting which antimicrobials will be active against *M. leprae*, our strategy in selecting agents for testing primarily involves selecting drugs found useful against cultivable mycobacteria and those that act at loci thus far unexploited in the therapy of leprosy. Because only antimicrobial agents with some bactericidal potential merit further investigation, our initial screening efforts utilize the kinetic technique at maximally tolerated doses. We further study active agents by the proportional bactericide technique and, at times, at lower concentrations and frequencies of administration. In recent years we found a number of cephalosporins, cephamycins, doxycycline and erythromycin inactive. We have had variously promising findings with cycloserine, aminoglycosides, certain dihydrofolate reductase inhibitors, cephradine, amoxicillin/clavulanic acid, ciprofloxacin and especially minocycline. The results and the status of our ongoing studies will be detailed. In addition, the ability of various dietary concentrations of dapson, rifampin, clofazimine and ethionamide to kill *M. leprae* as assessed by the proportional bactericide technique will be presented. The possible implications of those results to the therapy of leprosy in man will be discussed.

The neonatally thymectomized Lewis rat (NTLR) infected with *M. leprae* presents a situation analogous to human lepromatous leprosy, wherein viable persisting *M. leprae* remain despite therapy with all regimens of dapson and rifampin previously utilized. We have been studying the effect of other combination chemotherapy regimens in NTLR to assess their relative efficacy to prevent persists in established NTLR infections of  $10^7$  *M. leprae* or more. Regimens include: 1, larger doses of rifampicin alone and combined with dapson; 2, clofazimine and rifampicin; 3, dapson and ethionamide; and 4, rifampicin and ethionamide.

The results of these studies to date will be reviewed.

The standard means of laboratory monitoring of clinical trials in lepromatous leprosy have been the determination of the viability of  $5 \times 10^3$ – $10^4$  *M. leprae* obtained from biopsy material, usually skin, in the mouse foot pad. Attempts at monitoring trials utilizing thymectomized/irradiated mice, neonatally thymectomized Lewis rats (NTLR) and nude rats, as well as utilizing larger inocula in normal mice and some of these rodent systems are being employed in our laboratory and elsewhere. The current status of these studies will be reviewed.

## **18 Limited *in vitro* multiplication of *M. leprae*—application to screening potential anti-leprosy compounds. A M Dhople**

Inability to cultivate *Mycobacterium leprae* (*M. leprae*) *in vitro* has been a major bottleneck in leprosy research. Today, the leprosy bacillus remains the only bacterium causing disease in man that has not been cultured *in vitro* and until this is achieved, all studies on leprosy will remain at a serious disadvantage compared with other human bacterial infections. We have initiated studies in this direction and the preliminary findings are presented at this symposium.

The studies done so far by other investigators, though unsuccessful, dealt mainly with microscopic and/or macroscopic growth of *M. leprae* in a given medium. But we have adopted three biochemical indicators to follow the fate of *M. leprae* incubated in a given medium. The first one is adenosine triphosphate (ATP) content of *M. leprae*; because of its ubiquitous distribution, the quantitative measurement of this compound is a promising method for detecting and quantitating microorganisms. The second one is deoxyribonucleic acid (DNA) content of *M. leprae* because of its role in cell replication. The last one is the uptake of (3H) thymidine by *M. leprae* because of its role in the synthesis of DNA and also because of the evidence available on its relationship to



viability of *M. leprae*. In our studies, we have demonstrated that 17% of the total (3H) thymidine uptake by *M. leprae* is due to its incorporation into *M. leprae* DNA. Furthermore, we have observed that *M. leprae* possesses thymidine kinase but not thymidine phosphorylase, suggesting that thymidine is converted to thymidine monophosphate and thus, incorporated into *M. leprae* DNA.

Two kinds of culture media were selected. The first one is DH medium in which Dhople and Hanks had successfully achieved growth and subcultures of *M. lepraemurium*, and the second one is Mahadevan's conditioned medium using supernates of dorsal root ganglian cultures. The cultures containing *M. leprae* in these two media were incubated at 34°C. After an initial lag of 4–6 weeks, there was a definite multiplication of *M. leprae* in both the media. The maximum growth, as judged by all three of the above criteria, was obtained between 14 and 16 weeks. Even though the rate of multiplication was slow and the cell yield was very low, the harvested cells were shown to be *M. leprae* by several standard tests. The cells harvested from both the culture media after 16 weeks were used to inoculated freshly prepared respective media and the cultures were incubated at 34°C. During the 12 weeks of incubation there was a steady and constant decline of bacterial ATP, DNA and also (3H) thymidine uptake suggesting that metabolically the cells became totally inactive. The cells recovered at the end of 12 weeks failed to multiply in the foot pads of mice. Thus, it can be stated that there was a limited but definite multiplication of *M. leprae* in primary cultures but subcultures could not be achieved. Since then several modifications have been made in both the culture media to improve growth rates as well as cell yields.

Next, DH medium was employed to evaluate the effects of DDS and rifampicin. *M. leprae* were incubated in the presence of various concentrations of DDS and at periodic intervals, the cells were taken for ATP assays and (3H) thymidine uptake. No inhibitory effects were seen when the concentration of DDS was 10 ng/ml or less. At the end of 6 weeks, *M. leprae* became non-viable in the presence of 20 ng/ml DDS and this period decreased with the increasing concentration of DDS in the medium. *M. leprae* harvested at the end of 8 weeks of incubation were inoculated into the foot pads of mice to compare above *in vitro* results on viability. Similarly, using this method the MIC of rifampicin against *M. leprae* was found to be between 250 and 300 ng/ml. These studies are in progress.

## 19 Single bacterial cell mass analysis: a rapid test method in leprosy therapy control. U Seydel & B Lindner

To overcome problems arising from the *in vitro* non-cultivability of *M. leprae* we have started some time ago to develop an alternative technique to acquire fast and reliable information on the effectiveness of a chemotherapy by mass spectrometric analysis of a single *M. leprae* cells isolated from biopsies.<sup>1,2</sup> The information is derived from measurements of the intracellular concentrations of sodium and potassium ions and from the evaluation of so-called mass fingerprints which stem from fragment ions of the complex cell matrix.<sup>3</sup> All information is available within hours after the preparation of the samples from biopsies.

So far, it could be shown that the ratio of the intracellular sodium and potassium ion concentrations ( $\text{Na}^+$ ,  $\text{K}^+$ -ratio) is a sensitive indicator of the physiological state of a cell and that its value can be taken as a measure for the impairment of a cell following chemotherapy. From first evaluations of the time dependence of the  $\text{Na}^+$ ,  $\text{K}^+$ -ratio in a follow-up study it may be expected that the method can yield information on kinetics of drug interaction. The data extracted from mass fingerprint evaluation, furthermore, gives evidence for its applicability for monitoring the development of drug resistance.

In close cooperation with Dr A M Dhople (Melbourne, Florida/USA) a good agreement between the statements for his ATP-assay, the mouse footpad test and our measurements of the  $\text{Na}^+$ ,  $\text{K}^+$ -ratio was found.<sup>2</sup>

A particular advantage of the single cell mass spectrometry, however, is—beside the fact that all data are obtained from the analysis of only a few hundred cells—the possibility to get more detailed insight in the drug response of a cell population by analysing single cells and this way getting distributions of the respective data instead of averaged values.

## References

- <sup>1</sup> Lindner B, Seydel U. *J Gen Microbiol*, 1983; **129**, 51.
- <sup>2</sup> Seydel U, Lindner B, Dhople AM. *Int J Lepr*, 1985; **53**, 365.
- <sup>3</sup> Lindner B, Seydel U. *J Phys Colloq (France)*, 1984; **45** C-2, 785.

## 20 Metabolism in *M. leprae*: possible targets for drug metabolism. P R Wheeler

Metabolic activities in *M. leprae* which are essential for the growth and survival of the bacteria, and not present—or present but with completely different properties—in the host, are potential targets for anti-leprosy agents.

Much is known about the energy metabolism in *M. leprae*, including the dissimilation of carbon sources. However, most of the pathways are widely distributed amongst living organisms, and there often exist 'alternative pathways'. Thus energy metabolism may not be amenable to inhibition by anti-leprosy agents although two activities in *M. leprae*—glycosidases possibly involved in Hexuronate catabolism, and cytochrome o—are characteristically bacterial and specific inhibitors may be found there.

Generally, antibacterial drugs act against biosynthetic activities or replication (which can be seen as the culmination of many, co-ordinated biosynthetic activities in the bacterial cell). Studies of biosynthetic activities in *M. leprae* are fragmentary, but synthesis of the cell-wall can be discussed. In most respects, the wall of *M. leprae* is similar to that of other mycobacteria, so any agents developed which act on the cell wall of other mycobacteria should inhibit growth of *M. leprae*. Protein synthesis in *M. leprae* is characteristically bacterial, being inhibited by chloramphenicol. One amino acid not incorporated into protein is DOPA, yet this amino acid, as either L-DOPA or D-DOPA, is taken up and oxidized by *M. leprae*, interestingly an activity which appears restricted amongst the mycobacteria to *M. leprae*. The biological significance of DOPA oxidation is not known and it may be that in attempting to design agent against DOPA 'metabolism' an activity of no importance to the bacteria is being selected as a possible target.

Nucleic acid synthesis the target of many antibacterial drugs, and two agents effective against *M. leprae*, rifampicin and clofazimine, appear to affect these pathways. Thiosemicarbazones appear to inhibit mycobacterial ribonucleotide reductase, an enzyme for making nucleotides available for DNA synthesis. Much is known about the synthesis of nucleotides by *M. leprae*: it is doubtful whether de novo synthesis occurs in *M. leprae*, but the organisms scavenge purines very effectively. If *M. leprae* proves to require purines for growth, then there exists the possibility that drugs could be developed against purine scavenging in *M. leprae*. Indeed, pyrazolopyrimidines are known to inhibit growth of some pathogenic trypanosomidae which are dependent on preformed purines. The use of hyposanthine incorporation as a potential, general drug screening method will be briefly discussed.

Although the development of agents against folate metabolism will be discussed elsewhere, I will discuss the metabolic significance of inhibiting folate metabolism briefly in this talk.

## 21 Host-pathogen interaction—new *in vitro* drug test systems against *M. leprae*—their possibilities and limitations. P R Mahadevan

*Mycobacterium leprae* which has so far failed to grow even slowly *in vitro* or even metabolize actively on *in vitro* isolation, poses a problem for rapid drug sensitivity assay. The only drug test system that was possible till recently is using the growth potential of this bacterium in mouse footpad—an assay that would take at least 9 months to show drug sensitivity or resistance of *M. leprae* to the test compound. To overcome the disadvantages mentioned above, we have directed our attention to *M. leprae*-induced changes in host cells, as part of host-pathogen interaction. Having identified such changes it was possible to monitor such changes in presence or absence of drugs which would indicate inactivation or confirmed viability of *M. leprae* respectively. The indicator changes were involved in host cell membrane receptors, protein synthesis and activation of *M. leprae* metabolism.

Exploiting the above criteria, we have developed few *in vitro* assay systems—three of them are referred to as (a) Fc receptor assay, (b) FDA-EB assay and (c) Uracil uptake assay. There are others with potential use; they also will be described.

All these assay systems basically use cultured peritoneal macrophages from mice, exposed to the test drug in presence of phagocytosed *M. leprae*. The expected changes when live bacilli are present are monitored. If such changes do not occur in presence of a drug, the drug is considered as active.

Using all the three assay systems and some others, susceptibility of *M. leprae* to sulphone and rifampicin has been demonstrated and loss of viability of *M. leprae* in such experiments were also correlated with mouse foot pad tests. This correlation showed the validity of these test systems.

Some new compounds have been identified as potential anti-*M. leprae* agents by the above *in vitro* assay systems. One such compound is Brodimoprim. Its synergistic activity with dapsone against *M. leprae* was demonstrated and this has been confirmed in mouse foot pad.

Other drugs identified are Deoxyfructoserotonin, ciprofloxacin, Indole-2-carboxylic acid, Diflunisal and few other derivatives from the laboratories of Dr J K Seydel.

Two of the test systems were subjective, since it involved use of microscope by the investigator and counting. But both of these have been now confirmed by a more quantitative method. Fc receptor assay is demonstrated using I<sup>125</sup> labelled antibody coated SRBC. FDA-EB method by measuring fluorescence by spectrofluorimeter.

The advantages of these *in vitro* assay systems are (a) it is completed in less than 10 days, (b) *in vitro* MIC can be determined, (c) synergistic activity between two different drugs can also be established, (d) static or cidal effect can be assessed. Among the drawbacks (a) one needs at least 5–10 million *M. leprae* for each assay, as compared to  $1 \times 10^4$  in mice footpad. (b) As patients improve on drug therapy viability goes down thus to monitor viability one has to use higher number of bacilli and this may lead to ambiguous data.

However, we are in a better position now to identify potential anti-*M. leprae* compound much faster than we were 5 years ago. Information regarding the above assay systems, observations and conclusions will be presented and discussed.

## 22 Isolation of environment-derived *M. leprae* and its application in cultivation trials. J Kazda

It is well known that even in highly endemic areas, contact with leprosy patients cannot be established as a source of infection in a considerable proportion of new cases. In a study covering Africa, Asia and the United States, this contact could be established in 25 to 60%. Already at the IInd International Leprosy Congress in 1909 SAND postulated on the basis of epidemiological

observation in Norway, that leprosy is not transmitted by direct contact, but probably through some environmental medium, such as soil.

In samples collected in Bombay Leprosy Area an isolation of environment-derived *M. leprae* has been described recently. This strain of *M. leprae* was isolated from soil in foot-pad technique in mice. Besides biochemical properties specific for *M. leprae* (dopa oxidase and pyridine decolorization), the specific phenolic glycolipid I could be detected.

Using a direct inoculation of a suspension of the same soil sample in sphagnum nutritive substrate, used for cultivation trials with *M. leprae*, an additional mycobacterium, grown on conventional media, has been found together with *M. leprae*. This mycobacterium, identified as *M. intracellulare*, serotype Darden, caused an increase of pathogenicity of *M. leprae*, when inoculated in foot pads of nude mice. The swelling of foot pads, observed 4 to 6 months after inoculation, was accompanied with the development of cutaneous leproma in the dorsal site of the nude mice.

The first experiments with the influence of *M. intracellulare*, serotype Darden on the multiplication of *M. leprae in vitro* are described.

### **23 Investigations into the cultivation of *M. leprae*. A multifactorial approach. L Kato**

Culture media for *M. leprae* are proposed on the assumption that the mycobactin deficient *M. leprae* requires growth factors produced by leprosy-derived mycobacteria (LDM). *M. intracellulare* and *M. phlei*—both LDM—were used as donors of exochelins and/or mycobactins in the multifactorial media. The LDM—*M. phlei* and *M. intracellulare* were grown respectively for 10 and 20 days in Sauton medium or a basal medium without and with Tween 80 added. Autoclaved cultures were filtered and Na thioglycolate 1 g, thioctic acid 0.1 g, (NH<sub>4</sub>) SO<sub>4</sub> 2 g, MgSO<sub>4</sub> 0.1 g and ferric ammonium citrate 0.05 g were dissolved in 1 litre of the filtrates. The pH was adjusted to 5.8 with KH<sub>2</sub>PO<sub>4</sub>. Twenty ml media was distributed into each of 25 ml screw-cap tubes and autoclaved for 30 minutes. The media thus prepared contained sufficient amounts of exochelins and mycobactins to support growth of *M. paratuberculosis* ATCC 19698.

Host-grown *M. leprae* were inoculated into the multifactorial media and incubated at 34°C. Positive growth and subcultures were obtained from 3 out of 4 specimens in the exochelin-mycobactin enriched media.

Latency period of growth was estimated at 10–16 days and time of division at 8–12 days. Cells were long, acid-fast, arranged side by side or end to end, with a tendency to form long spiral cords or clumps when sedimented on siliconized slides. Pyridine extraction eliminated acid fastness, but not Gram positivity. Cultures did not grow on Dubos, Löwenstein or 7H10 media. In the footpads of mice they produce the disease characteristic of *M. leprae*. Subcultures remain dependent on the multi-factorial media, enriched with growth factors (exochelins and mycobactins) from the leprosy-derived cultivable mycobacteria.

## **SESSION III. DEVELOPMENTS AND FUTURE ASPECTS.**

*CHAIRMAN: ENNO FREERKSEN (FRG)*

### **24 Recent developments in the field of multidrug therapy and future research in chemotherapy of leprosy. J H Grosset**

The discovery of rifampicin together with the increasing prevalence of dapsone resistance were decisive factors to question the value of dapsone monotherapy and even of any drug monotherapy

in the treatment of leprosy. Thanks to the efforts of some leading personalities and of WHO a progressive move took place during the decade 1970–80 towards a multidrug therapy of leprosy as that was the case in the therapy of tuberculosis since the early fifties.

Besides to stop the transmission of the bacilli in the community, chemotherapy for leprosy as well as for tuberculosis has two objectives: (i) to prevent the selection of drug resistant mutants and (ii) to kill the drug sensitive organisms. To reach the first objective, a combination of drugs active against *M. leprae* should be given as long as the drug resistant mutants present at the beginning of treatment have not been eliminated. To reach the second objective one single sterilizing drug or a combination of sterilizing drugs should be given for a length of time sufficient to prevent the majority of relapses due to the regrowth of persisting organisms.

Although the more recent controlled clinical trials and field trials conducted in different parts of the world have demonstrated the high effectiveness of multidrug therapy, the precise length of time necessary to eliminate all drug resistant mutants is not yet known. This is therefore one of the priority of the research in chemotherapy of leprosy. While WHO and other organizations are working to solve the problem, it is necessary to use a three-drug combination throughout the whole course of chemotherapy for multibacillary leprosy, as recommended by WHO.

Another problem to be solved is the precise length of treatment necessary to prevent relapse after stopping treatment. Is the 2-year treatment too long or too short for all cases of multibacillary leprosy? What is the relationship between the presence and the number of persisters and the risk of relapse after stopping treatment? What is the effect of extending chemotherapy beyond the minimal course of 2 years on the number of persisters and on the relapse rate? What is the role of immunotherapy about that? These questions should be and will be answered in the future by research programmes. Meanwhile it is safe to follow the WHO recommendations that is to treat multibacillary patients for a minimum of 2 years or at best till the negativation of BI.

## **25 Strategies in the development of new drugs and drug combinations against leprosy demonstrated on the example of folate- and gyrase-inhibitors. J K Seydel, M Rosenfeld, M Sathish, R. Haller, M Kansy & G Hachtel**

The lack of an *in vitro* test system for *M. leprosy* is forcing us to think about new routes for the development and screening of potential antileprotic drugs and drug combinations. This is relevant for testing known antibacterials produced by pharmaceutical companies as well as for the development of new drugs against leprosy.

Mouse foot pad technique—besides being very time consuming—is only of limited value because of decisive differences in pharmacokinetics and metabolism of drugs in mice and man. This can lead to underestimation of the effectivity of the drug in mice (false negatives). In addition the observed effective dose is not relevant for the dose necessary for cure in man. These problems are discussed on the example of new quinolonic acid derivatives (Ciprofloxacin®, Ofloxacin®) and for new folate synthesis inhibitors developed in our laboratories. Test systems used are cell-free enzymes, cultivable mycobacterial strain, *M. leprae* suspensions (Dhople) and serum activity tests in human.

The new folate inhibitors are up to 300 times more effective against mycobacteria as compared to known folate inhibitors (trimethoprim, pyrimethamin). A strong synergism in combination with dapsone is observed.

According to the obtained results quinoline acid derivatives and combinations of the new inhibitors of bacterial folate synthesis are promising compounds for the treatment of leprosy.

## References

- Seydel JK, Wempe EG, Rosenfeld M. *Chemotherapy* 1983; **29**: 249.  
Seydel JK, Wempe EG. *Int J Lepr* 1982; **50**: 20.  
Rosenfeld M, Seydel JK. Proc. 14th Int. Congr. Chemotherapy, Kyoto, 1985: 5374.

## **26 Development of inhibitors of mycobacterial ribonucleotide reductase. K-J Schaper, J K Seydel, M Rosenfeld, J Kazda & H Schönenberger**

For several reasons there is an urgent need for new drugs for the chemotherapy of leprosy. Starting with the known but unsatisfactory activity of thiacetazone against *M. leprae* or the leprosy model strain '*M. lufu*', a screening of related thiosemicarbazones (TSCs) showed that 2-acylpyridine-TSCs are considerably more active against '*M. lufu*' than other TSCs lacking the basic N atom in the alpha-position. Literature results suggest that these metal ion chelators are acting as inhibitors of the iron containing bacterial enzyme ribonucleotide reductase.

The toxicity of acylpyridine-TSCs was considerably reduced by replacing their thioamide group by different N-heterocycles (new lead PH22). This exchange furthermore caused an increase of both antibacterial activity and chelating properties.

In accordance with the mode of action hypothesis of ribonucleotide reductase inhibition it was found in cell cultures that PH22 derivatives are very potent inhibitors of DNA synthesis. Interestingly a very pronounced synergism in antimycobacterial activity is observed on combination of PH22 with several drugs known to be inhibitors of the DNA synthesis pathway.

## **27 Successful regimens of definite duration in the treatment of leprosy. Theoretical considerations and practical results. S R Pattyn**

As we pointed out in the past the outlook of leprosy treatment was revolutionized by the discovery of the bactericidal activity of rifampicin (RMP) and the thioamides (THA). Based on theoretical considerations and results of experimental chemotherapy in the mouse, paucibacillary leprosy should be curable by a relatively short course regimen with a bactericidal drug in monotherapy. Multibacillary leprosy should be treated by combined therapy. Questions to be answered are: (a) what combination(s) of drugs, (b) at what frequency or intermittency should drugs be administered, (c) how long should treatment be pursued.

Precise definitions and criteria for cure have to be defined, the most important being the killing of all bacilli and the occurrence of absence of relapses, equally to be defined precisely.

The ideal treatment regimen is the one dose therapy, the 'therapia sterilisans magna'. Since this cannot be realized yet with the drugs available, treatment regimens approaching this goal have to be defined. Regimens should be efficacious and therefore supervisable and for this reason of short duration, eventually intermittent.

Since circumstances are largely different in the world, there is need for different regimens with known efficacy, to allow Public Health Authorities to make a rational choice of drug regimens that suit their possibilities at best.

The only method to measure the value of drug regimens is the conduction of prospective, eventually comparative, clinical trials. With this in mind we conducted over a number of years several prospective studies on the efficacy of different drug regimens in various forms of leprosy.

In PB leprosy we have studied the following regimens, the results of which will be illustrated and discussed.

1. RMP 900 mg 1/7 8 ×.
2. RMP 900 mg 1/7 10 ×.
3. RMP 900 mg 1/7 12 ×.
4. RMP 600 mg 1/7 10 ×.
5. RMP 600 mg 1/30 6 × (WHO regimen).

In MB leprosy we have studied regimens of:

1 year duration:

6 M RMP 600 7/7 ETH 500 7/7 DDS 7/7 + 6M DDS 100 7/7 with or without ETH 500 7/7.

of 6 months duration:

2W RMP 600 7/7 ETH 500 7/7 DDS 100 7/7 + 24W RMP 600 1/7 ETH 500 7/7 DDS 100 7/7.

of 3 months duration:

RMP 600 2/7 ETH 500 7/7 DDS 100 7/7.

The results will be illustrated and discussed.

## **28 Preliminary results of treatment of leprosy in the Netherlands with an alternative drug combination. D L Leiker**