CURRENT LITERATURE

This department carries selected abstracts of articles published in current medical journals dealing with leprosy and other mycobacterial diseases.

General and Historical

Mayama, A. [Current topics in Hansen's disease.] Iyodenshi To Seitai Kogaku **39** (1985) 1032–1037. (in Japanese)

Hansen's disease is one of six main tropic communicable diseases in African, Asian and Central-South American countries. It chiefly involves the peripheral nerves. Hansen's disease is much behind with its study due to the lack of a method of cultivation of *Mycobacterium leprae.*

In the past 40 years, remarkable progress of chemotherapy has been made for the disease, so that we can cure the patients without difficulty within a short period. More recently, a definite advance has been achieved in hansenology. The multiplication of Hansen's bacilli in the soles of the feet of the mouse, the experimental production of lepromatous leprosy in the armadillo, the introduction of chemotherapy using multiple drugs, and the development of molecular biologic approaches to the production of vaccine against Hansen's bacilli are important advances.

In Japan, there are about 8700 registered cases. All patients are under regular treatment at the 13 national leprosaria (7700 cases), three private leprosy hospitals (100 cases) and at home (900 cases). There may be 20 million estimated cases of Hansen's disease worldwide. The disease remains a serious problem in terms of public health and socio-economics in the developing countries. The need for a method of prevention continues to be very important for the control of Hansen's disease.—Author's English Abstract

Nebout, M., Husser, J. A. and Daumerie, D. [Problems in applying the practice of multidrug therapy in leprosy in West Africa.] Acta Leprol. **4** (1986) 473–478. (in French)

The implementation of multidrug therapy (MDT) in the states of West Africa oblige to analyze new restraints, in order to modify the existing health structures. The planning of Hansen's programs based on MDT needs to consider the technical and logistic parameters. Solutions are proposed for health workers' training course, flow chart, drug supply system and supervision system. The advocated method uses the existing resources, and aims at the integration into general health services, reinforced by specialized teams.—Authors' English Summary

Warzok, V. R., Thomas, P. K. and King, R. [Pathomorphology of leprosy.] Zentralbl. Allg. Pathol. **132** (1986) 3–9. (in German)

Leprosy is a widely distributed infectious disease in countries with tropic or subtropic climates and the most frequent treatable polyneuropathy. Because the import into countries with temperate climate should be considered, a review is given on different stages and forms of course. Special attention is paid to structural changes and to the significance of morphological studies in establishing the diagnosis.—Authors' English Abstract

Chemotherapy

Alvarenga, A. E. Report of the joint leprosy-tuberculosis project in Paraguay. Lepr. Rev. **57** Suppl. 3 (1986) 53–59.

On the basis of the agreement between the government of Paraguay and the German Leprosy Relief Association (DAHW),

a program is being developed in this country (Paraguay) for the eradication of leprosy and tuberculosis. The joint program started in 1979.

In both programs the same combined chemotherapy is being used, namely, Isoprodian-rifampin (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.

Until 31 December 1985, some 1623 cases of leprosy were admitted by the program. This represents 32% of the total registered cases in the country. Of these patients, 797 (49%) terminated treatment while 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 program also underwent a gradual development. The start was in Asunción and its neighboring populations as well as in settled groups of indigenous populations living in the Paraguayan chaco.

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 the program 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.

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.—Author's Abstract

Arora, S. K., Singh, G. and Sen, P. C. Side effects of levamisole. Indian J. Med. Sci. **40** (1986) 6–8.

Levamisole was tried in 30 leprosy cases in a dosage of 150 mg/day for 3 consecutive days every fortnight for 3 months. No severe side effect was seen. Mild degree side effects were seen in 50% of patients. Only 10% of patients had it regularly. We feel that levamisole is quite a safe drug. It can be used for long-term treatment for its immunostimulatory action.—Authors' Abstract

Baciewicz, A. M., Self, T. H. and Bekemeyer, W. B. Update on rifampin drug interactions. Arch. Intern. Med. **147** (1987) 565–568.

Rifampin, a potent inducer of the hepatic microsomal system, has been shown to cause clinically important interactions when combined with other drugs, including oral anticoagulants, oral contraceptives, digitoxin, methadone hydrochloride, sulfonylureas, and barbiturates. Additional literature on previously described interactions has been published recently on quinidine, glucocorticoids, digoxin, and theophylline. New rifampin interactions have been described for cyclosporine, ketoconazole, chloramphenicol, β-blockers, verapamil, and phenytoin. These interactions seem to be of clinical significance.—Authors' Abstract

Balakrishnan, S., Kumar, A., Rao, B. R. and Patro, T. P. Implementation of tests for monitoring drug compliance of leprosy out-patients under multi-drug therapy. Indian J. Lepr. **58** (1986) 555–559.

The field workers of two multidrug therapy (MDT) districts (Srikakulam in Andhra Pradesh and Ganjam in Orissa) were trained for implementation of the paper spot test together with pill/capsule count methods of monitoring the treatment compliance of leprosy outpatients receiving MDT. All the workers recognized the importance of implementing these methods and found them operationally feasible in the field. Of the 672 paucibacillary and 749 multibacillary cases monitored for drug compliance, 85%–88% cases showed regular compliance (more than 75% drug intake) to dapsone. The compliance to clofazimine among multibacillary cases was better (94% regular) than dapsone compliance. Both of these monitoring methods are advocated to be used on larger scale under our National Leprosy Eradication Programme.—Authors' Abstract

Becx-Bleumink, M. Operational aspects of the implementation of multidrug therapy at ALERT, Ethiopia. Lepr. Rev. **57** Suppl. 3 (1986) 115–123.

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 11 rural districts.

Leprosy diagnostic and treatment services are given in 292 centers; 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 centers 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 as 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 had 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 program 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 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 program, 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.—Author's Abstract

Boerrigter, G. and Ponnighaus, J. M. Preliminary evaluation of the effect of WHO-MDT on disabilities in leprosy patients in Malawi (Central Africa). Lepr. Rev. **57** Suppl. 3 (1986) 101–105.

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%).—Authors' Abstract

Chapon, F., Lechevalier, B., Da Silva, D. C., Rivrain, Y., Dupuy, B. and Deschamps, P. [Thalidomide induced neuropathy.] Rev. Neurol. (Paris) **141** (1985) 719–728. (in French)

Symptoms and signs in four patients with thalidomide-induced neuropathy developing during treatment of discoid lupus were limited for long period to distal paresthesiae with altered sensory conduction velocities. Semi-thin biopsy specimens of the distal sural nerve showed depopulation of myelinized fibers, mainly affecting those of large caliber, and signs of axonal degeneration. Study of dissociated fibers showed a high proportion of E fibers. Morphometry confirmed the axonal lesion. Ultrastructural examination demonstrated anomalies of axons of amyelinic fibers (vacuoles, lamellar figures) and of Schwann cells (stacked cytoplasmic prolongations), together with numerous collagen pockets, all nonspecific lesions. The disease course was slow, with disappearance of sensory symptoms in a few weeks in 3 of the 4 cases and normal clinical findings in 1 of the 4 patients 1 year after cessation of treatment. Definite correlations between the dose administered and the severity of the neuropathy could not be established. The still poorly understood mechanism of action is discussed.—Authors' English Summary

Depasquale, G. The Malta experience; Isoprodian-rifampicin combination treatment for leprosy. Lepr. Rev. **57** Suppl. 3 (1986) 29–37.

Since 1972, a total of 247 patients have been treated with a rifampin-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.—Author's Abstract

Dhir, R., Guha, P. K. and Singh, G. Short term chemotherapy of paucibacillary leprosy. Indian J. Lepr. **58** (1986) 549–554.

Twenty freshly diagnosed, previously untreated, adult male patients with paucibacillary leprosy were treated with multidrug therapy (MDT) according to WHO regimen (1982). The patients remained in the hospital throughout the course of the study. At the end of 6 months' treatment, they were evaluated clinicobacteriologically and histologically for evidence of disease activity. Active disease was present in 55% of the patients at the end of 6 months' treatment. Dapsone (DDS) had to be discontinued in one patient who developed hepatitis.—Authors' Abstract

Dietrich, M. and Wabitsch, R. Comparison of DDS with two combined chemotherapy regimens for multibacillary leprosy. Results after 3 years of treatment. A prospective randomized multicentre study. Lepr. Rev. **57** Suppl. 3 (1986) 60–62.

Confirmed cases of lepromatous or borderline lepromatous patients were randomized to receive one of the three following drug regimens: a) dapsone (DDS) 100 mg/day, b) DDS 100 mg/day + rifampin (RMP) 600 mg/day, c) RMP 600 mg/day + Isoprodian, 2 tablets/day. A complete physical checkup, 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.

Three hundred two patients were randomized in five different centers: 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 showed 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 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, BUN, and hematology serial examinations. Since 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 criterion for final judgment.—Authors' Abstract

Gelber, R. H. The use of rodent models in assessing antimicrobial activity against *Mycobacterium leprae.* Lepr. Rev. **57** Suppl. 3 (1986) 137–148.

The ability of antimicrobial agents to prevent multiplication of *Mycobacterium 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 foot pad infection, initially with the highest concentration of drug tolerated, orally if possible. Unfortunately, this techinque 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.—Author's Abstract

Greenwood, B. M., Greenwood, A. M., Bradley, A. K., Shenton, F. C., Smith, A. W., Snow, R. W., Williams, K., Eggelte, T. A., Huikeshoven, H. and de Wit, M. ELISA tests for dapsone and pyrimethamine and their application in a malaria chemoprophylaxis programme. Bull. WHO **64** (1986) 909–916.

Enzyme-linked immunosorbent assays (ELISAs) are described for determining levels of dapsone and pyrimethamine in

urine. Both assays have a sensitivity of about 20 μg/liter and are reproducible, but each produces some false-positives. The problem of false-positive reactions was partially obviated by requiring positive results in both assays. In a pilot study involving 50 children aged 3 months to 4 years who were given a single dose of Maloprim (pyrimethamine + dapsone), 75% were positive for dapsone 7 days after administration of the drug, while 25% were still positive 15 days after its administration. The corresponding proportions for pyrimethamine were 73% and 30%, respectively. Comparison of the results obtained in a larger chemoprophylaxis trial with those from the pilot study indicated that the assays described could be used to investigate whether antimalarials had been taken.—Authors' Abstract

Grosset, J. Recent developments in the field of multidrug therapy and future research in chemotherapy of leprosy. Lepr. Rev. **57** Suppl. 3 (1986) 223–234.

The discovery of rifampin 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 1970–1980 toward a multidrug therapy of leprosy as was the case in the therapy of tuberculosis since the early fifties.

Besides stopping the transmission of the bacilli in the community, chemotherapy for leprosy as well as for tuberculosis has two objectives: to prevent the selection of drug-resistant mutants and to kill the drug-sensitive organisms. To reach the first objective, a combination of drugs active against *Mycobacterium 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 priorities of the research in the 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 programs. Meanwhile, it is safe to follow the WHO recommendations, that is, to treat multibacillary patients for a minimum of 2 years or at best until the negativation of BI.—Author's Abstract

Iserson, K. V. Methemoglobinemia from dapsone therapy for a suspected brown spider bite. J. Emerg. Med. **4** (1985) 285–288.

Recent reports have suggested the use of dapsone for brown spider bites. This drug, for many years restricted to use in cases of leprosy and rare dermatologic disorders, has significant side effects that must be recognized. A case of methemoglobinemia is reported in one such patient treated with dapsone. The difficulty of making the diagnosis of brown spider bite is discussed.—Author's Abstract

Jenner, P. J. and Smith, S. E. Plasma levels of ethionamide and prothionamide in a volunteer following intravenous and oral dosages. Lepr. Rev. **58** (1987) 31–37.

Available evidence which may aid a decision concerning which of the thioamides, ethionamide or prothionamide, should be

recommended for use in the treatment of lepromatous leprosy is inconclusive. The drugs possess similar antimycobacterial activities, but earlier work has suggested that after oral dosage ethionamide may give rise to higher blood levels than prothionamide. We report on investigations designed to examine whether this finding is as a result of different systemic availabilities, by comparing blood levels following intravenous and oral administrations. We conclude that the drugs' pharmacokinetics are very similar, each having high bioavailabilities, and that other factors such as cost may be more important determinants as to which thioamide should be used.—Authors' Summary

Jopling, W. H. A report on two follow-up investigations of the Malta-Project. Lepr. Rev. **57** Suppl. 3 (1986) 47–52.

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.—Author's Abstract

Kalthoff, P. G. The use of MDT in the three western regions of Nepal. Lepr. Rev. **57** Suppl. 3 (1986) 106–114.

It has been proved that, even under very difficult field conditions as in Nepal, multidrug therapy (MDT) can be introduced in the field if there are detailed instructions available for the paramedical worker, 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 afterward 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 program in the future.—Author's Abstract

Kar, H. K., Bhatia, V. N., Vinod Kumar, C. H. D., Sirumban, P. and Roy, R. G. Evaluation of levamisole, an immunopotentiator, in the treatment of lepromatous leprosy. Indian J. Lepr. **58** (1986) 592–600.

Twenty subpolar lepromatous leprosy patients under multidrug therapy for a period of 1 to 3 years, who continued to be bacteriologically positive (BI 2 to 1 in Dharmendra's grade) were taken in the study. Ten cases (trial group) were given levamisole as an immuno-stimulator for 1 year along with chemotherapy. Another 10 cases (control group) continued to take chemotherapy alone. There was no conversion of Mitsuda reactivity in any of the cases from both groups. There was no improvement of leukocyte migration inhibition in either group. In both trial and control groups, statistically significant clinical and bacteriological improvements and an increase of E-rosette counts are found at the end of 1 year. However, only the bacteriological improvement in the trial showed statistical significance when the improvements were compared with those of the control group. No adverse effect due to levamisole therapy was encountered.—Authors' Abstract

Kartikeyan, S. and Bhalerao, V. R. Study of compliance of the patients in leprosy control programme in an urban slum. J. Postgrad. Med. **32** (1986) 127–130.

This study, conducted on defaulters among registered leprosy patients at Malavani, a slum area located in the western suburbs of Greater Bombay, showed that 46.94% of the 784 registered leprosy patients had defaulted in spite of routine defaulter action. This trend was among males, those diagnosed during mass surveys, and those in the 15–44 year age group. The reasons for dropout were mainly unsuitable clinic timings and a lack of knowledge on the part of the patient about the need for

continued and regular treatment. Social stigma was cited as a reason for default by only 7.39% of the interviewed patients.—Authors' Summary

Langhorne, P., Duffus, P., Berkeley, J. S. and Jesudasan, K. Factors influencing clinic attendance during the multidrug therapy of leprosy. Lepr. Rev. **57** (1986) 17–30.

Factors influencing clinic attendance during multidrug therapy (MDT) of leprosy were studied in a population of paucibacillary patients at Schieffelin Leprosy Research and Training Centre (SLR&TC) Karigiri in southern India. Information was gathered from patient records (293 patients) and by questionnaires (143 patients). Patients were grouped according to their long-term clinic attendance record. Factors associated with poor clinic attendance were detection by survey, poor attendance during dapsone monotherapy and longer periods of treatment with dapsone monotherapy prior to MDT, and absence from first or second clinics after registration for MDT. Factors associated with good clinic attendance were presence of deformity and voluntary presentation. Factors unrelated to clinic attendance were age, sex, clinic size, site or number of skin lesions and type of paucibacillary leprosy. The commonest reasons given for clinic absences were work and family commitments. Various schemes for predicting poor clinic attendance behavior were devised.—Authors' Summary

Leiker, D. L. On the epidemiology of leprosy in Malta. Lepr. Rev. **57** Suppl. 3 (1986) 38–41.

In 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 surburban 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 rifampin, and dapsone, 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.—Author's Abstract

Mahadevan, P. R., Jagannathan, R., Bhagaria, A., Vejare, S. and Agarwal, S. Host-pathogen interaction—new *in vitro* drug test systems against *Mycobacterium leprae*; possibilities and limitations. Lepr. Rev. **57** Suppl. 3 (1986) 182–200.

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 until recently is using the growth potential of this bacterium in the mouse foot pad—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 the presence or absence of drugs which would indicate inactivation or confirmed viability of *M. lepare,* 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 a 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 sulfone and

rifampin has been demonstrated, and loss of viability of *M. leprae* in such experiments was 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 a few other derivatives from the laboratories of Dr. J. K. Seydel.

Two of the test systems were subjective, since they involved use of a microscope by the investigator and counting. But both of these have now been confirmed by a more quantitative method. Fc receptor assay is demonstrated using I^{125}-labeled 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 1×10^4 in the mouse foot pad; b) as patients improve on drug therapy, viability goes down. Thus to monitor viability one has to use a higher number of bacilli and this may lead to ambiguous data.—Authors' Abstract

Mehta, J., Gandhi, I. S., Sane, S. B. and Wamburkar, M. N. Effect of clofazimine and dapsone on rifampicin (Lositril) pharmacokinetics in multibacillary and paucibacillary leprosy cases. Lepr. Rev. **57** Suppl. 3 (1986) 67–76.

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

The effect of dapsone and clofazimine when given separately and in combination was studied on rifampin pharmacokinetics in each group of 6 patients. Within-group comparison revealed that clofazimine reduced rifampin absorption significantly ($p < 0.01$) and prolonged the time to reach the peak serum concentration ($p < 0.01$). Since MCR and Ke 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 Cmax, although t 0.5 was increased significantly ($p < 0.02$) in RC group.

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

Of the three groups, except RC group, both RDC and RD groups were homogeneous. Ka, avd, Cmax and Auc/t 0.5 ratio of RC group were significantly different from those in RD group. While Ka and avd were significantly less ($p < 0.05$ and $p < 0.001$, respectively) and Cmax and Auc/t 0.5 ratio were significantly more ($p < 0.01$) in RC group. Since clofazimine reduced rifampin absorption, the difference in Ka and tp became more significant in the post-regimen phase ($p < 0.01$).—Authors' Abstract

Mester de Parajd, L. and Mester de Parajd, M. [Treatment of leprosy with human metabolites.] Acta Leprol. **4** (1986) 363–372. (in French)

We are interested for other human metabolites than deoxyfructo-serotonin (DFS), showing antileprosy activity. This is the case of deoxyfructo-5-hydroxytryptophane and of some liposoluble derivatives of DFS. The time of resorption and penetration into *Mycobacterium leprae* infected tissues is very different for these metabolites. For this reason the simultaneous application of these compounds may represent some advantage in the treatment of the multibacillary form of leprosy. The use of DFS together with the antileprosy diet "NAL" have the supplementary advantage to stabilize the DFS level in the serum during the treatment.—Authors' English Summary

Millan, J., Roux, G., Loko, S., Naudin, J. C., Boucher, P., Bodian, M., Camara, M., Moreira-Diop, T. and Grosset, J. [Multidrug therapy trial for leprosy in Senegal:

first results about hepatic tolerance of multibacillary patients—therapeutic proposals.] Acta Leprol. **4** (1986) 427–444. (in French)

The authors have studied tolerance of multibacillary patients in 4 multidrug therapy (MDT) regimens. These 4 regimens consist of: 1) One supervised part in which rifampin-ethionamide (RMP-ETH) combination is once-monthly; furthermore, in 2 of these regimens is included one "starter phase" with daily doses of that combination for 2 months. 2) One self-administered part during which clofazimine (CLO) is associated either with dapsone (DDS) for new cases or with ETH for relapses.

Out of 310 multibacillary patients, 7 cases of hepatitis occurred with or without icterus, but no death due to the treatment. Interruptions of MDT have been temporary and have been observed in 0.9% to 5.6% of the patients according to the therapeutic regimen. The SGOT was abnormally high in 16.3% of the patients before treatment. These pre-existing liver damages do not favor the appearance of intolerance disorders. During MDT, abnormal increases in SGOT are observed in 27% of the patients, but there is no exact correlation between the absorbed doses of ETH and the frequency of SGOT increases.

The clinical or biological evidence of liver damage occurs rather early (1st, 2nd month) in regimens with "starter phase," and later (4th–8th month) in those without "starter phase." But introduction of "starter phase" does not increase the global frequency of such intolerance accidents. ETH combined with RMP must be used under steady clinical and biological supervision.

Recalling the results of a previous survey, the authors consider that a long duration of MDT is not necessary. For the multibacillary leprosy treatment, they propose a diphasic regimen, more easily applicable in the field than the WHO protocols. In this diphasic regimen, the only part which must be supervised is the initial "starter phase" of 2 months. It consists of daily administration of 3 antibacillary drugs among which are RMP and ETH. The second phase is a relay treatment using 2 drugs, CLO combined with DDS or ETH, self-administered until smear negativity.—Authors' English Summary

Modderman, E. S. M. [Intramuscular administration of dapsone in leprosy: a new approach.] Pharma. Weekbl. [Sci.] **119** (1984) 564–572. (in Dutch)

Patient noncompliance and resistance are serious problems in the treatment of leprosy with dapsone tablets. The object of the dapsone project at the Department of Biopharmaceutics of the University of Amsterdam is the development of a long-acting injection of dapsone which can be administered intramuscularly, once a month. Such a preparation would fit in the new WHO programs for monthly supervised administration of antileprosy drugs.

Possibilities for the formulation of such an injection were investigated. In trials with healthy volunteers and with leprosy patients, intragluteal injections of aqueous dapsone suspensions were administered. It was concluded that these preparations are suitable for monthly injection in women; whereas the same preparation gives a less-acceptable sustained release in men. Explanations and solutions for this phenomenon are discussed.—Author's English Abstract

Onsun, N., Saylan, T. and Pattyn, S. R. Combined chemotherapy of multibacillary leprosy of 6 months' duration. Lepr. Rev. **57** Suppl. 3 (1986) 124–126.

A treatment regimen of 6 months' duration and composed of 2 weeks daily rifampin (RMP) 600 mg, prothionamide (PRO) 500 mg, and dapsone (DDS) or clofazimine (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 multibacillary 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.—Authors' Abstract

Parikh, D. A., Ganapati, R. and Revankar, C. R. Thalidomide in leprosy—study of 94 cases. Indian J. Lepr. **58** (1986) 560–566.

Thalidomide has a beneficial effect on type 2 lepra reaction, especially chronic and recurrent reaction. It helps to minimize steroid dependency. Thalidomide was given to 94 cases of type 2 lepra reaction who had not responded to steroids or had repeated reactions. This clinical data was analyzed regarding clinical improvement, relapse of reaction, side effects of the drug, etc. The analysis showed that all the patients improved remarkably, and steroids could be withdrawn. If the competence of staff using this drug is enhanced, morbidity due to leprosy can considerably be reduced.—Authors' Abstract

Pattyn, S. R. Activity of ofloxacin and pefloxacin against *Mycobacterium leprae* in mice. (Letter) Antimicrob. Agents Chemother. **31** (1987) 671–672.

The anti-*Mycobacterium leprae* activity of the drugs was determined by the proportional bactericidal test. Ofloxacin was bactericidal against *M. leprae* when administered five times a week at dosages of 150, 100, and even 50 mg/kg (body weight). Pefloxacin showed no activity when administered at 100 mg/kg 5 days a week or at 150 mg/kg at lower frequencies. However, the drug showed activity at 300 mg/kg three times a week. The activity vanished when frequency of administration was more spaced. On the basis of these results, studies with ofloxacin should be undertaken in humans to determine its usefulness in the treatment of human leprosy.—(*From* the Letter)

Pattyn, S. R. Efficacy of different regimens in multibacillary leprosy. Lepr. Rev. **57** Suppl. 3 (1986) 264–271.

As we pointed out in the past the outlook of leprosy treatment was revolutionized by the discovery of the bactericidal activity of rifampin (RMP) in 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.—Author's Abstract

Pattyn, S. R., Husser, J. A., Janssens, L., Grillone, S. and Bourland, J. [Inadequate treatment in multibacillary leprosy and incubation times for relapses.] Acta Leprol. **4** (1986) 495–499. (in French)

Among a population of over 500 paucibacillary (PB) patients treated with different regimens, 6 multibacillary (MB) relapses were detected; 5 in patients erroneously classified as PB but in reality MB with a low bacterial load, 1 patient was PB at the start. Treatment regimens had been: 10 weekly doses of rifampin (RMP) either 600 mg (1 case) or 900 mg (1 case), 2 successive doses of RMP 1500 mg (1 case), a single dose of RMP 40 mg/kg (3 cases). Four MB patients with proven dapsone resistance relapsed after a single dose of RMP either 20 mg/kg (1 case) or 40 mg (3 cases). The two strains isolated were RMP sensitive. Seven of the 10 relapses appeared within 24 months after start of treatment.—Authors' English Summary

Pattyn, S. R., Husser, J. A., Janssens, L. and Nollet, E. [Histologic evolution of paucibacillary leprosy during treatment with various therapeutic regimens.] Acta Leprol. **4** (1986) 501–504. (in French)

Histopathological examination of skin biopsies from paucibacillary patients during the first 12–18 months of treatment did not reveal any significant difference in the time necessary for disappearance of the lesions. The regimens studied were: Dapsone (DDS) 100 mg 7/7, rifampin (RMP) 600 mg 1/30 6×, RMP 600 mg 6/6 6×, RMP 900 mg 1/7 8× and 12×, RMP 1500 mg 1× and 1 year of DDS, RMP 4 mg/kg 1×.—Authors' English Summary

Prabhakaran, K., Harris, E. B., Sanchez, R. M. and Hastings, R. C. β-Lactamase synthesis in *Mycobacterium leprae*. Microbios **49** (1987) 183–188.

β-Lactam antibiotics are not active against *Mycobacterium leprae*. The enzyme β-lactamase mediates the most common form of bacterial resistance to penicillins and cephalosporins. Cell-free extracts of purified suspensions of *M. leprae* were examined for β-lactamase. The bacteria were prepared from the tissues of experimentally infected nine-banded armadillos. Most of the suspensions were inactive. However, the bacteria obtained from the tissues of armadillos treated with penicillin G benzathine (bicillin) 6 months or more prior to sacrifice had β-lactamase. If the organisms had been exposed to the antibiotic only for a few days, they were negative. Attempts to induce β-lactamase in the bacteria *in vitro* did not succeed. Interestingly *M. leprae* separated from untreated armadillos, infected with the bacilli derived from treated animals, contained the enzyme activity. Apparently, the *M. leprae* genome contains the operon for β-lactamase, and once it is stimulated to express the enzyme, it continues to do so even after the inducer is withdrawn.—Authors' Abstract

Ravettini, B. and Achenbach, R. [Combined and supervised treatment of leprosy—two years of follow-up.] Rev. Argent. Dermatol. **68** (1987) 39–47. (in Spanish)

The WHO short-term multiple drug regime was applied over the minimum period to 46 multibacillary and 28 paucibacillary patients: 58.6% multibacillary continued treatment, 10.9% abandoned it, and 17.4% suspended it mostly due to digestive intolerance. Clinical improvement of the lesions was observed. Reactions were seen during the first year, with a sharp decrease during the second year. Bacteriological values decreased without becoming negative. The period of time required to reach negative values in nasal mucus was longer than for other treatments; 85.7% paucibacillary patients were released; one relapsed after 14 months. Early improvements were observed, tolerance was good and no exacerbations were seen. Response in the BT group was somewhat slow. One patient from each group showed side effects in the form of toxic hepatitis forcing an interruption of treatment.—Authors' English Summary

Revankar, C. R., Mahadevan, P. R. and Ganapati, R. A comparative study of the efficacy of WHO and IAL multidrug therapy regimens for leprosy—an *in-vivo* and *in-vitro* study. Indian J. Lepr. **58** (1986) 543–548.

In the absence of definite evidence on the utility of intensive therapy with rifampin in multibacillary leprosy cases, a laboratory-based investigation was undertaken basically to compare the efficacy of WHO and IAL regimens. In each group four untreated BL-LL patients were included and their skin biopsies were subjected for viability test in both *in vitro* and *in vivo* systems. A consistent fall in BI with good clinical improvement was observed in both the groups. However, good viability was maintained until about the third pulse dose in WHO group; whereas with the IAL group a rapid fall in viability was observed after the intensive phase. Viable bacilli were seen even after 12, 15, 18 and 24 doses in both groups. These findings question the need for the additional 21 doses of rifampin in the IAL schedule. However, such studies are to be repeated on larger samples.—Authors' Abstract

Schaper, K.-J., Seydel, J. K., Rosenfeld, M. and Kazda, J. Development of inhibitors

of mycobacterial ribonucleotide reductase. Lepr. Rev. **57** Suppl. 3 (1986) 254–264.

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 *Mycobacterium 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.—Authors' Abstract.

Seydel, J. K., Rosenfeld, M., Sathish, M., Wiese, M., Schaper, K.-J., Hachtel, G., Haller, R., Kansy, M. and Dhople, A. M. Strategies in the development of new drugs and drug combinations against leprosy, demonstrated on the example of folate and gyrase inhibitors. Lepr. Rev. **57** Suppl. 3 (1986) 235–353.

The lack of an *in vitro* test system for *Mycobacterium leprae* 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.

The 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 humans.

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.—Authors' Abstract

Subcommittee on Clinical Trials of the Chemotherapy of Leprosy (THELEP) Scientific Working Group of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. Characteristics of patients in the THELEP trials of chemotherapy of leprosy at Bamako and Chingleput. Lepr. Rev. **58** (1987) 7–16.

The characteristics evident before beginning treatment of 215 lepromatous patients admitted to the THELEP clinical trials of combined chemotherapy at Bamako and Chingleput, including age, sex, BI, LIB, $\log_{10}$ of the number of acid-fast bacilli per gram tissue, clinical classification, and histopathological classification, have, in general, been found to be uniformly distributed between treatment centers and among the regimens within each center. Thus, there appears little likelihood that the results of treatment by the trial regimens will have been influenced by any of these characteristics. Except for the clinical and histopathological classifications, which did not agree more frequently than predicted by chance, the expected interrelationships among these characteristics were demonstrated.—Authors' Summary

Waters, M. F. R., Ridley, D. S. and Ridley, M. J. Clinical problems in the initiation and assessment of multidrug therapy. Lepr. Rev. **57** Suppl. 3 (1986) 92–100.

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, since 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.—Authors' Abstract

Wheeler, P. R. Metabolism in *Mycobacterium leprae*: possible targets for drug action. Lepr. Rev. **57** Suppl. 3 (1986) 171–181.

Metabolic activities in *Mycobacterium 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 antileprosy 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 among living organisms, and there often exist "alternative pathways." Thus energy metabolism may not be amenable to inhibition by antileprosy agents although two activites 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 coordinated 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 among the mycobacteria to *M. leprae.* The biological significance of DOPA oxidation is not known, and it may be that in attempting to design an 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,* rifampin 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.—Author's Abstract

Clinical Sciences

Ashamalla, L. Immunologic aspects of leprosy. Int. J. Dermatol. **25** (1986) 452–455.

Twenty-seven cases of leprosy from Egypt were examined. Monocytes were found to be high in peripheral blood in lepromatous cases. The percentage of small lymphocytes in blood was increased in tuberculoid patients. The immunoglobulins were higher in all types of leprosy patients than in normal persons.—Author's Abstract

Bobhate, S. K., Kedar, G. P., Kher, A. V. and Grover, S. Metastatis of malignant plantar ulcer in lymph nodes in femoral triangle—a case report. Indian J. Lepr. **58** (1986) 630–631.

A case report of secondary metastasis of squamous cell carcinoma of plantar ulcer in lymph nodes in femoral triangle in a leprosy patient is presented.—Authors' Abstract

Guha, P. K., Pandey, S. S., Singh, G. and Kaur, P. Family studies of leprosy cases. Indian Med. Gaz. **119** (1985) 148–149.

First degree relatives among the intrafamilial contacts of 400 leprosy patients were examined to detect any evidence of the disease in them. Type of leprosy detected in contacts in relation to that in the index cases has been analyzed in order to evaluate the role of genetic factors in determining the type of leprosy one suffers from. Observations in this study, however, do not tend to indicate the existence of a genetic diathesia.—Authors' Summary

Gupta, C. M., Tutakne, M. A. and Bhate, R. D. A study of palmar ridge malformation in leprosy. Indian J. Lepr. **58** (1986) 584–591.

Palmar ridge malformation of 150 male leprosy patients (50 multibacillary and 100 paucibacillary) were compared with matched controls. A significantly high incidence of ridge malformation was found on the palms of multibacillary leprosy patients. The acquired ridge atrophy was found in 32% multibacillary leprosy, 4.5% paucibacillary leprosy, and 0% controls. The congenital ridge dissociation was found in 46% multibacillary leprosy, 20% paucibacillary leprosy, and 22% controls. The difference is statistically significant.—Authors' Abstract

Jagirdar, P. C. The usefulness of acupuncture in leprosy. Indian J. Lepr. **58** (1986) 618–622.

The damage to motor nerves in leprosy causes imbalance at various joints, and these postural alterations result in various deformities. Active exercises which can prevent disuse atrophy of muscles are not possible when the muscles are completely paralyzed. Needleless electroacupuncture produces electric impulses similar to nerve impulses. Electroacupuncture done at the correct acupuncture points can give active exercises to the paralyzed muscles and thus prevent disuse atrophy of the paralyzed muscles. Electroacupuncture can serve as the most effective physical therapy to prevent and treat early deformities such as claw hand, foot drop, trophic ulcer, etc. Acupuncture can give relief from the neuritic pain in leprosy.—Authors' Abstract

Jain, A. P., Gupta, O. P., Jajoo, U. N. and Kumar, J. Study of autoantibodies in lepromatous leprosy in rural central India. Indian Med. Gaz. **119** (1985) 236–237.

Autoantibodies, i.e., rheumatoid factor in 40% (20/50), antinuclear factor in 10% (5/50), antithyroid (microsomal and mitochondrial) in 8% (4/50), and antisperm antibodies in 18% (9/50) were present in 30 LL and 20 ENL patients. None of these had any clinical expression, i.e., arthritis, SLE, thyroid, sterility and gynecomastia, respectively. Variation in clinical picture had no effect on these autoantibodies.—Authors' Summary

Miller, R. A., Wener, M. H., Harnisch, J. P. and Gilliland, B. C. The limited spectrum of antinuclear antibodies in leprosy. J. Rheumatol. **14** (1987) 108–110.

Sera from 46 consecutive patients with leprosy were collected and tested against an extensive panel of defined nuclear antigens. Antinuclear antibodies (ANA) were present in 16% of patients, but the titer was uniformly low and there was no consistent fluorescence pattern. None of the ANA positive sera contained antibodies which reacted with native DNA, or which were directed against histones, centromeres, SSB, Sm, or ribonucleoprotein. These more specific autoantibody assays thus retained their clinical utility in the differential diagnosis of rheumatologic complaints in patients with leprosy.—Authors' Abstract

Naafs, B., Lyons, N. F., Matemera, B. O. and Madombi, L. The "Ellis" and "Ryrie" tests. Lepr. Rev. **58** (1987) 53–60.

Two simple non-time-consuming tests for the detection of reaction and guidance of reactional therapy in leprosy patients are described and analyzed. The tests showed to be useful both in the hospital and in the field.—Authors' Summary

Nigam, P., Pant, K. C., Kapoor, K. K., Kumar, A., Saxena, S. P., Sharma, S. P., Mukhija, R. D., Gupta, A. K. and Dubey, A. L. Histo-functional status of kidney in leprosy. Indian J. Lepr. **58** (1986) 567–575.

A study of 64 cases of leprosy (44 lepromatous and 20 nonlepromatous) revealed that the duration of the disease has a significant relationship with renal involvement ($\chi^2 = 16.9$, $p < 0.001$). Proteineuria, microscopic hematuria, granular and hyaline casts are mainly seen in lepromatous cases, especially with lepra reaction (100%), while few of the nonlepromatous (2%) cases may show these abnormalities. Impaired renal functions are mostly observed in lepromatous leprosy (62.9%), especially those with lepra reaction (100%), while 2% of nonlepromatous cases have these impaired renal functions. Histopathological studies revealed nonspecific changes in 44.4% of the cases and those of chronic pyelonephritis in 15.5% of the cases. Renal amyloidosis is a less-common occurrence (4.4%). The specific lesion, that is "leproma kidney," is rare and was seen in one patient only. Acid-fast bacilli could not be seen in any of the kidney tissue. It is, therefore, concluded that the renal involvement in the form of inflammatory lesions and nonspecific changes in the glomeruli and tubules are very common in lepromatous leprosy, especially during the reactive phase.—Authors' Abstract

Parikh, A. A. and Shah, B. H. Tetanus in a case of lepromatous leprosy. Indian J. Lepr. **58** (1986) 628–629.

A patient of lepromatous leprosy had neuropathic plantar ulcer of 6 months' duration. He developed "tetanus." We are reporting this case with a review of literature, as there is a dearth of published literature on this association of leprosy and tetanus.—Authors' Abstract

Ramachandran, A. and Seshadri, P. S. Multiple relapses in borderline leprosy—a case report. Indian J. Lepr. **58** (1986) 623–625.

A case of borderline lepromatous leprosy, with a history of 5 years' duration of disease, was first seen in 1971 and treated with graded doses of dapsone in the outpatient clinic of the Institute. He became inactive and bacteriologically negative in 3 years. While continuing on dapsone therapy, he relapsed into active dapsone-resistant leprosy 3½ years later. He was admitted to the hospital and given rifampin 600 mg daily for 15 days along with dapsone 100 mg daily. He again became inactive and bacteriologically negative within 3 years. Three years later under regular dapsone therapy he relapsed again for the second time into active BT leprosy, but remained bacteriologically negative. He was given three drug regimen subsequently and became clinically inactive within 15 months.—Authors' Abstract

Sarate, G. S., Kulkarni, S. S., Pathak, R. G. and Joshi, A. V. Anaesthetic complications in a leprosy patient. Indian Med. Gaz. **119** (1985) 275–277.

Involvement of the autonomic nervous system is known to occur in leprosy. This can result in an unstable cardiovascular system. A case of lepromatous leprosy is reported here who went into cardiac arrest

and developed pulmonary edema resistant to treatment after induction of anesthesia. The possibilities are discussed on the basis of involvement of autonomic nervous system and also pathophysiological changes in the blood vessels that are reported to occur in this disease.

The lethal potential of such involvement imposes yet another burden of alertness on the clinician when he prescribes respiratory and cardiovascular depressants or administers anesthesia to a leprosy patient.—Authors' Summary

Sheriff, D. S. Semen analyses in Hansen's disease. Trans. R. Soc. Trop. Med. Hyg. **81** (1987) 113–114.

Disturbances in semen quality can be induced by exogenous factors including trivial illnesses like throat infection and viral infection with fever. Hansen's disease presents with problems such as long-term dependence on drugs, hyperthermia, hemolysis and anxiety due to social stigma. In the present study, the semen quality of patients with tuberculoid-type leprosy was studied during and after withdrawal of therapy. Patients generally had low sperm counts and spermatozoal motility was low with a greater number of abnormal forms compared with control subjects. Patients did not show a circannual variation in their sperm count as was observed in controls. The patients were oligozoospermic during their first year of medication, showing improvement in subsequent years.—Author's Abstract

Singh, K. An unusual bullous reaction in borderline leprosy. Lepr. Rev. **58** (1987) 61–67.

A 45-year-old male patient suffering from borderline lepromatous leprosy with reaction developed round or irregular, well-defined, large, tense bullae on existing leprosy lesions. There was deposition of IgG, IgM, IgA and fibrin along the basement membrane. It was not a bullous drug eruption due to either rifampin, dapsone or clofazimine, but a component of leprosy reaction. Difficulties in classifying as either type 1 or type 2 reaction are discussed.—Author's Summary

Uplekar, M. W. and Antia, N. H. Clinical and histopathological observations on pure neuritic leprosy. Indian J. Lepr. **58** (1986) 513–521.

Pure neuritic leprosy is a well-accepted clinical entity. In the absence of skin lesions there is a greater possibility of missing the diagnosis of leprosy due particularly to a wide variety of pure neural manifestations that may mimic other peripheral neuropathies. Histopathological studies of pure neuritic leprosy have received less attention for ethical reasons and limitations of surgery. Classification of pure neuritic leprosy poses problems since the histological spectrum (e.g., Ridley-Jopling scale) is based chiefly on the skin picture. Review of the past literature shows conflicting reports about comparability of the skin and nerve pictures. This paper presents clinical and histopathological observations on 12 patients with pure neuritic leprosy, the interesting observations being that all the patients showed lepromin positivity and a narrower histological spectrum, ranging from TT to BB only.—Authors' Abstract

Valdés-Portela, A. [Detection of immune complexes in lepromatous leprosy. I. Appraisal of precipitation technique with polyethylene glycol.] Rev. Cubana Med. Trop. **37** (1985) 295–299. (in Spanish)

The precipitation technique with polyethylene glycol for measuring circulating immune complexes in patients suffering from lepromatous leprosy is appraised. A highly significant difference between patients and controls is observed. Results obtained suggest that this technique is a simple and useful one to determine circulating immune complexes in leprosy patients.—Author's English Summary

Wallach, D., Bussel, A., Koch, P., Pennec, J. and Cottenot, F. Plasma exchange in severe erythema nodosum leprosum. Int. J. Artif. Organs **9** (1986) 183–188.

Four patients with severe erythema nodosum leprosum (ENL) were treated by plasma exchange and/or fresh frozen plasma infusions after failure of classical therapy. After the procedures, the patients im-

proved rapidly; with a follow-up between 4 and 7 years after the last plasma exchange, no clinical relapse was noted. The replacement fluids were variable; the most beneficial procedure seemed to be plasma exchange replaced with fresh frozen plasma. Elimination of circulating immune complexes or replacement of a lacking plasma factor are possible mechanisms of action. Plasma exchange may also work like a regulator of immune mechanisms, since it has been shown that there is a depression of suppressor cells in ENL.—Authors' Abstract

Wariyar, B. and Adams, L. M. Hansen's disease (leprosy) revisited. Nebr. Med. J. **71** (1986) 357–358.

This report is presented for its medical interest and curiosity in this part of the world. Since many of us now do see South East Asian patients in our practice, it is prudent that we keep leprosy at the back of our minds when they present with varying peripheral neurodermatologic manifestations. Following confirmation of the diagnosis, the family and close contacts should be screened.—Authors' Conclusion

Immuno-Pathology

Anonymous. Delayed-type hypersensitivity in human volunteers immunized with a candidate leprosy vaccine. Bull. Pan Am. Health Organ. **20** (1986) 88–91.

The present study suggests that strong DTH reactivity can be induced by *Mycobacterium leprae* in man with doses that do not produce unacceptable side effects. The next step is to carry out similar studies in leprosy-endemic areas. Such studies would record the delayed-type hypersensitivity (DTH) reactions immediately after vaccination as well as the duration of the sensitization afforded by the vaccine. These studies should also compare the efficacy of *M. leprae* alone versus the efficacy of a combined *M. leprae* + BCG vaccine, since Convit, *et al.* have found that the combined vaccine is able to restore DTH in unresponsive, indeterminate, and lepromatous patients. This suggests that leprosy could be one of the few infectious diseases for which a vaccine might be both prophylactic and immunotherapeutic.—(*From* the Conclusion)

Bottasso, O. A., Hinrichsen, L., Morini, J. C. and Rabasa, S. L. [Genetic component of the immune response to *Mycobacterium leprae* in healthy individuals.] Medicina (B. Aires) **46** (1986) 713–718. (in Spanish)

The cutaneous challenge with heat-killed *Mycobacterium leprae* in persons without leprous infection produces in most cases after 21–28 days, a late nodular reaction called the Mitsuda reaction (MR). This represents the host's ability to elicit an immune response to an immunizing dose of *M. leprae.* Because MR shows a familial association, the genetic component of this character was investigated.

A total of 116 adults, males and females, all of them vaccinated with BCG in their childhood and without previous challenges with *M. leprae,* were studied. The average age was 19 ± 11 years ($\bar{x}$ ± S.D.). The sample was distributed in 40 sibships with 2 to 7 members per group. MR was carried out by injecting 0.1 ml of 4×10^7 *M. leprae*/ml, and assessed 21 days after challenge. It was expressed as the mean of the major diameter plus the minor diameter. No correlation between members' ages and MR size was found. Histologically, a typical granulomatous reaction was observed.

As a qualitative measure, MR can be negative or positive, so heritability (h^2) of the reaction as a threshold character was determined, according to the incidence of nonreactive individuals in general populations (13/116, $q_g = 0.112$) and the incidence in the relatives of a propositi (6/17, $q_r = 0.353$); h^2 was calculated by two methods, Falconer 0.98 ± 0.3 and Edwards 1.19 ± 0.3. As a

quantitative measure, only positive responses could be studied because the negative ones were outside the normal distribution. Consequently, 88 persons with positive MR from 32 sibships were analyzed. In order to obtain a homogeneous variation, the MR quantitative estimations were transformed in $\log_{10}$. The intraclass correlation coefficient obtained was 0.47, expressing that in each sibship around 50% of genetic identity for MR exists. Otherwise, h^2 was also estimated by the regression of sibs on parents. In 25 of 32 sibships, MR was also studied in both parents, and 19 families were selected in which both parents gave positive responses. The results obtained show that an important additive variance may be working ($b = 0.35 \pm 0.13$; $p < 0.02$). Although MR presents a high h^2 as a threshold character, it must be stressed that estimation methods are based on the influence of additive effects of several loci in absence of dominance; therefore, if the variance component due to dominance deviation from additivity is not negligible, this estimate is somewhat overestimated.

We can conclude that in the heritability of MR, two different genetic components are present. As a threshold character, this high h^2 indicates that this reaction could be controlled and/or regulated by few genes with high penetrance. In the case of a positive reaction, these genes could turn on a more polygenic additive system, and together with additional environmental influences, could justify the variation in the response.—Authors' English Summary

Buchanan, T. M., Nomaguchi, H., Anderson, D. C., Young, R. A., Gillis, T. P., Britton, W. J., Ivanyi, J., Kolk, A. H. J., Closs, O., Bloom, B. R. and Mehra, V. Characterization of antibody-reactive epitopes on the 65-kilodalton protein of *Mycobacterium leprae*. Infect. Immun. **55** (1987) 1000–1003.

Twenty-three monoclonal antibodies (MAbs) prepared in 7 different laboratories were studied, all of which recognized the 65-kilodalton (kD) protein of *Mycobacterium leprae* as determined by Western blotting or gel immunoradioassay or both. Fourteen of the MAbs recognized different epitopes, as evaluated by cross-competition studies using radiolabeled MAb and unlabeled inhibitors; the species specificity of these epitopes was defined by nitrocellulose dot blot immunoassays with bacterial sonic extract antigen preparations from 23 species of mycobacteria. Each of the 14 distinct MAbs recognized a 65-kD protein produced by a lysogenized *Escherichia coli* Y1089 host containing cloned rDNA which included the gene for the *M. leprae* 65-kD protein. Of the 14 distinct MAbs, 1 recognized an epitope found only on *M. leprae*, and the others recognized epitopes present on as few as 8 or as many as all 23 of the mycobacterial species studied. Identification of these distinct 65-kD protein epitopes and use of the MAbs which recognize them should assist future structural studies of this protein and characterization of the T-cell reactive and serodiagnostically useful portions of the molecule.—Authors' Abstract

Drosos, A. A., Brennan, P. J, Elisaf, M. S., Stefanou, S. G., Papadimitriou, C. S. and Moutsopoulos, H. M. Specific antigen and antibody to *Mycobacterium leprae* in the cryoprecipitate of a patient with Lucio phenomenon. Rheumatol. Int. **6** (1986) 93–94.

Using a sensitive and specific enzyme-linked immunosorbent assay (ELISA) we showed that the cryoglobulins of a patient with Lucio phenomenon contain phenolic glycolipid-I antigen and a specific antibody.—Authors' Summary

Emmrich, F., Thole, J., van Embden, J. and Kaufmann, S. H. E. A recombinant 64 kilodalton protein of *Mycobacterium bovis* bacillus Calmette-Guerin specifically stimulates human T4 clones reactive to mycobacterial antigens. J. Exp. Med. **163** (1986) 1024–1029.

A recombinant 64 kD protein of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) (antigen A), which amounted to ~2% of an *Escherichia coli* lysate, was tested for its capacity to simulate human T4 clones reactive to mycobacterial proteins. Two out of four crossreactive clones, established from a patient with tuberculoid leprosy, which could be stimulated by protein preparations of *M. leprae* and *M. tuberculosis*,

and by particulate *M. bovis* BCG were also reactive to antigen A without further enrichment from *E. coli* lysate. In addition, BCG-reactive T-cell clones from two of three healthy PPD′ donors reacted with antigen A. This finding shows that human T-cell clones may be useful for probing gene-cloned proteins of potential value for vaccination against diseases where protection is mediated exclusively by T cells.—Authors' Summary

Harboe, M. and Ivanyi, J. Analysis of monoclonal antibodies to *Mycobacterium leprae* by crossed immunoelectrophoresis. Scand. J. Immunol. **25** (1987) 133–138.

Monoclonal antibodies to *Mycobacterium leprae* were characterized in crossed immunoelectrophoresis and showed markedly different patterns of reactivity with *M. leprae* lines 2, 7, and 11, respectively. Line 7 corresponds to a cell wall-associated macromolecular complex containing lipid, polysaccharide, and two distinct 36 kD and 65 kD proteins.—Authors' Abstract

Howard, M. K., Gull, K. and Miles, M. A. Antibodies to tubulin in patients with parasitic infections. Clin. Exp. Immunol. **68** (1987) 78–85.

Sera from a total of 268 patients with protozoan, helminth, bacterial (leprosy and tuberculosis) infections or appropriate controls were assayed for anti-tubulin antibodies in an indirect enzyme-linked immunosorbent assay (ELISA), using purified tubulin as antigen. Levels of serum anti-tubulin antibody were significantly elevated in 67% of patients with visceral leishmaniasis, in 60% of patients with cutaneous leishmaniasis, in 89% of patients with onchocerciasis, in 100% of patients with schistosomiasis, and in 94% of patients with leprosy. Little or no increase in anti-tubulin antibody levels was seen in sera from patients with malaria (*Plasmodium vivax*) or tuberculosis.—Authors' Summary

Hussein, S., Curtis, J., Akuffo, H. and Turk, J. L. Dissociation between delayed-type hypersensitivity and resistance to pathogenic mycobacteria demonstrated by T-cell clones. Infect. Immun. **55** (1987) 564–567.

One Lyt-2+ clone and 14 T-helper clones (Lyt-1+ L3T4+ Lyt-2−) were isolated from *Mycobacterium lepraemurium*-infected BALB/c and C57BL/6 mice. All of the clones were tested for their ability to transfer delayed-type hypersensitivity adoptively, and six clones were tested for their ability to transfer resistance. It was found that four L3T4+ clones that transferred delayed-type hypersensitivity responses locally and one L3T4+ clone that did not had no effect on resistance. The Lyt-2+ clone transferred increased resistance locally (77% reduction in the numbers of organisms recovered from the infection site) but did not transfer delayed foot pad responses.—Authors' Abstract

Hussein, S., Curtis, J., Griffiths, D. and Turk, J. L. Study of DTH and resistance in *Mycobacterium lepraemurium* infection using a T-cell line isolated from mice infected with *Mycobacterium bovis* (BCG). Cell. Immunol. **105** (1987) 423–431.

A T-cell line of mixed phenotype (60% L3T4+, 40% Lyt-2+) was isolated from mice infected with *Mycobacterium bovis* (BCG). This line responded to *M. lepraemurium* and BCG but not to *M. leprae* and produced TCGF spontaneously. It also produced factors which stimulated macrophages to secrete hydrogen peroxide and superoxide anion. *In vivo* studies showed that only L3T4+ cells were required to transfer DTH responses and that Lyt-2+ cells suppressed this response. Both L3T4+ and Lyt-2+ cells were required to inhibit *M. lepraemurium* multiplication *in vivo*.—Authors' Abstract

Husson, R. N. and Young, R. C. Genes for the major protein antigens of *Mycobacterium tuberculosis*: the etiologic agents of tuberculosis and leprosy share an immunodominant antigen. Proc. Natl. Acad. Sci. U.S.A. **84** (1987) 1679–1683.

Mycobacterium tuberculosis genes encoding immunologically relevant proteins were isolated by systematically screening a λgt11 recombinant DNA expression library with a collection of murine monoclonal antibod-

ies directed against protein antigens of this pathogen. These antibodies, previously characterized by a World Health Organization workshop on monoclonal antibodies against mycobacteria, were used to isolate DNA sequences encoding five major protein antigens of this pathogen. To evaluate the extent of crossreactivity between these *M. tuberculosis* antigens and antigens of *M. leprae,* recombinant antigens were probed with monoclonal antibodies directed against the protein antigens of these bacilli. One of the antigens, a 65-kD protein, has determinants common to *M. tuberculosis* and *M. leprae.* We find not only that this antigen is recognized by mouse monoclonal antibodies but that it is the major protein recognized by anti-*M. tuberculosis* rabbit sera. The 65-kD proteins of *M. tuberculosis* and *M. leprae* appear to play a role in the humoral and cell-mediated immune response to these pathogens.—Authors' Abstract

Kaldany, R.-R. J., Maasho, K., Ohman, R., Reitz-Vick, D., Britton, S. and Lefford, M. J. Methods for the detection of a specific *Mycobacterium leprae* antigen in the urine of leprosy patients. Scand. J. Immunol. **25** (1987) 37–43.

Two methods for detecting the phenolic glycolipid, PGL-I, a *Mycobacterium leprae*-specific molecule, in the urine of leprosy patients are described. Both methods rely on the 100-fold preconcentration of the urine, which can be accomplished by a single-step ultrafiltration procedure. The equivalent of approximately 2.5 μg of PGL-I/ml was detected in the urine of LL patients with an inhibition ELISA. The second method, a direct dot-blot assay on nitrocellulose paper, was much simpler and more sensitive. As little as 3 ng of antigen was detected by the dot-blot technique. PGL-I was detected in the urine of LL patients.—Authors' Abstract

Kaplan, G., Gandhi, R. R., Weinstein, D. E., Levis, W. R., Patarroyo, M. E., Brennan, P. J. and Cohn, Z. A. *Mycobacterium leprae* antigen-induced suppression of T cell proliferation *in vitro.* J. Immunol. **138** (1987) 3028–3034.

The extent to which *Mycobacterium leprae* and its products induced suppression of T lymphocyte proliferation *in vitro* was evaluated. *M. leprae* antigens suppressed T-cell proliferation in response to mitogens and antigens in both lepromatous and tuberculoid patients, as well as controls never exposed to *M. leprae* or *M. leprae*-endemic areas. Both soluble and particulate fractions of *M. leprae* were found to suppress proliferation in a dose-dependent manner. The extent of suppression was inversely related to the proliferative response of the donors mononuclear cells to *M. leprae.* Evidence indicates that *M. leprae* contain both stimulatory and suppressive molecules for T cells. One such suppressive antigen, lipoarabinomannan (LAM)-B of *M. leprae,* also suppressed the proliferative response of tuberculoid patients. Suppression was also observed with the LAM-B of *M. tuberculosis.* The suppressive effects observed were not due to the toxicity of the antigen. Some of the suppressive activity was mediated by T8+ suppressor cells, and was expressed in both lepromatous and tuberculoid patients. We suggest that previous sensitization to *M. leprae* and other crossreactive mycobacterial antigens determines the sensitivity of T cells to the suppressive effects of *M. leprae* antigens.—Authors' Abstract

Kim, H. B. and Kim, M. Y. [A study of the histopathologic changes of the nasal mucosa in patients with leprosy.] Korean J. Dermatol. **24** (1986) 43–48. (in Korean)

The route of transmission of leprosy is still unclear. Recently some authors proposed the possibility that the bacilli discharged from nasal mucosa might be the source of infection in leprosy. This study was undertaken to evaluate the histopathologic findings of nasal mucosa in 24 fresh, untreated patients with leprosy using hematoxylin and eosin (H&E) and Ziehl-Neelsen staining.

Among 24 cases, under clinical observation, 10 cases (41.7%) complained of nasal stuffiness, 8 cases (33.3%) had congestion, 1 case (4.2%) was crusted, and 1 case (4.2%) was ulcerated. The bacterial index of nasal mucosal smears showed that 15 cases were positive; 5 cases were 1+, 7 cases were 2+, 2 cases were 3+, and 1 case was 4+. Under H&E stain, the histopathologic findings of the inferior turbinate of the nasal

mucosa showed that 22 cases had cystic dilatation of mucous glands, 19 cases had granulomas with foamy cells, and 14 cases had epidermal atrophy.

Under Ziehl-Neelsen stain, the bacterial index of the nasal mucosal biopsy showed that 21 cases were positive; 3 cases were 3+, 7 cases were 4+, 6 cases were 5+, and 5 cases were 6+. The histopathologic findings of the nasal mucosa were similar to the skin lesions of leprosy.—Authors' English Abstract

Koster, F. T., Scollard, D. M., Umland, E. T., Fishbein, D. B., Hanly, W. C., Brennan, P. J. and Nelson, K. E. Cellular and humoral immune response to a phenolic glycolipid antigen (PhenGL-I) in patients with leprosy. J. Clin. Microbiol. **25** (1987) 551–556.

The ability of phenolic glycyolipid-I (PGL-I) of *Mycobacterium leprae* to stimulate *in vitro* lymphocyte proliferation (LP) was tested in cultures of peripheral blood cells from 42 patients with leprosy in Chicago and Thailand, 9 individuals with household contact in Thailand, and 10 unexposed North American controls. Only 10 responders (24%) were found among the patients, and the degree of LP was small. Responders were found among patients with lepromatous (18%) or tuberculoid (30%) leprosy without relation to age, complications, duration of treatment, or lepromin responsiveness. The specificity of the response was supported by a lack of response to two other glycolipids, by responses by T cells but not B cells, and by the observation that 3 of 4 responders tested maintained their responses to PGL-I for at least 1 year. Serum immunoglobulin M (IgM) and IgG antibodies were measured in the same patients by using PGL-I or its terminal monosaccharide conjugated to a bovine serum albumin carrier in an enzyme-linked immunosorbent assay. The presence of IgM antibody correlated negatively with LP to lepromin and to PGL-I in patients with tuberculoid leprosy. We conclude that circulating T cells from some leprosy patients proliferate in the presence of PGL-I *in vitro,* but the response is weak, possibly due to concomitant suppression or inhibition. The predominance of IgM antibody to PGL-I may be related to a lack of a T-helper-cell-mediated switch to IgG antibody response.—Authors' Abstract

Kumar, B., Vaishnavi, C., Ganguly, N. K. and Kaur, S. Macrophage chemotaxis in *Mycobacterium leprae* infected mice. Indian J. Med. Res. **85** (1987) 125–129.

Mycobacterium leprae obtained from untreated lepromatous patients were inoculated into the foot pads of Swiss albino mice (normal and immunosuppressed). Uninfected controls (both normal and immunosuppressed) were also included in the study. Macrophage chemotaxis was studied in the infected mice using three leukoattractants—zymosan-activated serum, bacterial filtrate and casein—at 3, 6, and 9 months post-inoculation with *M. leprae* in the infected groups and at a corresponding period in the uninfected controls. No significant difference in macrophage chemotactic response was observed in the infected groups as compared to controls.—Authors' Abstract

Lee, C. W., Lee, H. Y., Son, S. J. and Kim, D. I. [*In situ* characterization of immune cells in the annular lesions of leprosy.] Korean J. Dermatol. **24** (1986) 49–54. (in Korean)

To characterize the immunopathologic phenotype of the cells in the lesional tissue and to further examine the mechanism of the development of the annular lesions in leprosy, we have studied immune cells (T lymphocytes and subsets, Langerhans' cells, and HLA-DR antigen expressing cells) at different anatomical sites—inside the lesion, at the active border of the lesion, and outside the lesion in apparently normal skin—in the annular lesions of leprosy. We took biopsy specimens from four patients of the BT type, then processed the specimens by the staining method of indirect immunoperoxidase with monoclonal antibodies. In the active border of the lesions, the number of T cells was over 50% of the total cells infiltrated in the dermis. Helper T cells were dominant in number, and about three fourths of the cells were positive for HLA-DR staining. In two patients there was expression of DR antigen on the surfaces of the keratinocytes in the epidermis, in con-

trast to the findings inside the lesion where even the intensities were not strong. At the inside of the annular lesions, T cells were about 40% and the ratio of helper/suppressor T cells was approximately 1:1. However, HLA-DR positive immune cells were not more than 10% of the total infiltrates. Langerhans' cells were increased in number and in size, both in the border or at the inside of the annular lesions.

With these results, we presume that the T-cell-mediated immune responses against *Mycobacterium leprae* may play an important role in the formation and extension of the annular lesions in leprosy.—Authors' English Abstract

Mackworth-Young, C., Sabbaga, J. and Schwartz, R. S. Idiotypic markers of polyclonal B cell activation; public idiotypes shared by monoclonal antibodies derived from patients with systemic lupus erythematosus or leprosy. J. Clin. Invest. **79** (1987) 572–581.

We investigated idiotypic markers of monoclonal antibodies derived from patients with polyclonal B-cell activation. Four monoclonal antibodies with different ligand-binding specificities derived from a patient with lepromatous leprosy and three monoclonal anti-DNA antibodies from two patients with SLE were studied. Three new public idiotopes, which were common to monoclonal antibodies from all three patients, were defined by five polyclonal rabbit antiidiotypes, two monoclonal mouse antiidiotopes, and a monoclonal mouse antibody against a synthetic peptide that contains residues of the heavy chain CDR-I of a monoclonal lupus anti-DNA antibody. The antibody against the synthetic idiotype was found to react with native immunoglobulins in solution. One idiotope was found to be consistently immunogenic in all animals tested. Since the three patients are of different ethnic origins, these shared idiotypes are probably encoded by germline V genes. These genes may be recurrently expressed in states of polyclonal B-cell activation, regardless of etiology. The results suggest that some autoantibodies arise by expansion of a pool of precursors in the normal antibody repertoire.—Authors' Abstract

Maeda, M. and Narita, M. Affinity of *Mycobacterium leprae* with Lewis rat schwannoma cell line (Lewis TC 98). Lepr. Rev. **58** (1987) 39–51.

The possible affinity of *Mycobacterium leprae* with Lewis TC 98 cell line established from Lewis rat spinal schwannoma tissue was investigated. Lewis TC 98 cells phagocytosed *M. leprae* well, showing a phagocytic index of over 60% after 5-hr exposure at a mycobacterium-to-cell ratio = 25, higher than C6 cells from rat glioma and still more higher than cells from human neuroblastoma. In a comparative study with three kinds of inocula, i.e., live *M. leprae,* heat-damaged *M. leprae,* and *M. lepraemurium,* only live *M. leprae* revealed a high affinity with Lewis TC 98 cells. Also, the phagocytic activity to *M. leprae* of Lewis TC 98 cells was not affected by changing the condition of the cell growth with low doses of fetal bovine serum (FBS) in a culture medium. These results may suggest the special affinity of *M. leprae* with Schwann cells and the possible presence of a receptor with reactivity to live *M. leprae,* presumably existing on cell surfaces of Lewis TC 98 cells. However, two rabbit antisera against Lewis TC 98 cell surface antigens could not block the interaction between Lewis TC 98 cells and *M. leprae.*—Authors' Summary

Narayanan, R. B., Ramu, G., Sinha, S., Sengupta, U. and Gupta, C. M. Immunohistologic comparison between armadillo-derived leprosin and standard lepromin skin tests in leprosy patients. Int. Arch. Allergy Appl. Immunol. **82** (1987) 202–207.

A comparison was made on the *in situ* immunological characteristics of dermal infiltrates of early (24-hr) and late (3–4 weeks) skin reactions in leprosy patients. The skin reactions were induced by armadillo-derived leprosin coupled to liposomes and standard Dharmendra lepromin. Most lymphocytes in the early reaction induced by both antigens were positive for Leu4, Leu3a, OKT8, and Ia-like antigens, indicating thereby the presence of activated T cells. The ratio of Leu3a/OKT8+ cells were similar. In the late reaction elicited by both antigens, the lymphocytes in the granu-

lomas were predominantly activated T lymphocytes expressing Leu4, Leu3a, OKT8, and Ia-like antigens. Leu3a+ cells were scattered diffusely amid the epithelioid cells. In contrast, the OKT8+ cells were present mainly as "a ring" in the periphery of the granuloma. A similar ratio of Leu3a+/OKT8+ cells was observed in these granulomas. Macrophages in the granulomas expressed Ia-like antigens. These observations indicate that the immunological characteristics of dermal infiltrates in the skin reaction induced by armadillo-derived leprosin coupled to liposomes and standard Dharmendra lepromin appear to be identical.—Authors' Abstract

Rao, T. D. and Rao, P. R. Tγ, Tμ and B lymphocytes in erythema nodosum leprosum reactions of leprosy. Indian J. Lepr. **58** (1986) 601–608.

Enumeration of subpopulation of T cells with receptors for Fc portion of IgG (Tγ) and Fc portion of IgM (Tμ) and B lymphocytes in the peripheral blood of 39 lepromatous, 44 ENL, and 22 post-ENL patients was undertaken. ENL patients showed a significantly decreased Tγ cell percentage than lepromatous and post-ENL patients. Although Tμ cell percentage was lowered in ENL patients, a relatively elevated Tμ/Tγ ratio was found than in lepromatous and post-ENL patients, indicating elevation of helper activity in ENL. B cells did not register any change in the three stages.—Authors' Abstract

Rotberg, A. The "Hansen-anergic fringe." Acta Leprol. **4** (1986) 347–354.

The Hansen-anergic fringe (HAF) of the population is the minority genetically incapable of developing that variety of specific immunity to *Mycobacterium hansenii* which leads to the positivity of the Mitsuda reaction. In spite of the fact that the HAF was identified as early as 1937, its real proportion in the human species is still not exactly known.

Considering that *M. tuberculosis* has been proved to induce Mitsuda-positivity in the genetically capable majority, studies with the tuberculin reactions and cross-stimulation with BCG should be instrumental for the determination of the width of the HAF in various races and different endemic and nonendemic areas. In Brazil it stands around 20%–25%, but more accurate studies are necessary. Between the HAF and the Mitsuda-positive or potentially positive majority there is a zone of "intermediate reactivity" which also needs quantification.

The specific HAF reacts normergically to all other tests so far investigated, and has not yet been associated with any other kind of immunodeficiency. Also, it has not been associated with any of the known genetic markers, although HLA studies seem promising. If its postulated genetic nature is confirmed, the possibility of effective vaccination will be doubtful.—Author's Summary

Schmutzhard, E., Fuchs, D., Hausen, A., Reibnegger, G. and Wachter, H. Is neopterin—a marker of cellmediated immune response—helpful in classifying leprosy? E. Afr. Med. J. **63** (1986) 577–580.

The urinary excretion of neopterin, an indicator of cellular immune response, was measured in patients suffering from leprosy. No difference was found between the BT + T group and BL + L group. All age groups presented with similar urinary neopterin excretion. There was no correlation apparent between the duration of disease and urinary neopterin excretion. Whether the most recent skin smear was positive or negative, did not make any difference. Similarly, prior medication did not play any role in influencing urinary neopterin excretion.—Authors' Summary

Senba, M., Fukushima, N. and Toda, T. A rapid silver staining method for identification of *Mycobacterium leprae* in histologic sections. Tohoku J. Exp. Med. **150** (1986) 363–364.

A few methods have been reported for the purpose of staining *Mycobacterium leprae* in paraffin sections, including the Fite oil fuchsin method, auramine-rhodamine method, and the Blanco-Fite silver method. Among these staining techniques, the Fite oil fuchsin method and the auramine-rhodamine methods are popular. However, the Blanco-Fite silver method takes approximately 20 days. Therefore, we developed a

new procedure for the rapid identification of *M. leprae* in paraffin sections using another silver solution and found that the procedure gave stable and satisfactory results. This new method has proved superior to others in demonstrating a reliable staining for *M. leprae.*—Authors' Abstract

Sengupta, U., Sinha, S., Ramu, G., Lamb, J. and Ivanyi, J. Suppression of delayed hypersensitivity skin reactions to tuberculin by *M. leprae* antigens in patients with lepromatous and tuberculoid leprosy. Clin. Exp. Immunol. **68** (1987) 58–64.

Delayed hypersensitivity skin reactions to tuberculin when injected alone or in mixture with antigens of *Mycobacterium leprae* were examined in leprosy patients and in healthy controls. The tuberculin reaction was significantly inhibited in more than one half of both LL and BT patients by the soluble extract of *M. leprae* (leprosin), the leprosin-derived 12 kD protein, or leprosin depleted of the 12 kD antigen. However, suppression was not found in healthy controls from a leprosy-endemic region. These results suggest that multiple *M. leprae*-specific antigens have an immunoregulatory function. Since suppression was demonstrable not only in LL (leprosin-anergic) but also in BT (leprosin-responder) patients, it is of interest that the "mixed" skin test can discriminate the immune status of at least certain BT patients from that of the infected but self-healing healthy controls. Corollary lymphocyte cultures failed to show any suppression by leprosin of the lymphoproliferative responses to tuberculin.—Authors' Summary

Microbiology

Cocito, C. and Delville, J. Biological, chemical, immunological and staining properties of bacteria isolated from tissues of leprosy patients. Eur. J. Epidemiol. **1** (1985) 202–231.

Two kinds of microorganisms are found in tissue of leprosy patients: *Mycobacterium leprae* (ML) and leprosy-derived corynebacteria (LDC). ML from untreated patients has an alcohol-acid-fastness, which is lost upon treatment with antibiotics and immune response (tuberculoid leprosy). Vulnerable ML thus produced can be reversibly de-stained by organic solvent: in tissue sections from tuberculoid and treated patients, more bacteria are, thus, revealed by the Wade-Fite than by the Ziehl-Neelsen procedure.

Organisms of genera *Corynebacterium, Mycobacterium,* and *Nocardia* (CMN group) have DNA with %GC contents of 50–70, 69–72, and 68–70, respectively. GC values of DNA from ML and LDC are close to 56%. DNA from different LDC strains display high homology among them and low homology with reference corynebacteria.

CMN cell wall consists of interconnected peptidoglycan and polysaccharide-mycolate complex. Peptidoglycan of LDC (and known CMN) has the polysaccharide backbone linked to a tetrapeptide of L-Ala, D-Glu, m-DAP (meso-diamino-pimelate), D-Ala. In ML, L-Ala is replaced by glycine. Mycobacterial wall polysaccharides (that of ML is unknown) are branched arabinogalactans with end arabinoses linked to C70 to C90 mycolates. LDC peripheral polysaccharides are arabinogalactomannans with arabinose and mannose lateral strands. Mycolic acids of LDC are of corynomycolic type (C32, C34, and C36 with 1–4 double bonds) and those of ML are of mycobacterial type.

Components of CMN wall and cytoplasm are immunologically active as antigens (polysaccharides, proteins), haptens (lipids), and adjuvants (peptidoglycans). Strong intrageneric and weak intergenera cross-reactions are observed among CMN bacteria: LDC preparations, however, cross-react strongly with ML and mycobacteria, and weakly with reference corynebacteria. LDC in leprosy tissues can, thus, be re-

vealed as well by fluorescent anti-LDC antisera as by anti-ML antisera. The main cross-reacting component is antigen M1 of LDC, which corresponds to antigens Ag7 of ML and Ag60 of BCG, the active components of lepromin and tuberculin (known reagents for cutaneous tests). Antigen M1 has a polysaccharide moiety crossreacting with the wall polysaccharide of LDC. Immunological reactivity in leprosy apparently is directed toward the polysaccharide moiety during the tuberculoid phase, and the polypeptide moiety during the lepromatous phase.

Immunological kinship of LDC and ML suggests their possible cooperation in leprosy development. Injection of small number of LDC in one foot pad of mice, which were challenged in both foot pads with ML, produced a faster proliferation of ML suggestive of synergism. Leprosy is thus a disease produced by ML, organisms of uncertain taxonomic position, possibly helped by LDC, a unique group of corynebacteria which are well characterized both biochemically and immunologically.

LDC have now been renamed *Corynebacterium tuberculostearicum* sp. nov. (Intl. Comm. Syst. Bacteriol., 1985), as proposed by L. Barksdale, on the basis of their property to produce tuberculostearic acid. A collection of these organisms is deposited at the American Type Culture Collection.—Authors' Abstract

Dhople, A. M. and Green, K. J. Limited *in vitro* multiplication of *Mycobacterium leprae*: application to screening potential antileprosy compounds. Lepr. Rev. **57** Suppl. 3 (1986) 149–162.

Inability to cultivate *Mycobacterium 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 ^{3}H-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 ^{3}H-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 inoculate 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 ^{3}H-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 multipli-

cation 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 dapsone (DDS) and rifampin. *M. leprae* were incubated in the presence of various concentrations of DDS and at periodic intervals, the cells were taken for ATP assays and ^{3}H-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 nonviable 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 rifampin against *M. leprae* was found to be between 250 and 300 ng/ml. These studies are in progress.—Authors' Abstract

Franzblau, S. G., Takeda, T. and Nakamura, M. Mycobacterial plasmids: screening and possible relationship in antibiotic resistance in *Mycobacterium avium/Mycobacterium intracellulare*. Microbiol. Immunol. **30** (1986) 903–907.

Forty-eight mycobacterial isolates were screened for plasmid DNA by a modified Kado and Liu procedure. Plasmid DNA was not detected in single clinical isolates of *Mycobacterium kansasii* and *M. scrofulaceum* or in a single isolate of *M. lepraemurium*. Also negative were five unidentified isolates isolated from human leprosy nodules at the Foundation for Medical Research (FMR), Bombay, India, including rifampin and DDS-resistant strains and two mycobacterial contaminants from *M. leprae* experimentally infected armadillo and nude mouse. In addition five slow-growing, acid-fast environmental isolates and single reference strains of *M. avium* and *M. intracellulare* failed to yield plasmid DNA. Thirty-one *M. avium/intracellulare* clinical isolates from Hiroshima were analyzed for plasmid content, antibiotic sensitivity, and colonial morphology. Of the 16 plasmid-positive isolates (1–3 plasmids), 15 had MICs of ≥ 100 μg/ml for kanamycin, streptomycin, and rifampin and, with one exception, produced translucent or rough colonies. In contrast, the 15 plasmid-negative isolates demonstrated a range of sensitivities to these antibiotics with only two isolates showing high-level resistance to all three drugs. Most of these isolates produced opaque colonies. The results remain inconclusive with regard to the mechanism and coding site of drug resistance, but the pattern of resistance in plasmid-positive isolates is suggestive of a permeability barrier.—Authors' Summary

Kato, L. Investigations into the cultivation of *Mycobacterium leprae*; a multifactorial approach. Lepr. Rev. **57** Suppl. 3 (1986) 209–219.

Culture media for *Mycobacterium leprae* are proposed on the assumption that the mycobactin-deficient *M. leprae* require 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, $(NH_4)SO_4$ 2 g, $MgSO_4$ 0.1 g, and ferric ammonium citrate 0.05 g were dissolved in 1 liter of the filtrates. The pH was adjusted to 5.8 with KH_2PO_4. Twenty ml media was distributed into each of 25 ml screw-cap tubes and autoclaved for 30 min. 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 foot pads of mice they produce the disease characteristic of *M. leprae.* Subcultures remain dependent on the multifactorial media, enriched with growth factors (exochelins and mycobactins) from the leprosy-derived cultivable mycobacteria.—Author's Abstract

Kazda, J., Ganapati, R., Revankar, C., Buchanan, T. M., Young, D. B. and Irgens, L. M. Isolation of environment-derived *Mycobacterium leprae* from soil in Bombay. Lepr. Rev. **57** Suppl. 3 (1986) 201–208.

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 II 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 *Mycobacterium leprae* has been described recently. This strain of *M. leprae* was isolated from soil by the 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.—Authors' Abstract

Ramasesh, N., Hastings, R. C. and Krahenbuhl, J. L. Metabolism of *Mycobacterium leprae* in macrophages. Infect. Immun. **55** (1987) 1203–1206

The incorporation of ^{14}C-labeled palmitic acid ([U-^{14}C]PA) into the phenolic glycolipid-I (PGL-I) fraction of *Mycobacterium leprae* was studied in a murine macrophage system *in vitro.* Peritoneal macrophages from Swiss Webster mice were infected with fresh viable or Formalin-killed *M. leprae* harvested from infected foot pads of *nu/nu* mice, and [U-^{14}C]PA was added to the culture medium. Labeled glycolipid synthesized by live *M. leprae* was fractionated on a Florisil-silicic acid column and identified as PGL-I by using thin-layer chromatography and localization on a polysulfone membrane with an anti-PGL-I monoclonal antibody. Increased incorporation of [U-^{14}C]PA into the PGL-I fraction was observed in macrophages infected with only live *M. leprae.* Treatment of the infected macrophages with rifampin caused a significant reduction in the incorporation of palmitic acid into PGL-I. These preliminary studies suggest that PGL-I synthesis can be used to quantitate the metabolism of *M. leprae* in macrophages *in vitro.*—Authors' Abstract

Seydel, U. and Lindner, B. Single bacterial cell mass analysis: a rapid test method in leprosy therapy control. Lepr. Rev. **57** Suppl. 3 (1986) 163–170.

To overcome problems arising from the *in vitro* noncultivability of *Mycobacterium 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 single *M. leprae* cells isolated from biopsies. 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. 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 (Na^+,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 Na^+,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, U.S.A.), a good agreement between the statements for his ATP-assay, the mouse foot pad test, and our measurements of the Na^+,K^+-ratio was found.

A particular advantage of the single cell mass spectrometry, however, is—besides 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 analyzing single cells and this way getting distributions of the respective data instead of averaged values.—Authors' Abstract

Experimental Infections

Mathur, I. S., Gupta, H. P., Srivastava, S. K., *et al.* Evaluation of subdermal biodegradable implants incorporating rifampicin as a method of drug delivery in experimental tuberculosis of guinea pigs. J. Med. Microbiol. **20** (1985) 387–392.

Conventional chemotherapy of tuberculosis and leprosy requires rifampin (RMP) to be administered orally. The long period of treatment and adverse side effects of the drug lead to poor compliance. To overcome this, subdermal implants incorporating RMP in pure and micro-encapsulated forms with biodegradable material were used as a new drug delivery system in experimental tuberculosis of guinea pigs. Two experiments were performed with 45-mg and 100-mg drug implants. Progress of infection was followed at intervals by studying necropsy scores and weights of the organs of predilection, and levels of the drug in the blood were determined. There was a constant and sustained release of the drug in therapeutic concentrations for 30 and 50 days until the implants were completely assimilated without causing any damage to the implant site. The importance of multiple implants at long intervals is discussed.—(*From* Excerpta Medica)

Vaishnavi, C., Kaur, S., Kumar, B., Kaur, H. and Ganguly, N. K. Circulating immune complexes in normal and immunosuppressed mice infected with *Mycobacterium leprae.* Indian J. Lepr. **58** (1986) 522–529.

Mycobacterium leprae infection was produced through the foot pads in normal and immunosuppressed mice. Circulating immune complexes were detected by specific binding test and by conglutinin binding assay for specific and total immune complexes, respectively, in the sera of these mice during different periods of infection. Out of the total 30 samples tested from the infected groups, 3 were positive by specific binding test and 5 by conglutinin binding ELISA. The implications of the findings in relation to human leprosy are discussed.—Authors' Abstract

Epidemiology and Prevention

Bona, S. H., da Fonseca, A. deP. M., Lima da Silva, A. C. and da Costa, R. J. [Acid-fast bacilli in *Culex fatigans.*] An. Bras. Dermatol. **60** (1985) 163–170. (in Portuguese)

The authors examined 194 *Culex fatigans*—3 male and 191 female—from 100 homes of cases of Hansen's disease (L and B forms) and 42 *Culex*—8 male and 34 female—from 19 homes of non-treated patients from December 1981 through December 1982 in the city of Teresina, state of Piauí, Brazil. The objective was to evaluate the degree of contamination of the insects with acid-fast bacilli and to compare the results with those obtained from 264 *Culex*—18 male and 246 female—caught in 100 homes of healthy persons. Ziehl-Neelsen preparations were made from salivary glands, guts, feces, and ovaries of the insects. Numerous bacilli were found in about 90% of the examined slides; the control group (from the houses of healthy persons) had the same positivity. Further studies are needed to identify the bacilli.—Authors' English Summary

Brightmer, I. Leprosy in Cross River State, Nigeria. Lepr. Rev. **58** (1987) 69–78.

This spatial study of leprosy was stimulated by reports of medical workers in the state that serious and advanced cases of the disease are appearing, and that possibly there are pockets of infection which need to be identified and taken into account by the state's leprosy control program.

A preliminary examination of the spatial pattern of leprosy in the state is presented here, and shows higher prevalence rates for the disease in the areas of sparsest population. The work is continuing, and will attempt to explain these distribution features.

An account is given of some prevailing local attitudes to leprosy and their consequences for the control of the disease. The structure of the control service (state and federal) is outlined, and constraints limiting its effectiveness are discussed.—Author's Summary

Diop Mar, I., Wone, I. and Millan, J. [The epidemiology of leprosy.] Med. Afr. Noire **32** (1985) 295–309. (in French)

Despite all efforts to fight leprosy, its endemic status remains stable. Modern leprosy epidemiology studies the triad: causative agent–host–environment, on the community level, searching for existing interactions between the human system and the ecosystem in order to determine all factors that may be used for the prevention of the disease.—(*From* Excerpta Medica)

Kalyankar, S. D., Vyas, G. H., Kotambe, D. S. and Rode, C. R. A preliminary report on *Mycobacterium leprae* in Aurangabad. G. Mal. Infet. Paras. **37** (1985) 764–768.

A survey of leprosy as carried out in Aurangabad, Maharashtra State, India, shows that the disease is very common in the advanced age groups from 25 to 60 years and older. It also shows that it is very common among the economically poorer section. This study revealed a prevalence rate of 3.2 in Aurangabad district for the year 1979–1980. The survey revealed that the behavior of this society toward leprosy patients is not very good.—Authors' Summary

Mishra, B., Ramu, G., Chauhan, V. S., Kushwaha, S. S. and Dwivedi, M. P. Leprosy in eastern region of Rajasthan. Indian J. Lepr. **58** (1986) 576–583.

Total population survey covering 28,055 persons living in 34 villages of eastern Rajasthan was carried out. Out of 28,055 persons, 20,276 (72.27%) were actually examined and 218 cases of leprosy were detected. Prevalence of leprosy was found to be 10.75/1000, which is very high and contrary to earlier observations regarding leprosy prevalence in Rajasthan. The leprosy problem in Rajasthan has been discussed.—Authors' Abstract

Shao, K., *et al.* [Epidemiological changes of leprosy in Fujian Province.] Chin. J. Prev. Med. **20** (1986) 73–75. (in Chinese)

Repeated surveys have found 27,058 leprosy cases. There were 2155 cases of leprosy by the end of 1984, with the prevalence rate of 0.08%, 87.5% lower than the year of the highest prevalence rate. The annual detection rate dropped by 96%, while the annual incidence rate of the disease (in 1978) fell by 93%. With the large drop of the above morbidity rates, shrinkage of the epidemic areas, and the remarkable decrease of disease incidence among the children, it shows that leprosy has been better controlled. Furthermore, the shorter disease period, among the newly discovered cases, decrease in the ratio of deformity and disability cases, and the rise in the proportion of patients with few bacilli and with single skin lesions all indicate that notable progress has been made in leprosy control in the province.—Authors' English Summary

Shields, E. D., Russell, D. A. and Pericak-Vance, M. A. Genetic epidemiology of the susceptibility to leprosy. J. Clin. Invest. **79** (1987) 1139–1143.

To test the hypothesis that genetic factors are operative in the predisposition to leprosy (Hansen's disease) in humans, a genetic epidemiologic investigation was performed on 269 leprosy kindreds containing 552 affected individuals from an isolated population in Papua New Guinea. The community, and not the family, was the basic social unit. Leprosy, an infectious disease, was not communal but strongly familial within the Karimui. Segregation analysis, to determine whether a major gene for the susceptibility to leprosy was segregating within a single multi-generational kindred, could not differentiate between a Mendelian genetic and a purely environmental hypothesis. The composite kindred data, however, suggest a genetic hypothesis for the non-immunologically induced susceptibility to leprosy per se. Within familial kindreds leprosy invariably emanated from a common ancestral sibship, and risk was associated with the closeness of kin but not with infectivity or severity.—Authors' Abstract

Rehabilitation

Geary, N. P. J. and Klenerman, L. The insensitive foot; Northwick Park experience. Lepr. Rev. **58** (1987) 79–84.

The problems of the pathogenesis and management of plantar ulceration in diabetic subjects and patients with leprosy are compared. Use of the total contact cast for treatment of an ulcer and methods for prevention of recurrence by appropriate insoles in footwear are described.—Authors' Summary

Palande, D. D. Surgical correction of intrinsic minus fingers. Indian J. Lepr. **58** (1986) 537–542.

Today we have many different surgical procedures available to correct deformity and disability of intrinsic minus fingers. Starting with tendon transfers inserted into the lateral bands of the extensor expansion in each finger, the evolution of different surgical methods for correcting intrinsic minus fingers is discussed in the light of post-operative problem situations and drawbacks that the author has come across in the last 15 years. It is affirmed that each procedure is meant for a particular hand in a particular situation, and that it is necessary for a reconstructive surgeon to evaluate each hand on its own needs and deficits so as to decide on the choice of a particular type of surgical procedure required.—Author's Abstract

Palande, D. D. Surgical correction of thumb with ulnar or ulnar median paralysis. Indian J. Lepr. **58** (1986) 530–536.

The commonest deformities and disabilities of the thumb seen in this country are secondary to ulnar and median nerve paralysis because of leprosy. The small muscles of the thumb supplied by these nerves are paralyzed, causing instability, imbal-

ance of muscular forces, deformities, and disabilities. The clinical features of these together with principles of the various methods of surgical correction and their evaluation are presented and discussed in this paper.—Author's Abstract

Shah, A. and Bhagat, N. Rehabilitation surgery for leprosy handicapped at a rural leprosy colony. Int. J. Rehab. Res. **8** (1985) 345–347.

Rehabilitation of leprosy handicapped does not end by providing them food, shelter, and clothes but is the beginning of a long-term care in which rehabilitation surgery forms an integrated part of the program.—(*From* the Author's Conclusion)

Other Mycobacterial Diseases and Related Entities

Ahrens, E. M., Meckler, R. J. and Callen, J. P. Dapsone-induced peripheral neuropathy. Int. J. Dermatol. **25** (1986) 314–316.

A young man with dermatitis herpetiformis developed fatigue and neurologic complaints 4 years after he began oral dapsone therapy. Neurological examination and nerve conduction studies confirmed the presence of a combined motor and sensory peripheral neuropathy. The symptomatic improvement reported by the patient was supported by improvement in nerve conduction studies after cessation of dapsone therapy. Substitution of sulfapyridine did not adversely affect the resolution of his neuropathy.—(*From* Excerpta Medica)

Ausina, V., Condom, M. J., Mirelis, B., *et al.* *In vitro* activity of clofazimine against rapidly growing nonchromogenic mycobacteria. Antimicrob. Agents Chemother. **29** (1986) 951–952.

The *in vitro* activity of clofazimine against 80 isolates of rapidly growing nonchromogenic mycobacteria was studied by an agar dilution method. The drug inhibited 96% of strains tested at concentrations ≤ 1 μg/ml, and it appears to be an agent of potential efficacy against *Mycobacterium fortuitum* and *M. chelonei.*—(*From* Excerpta Medica)

Brand, P. W. Biomechanics of tendon transfers. In: *Tendon Surgery in the Hand.* Hunter, J. M., Schneider, L. H. and Mackin, E. J., eds. St. Louis: The C. V. Mosby Company, 1987, pp. 395–409.

The success of any operation for tendon transfer depends on five considerations: 1) the capability of the muscle that is selected for transfer; 2) the leverage, or moment arm, of the tendon at each of the joints that it crosses; 3) the overall balance of the hand as it is altered by the transfer; 4) the passive drag and friction that resists the tendon and joint movement; and 5) the motivation and re-education of the patient to use the transferred muscle for its new function.—(*From* the Chapter)

Brand, P. W. and Coleman, W. C. A standard for dorsal-plantar and lateral radiographic projections of the feet. Orthopedics **10** (1987) 117–120.

Several attempts have been made to persuade physicians to establish standardized projections of foot radiographs. However, standards have not been widely accepted. Further advancement toward quantification of deformity and surgical criteria is delayed due to a lack of universally accepted technique. Procedures for obtaining two precise projections of the foot are described.—Authors' Abstract

Brand, P. W., Thompson, D. E. and Micks, J. E. The biomechanics of the interphalangeal joints. In: *The Interphalangeal Joints; Vol. 1. The Hand and Upper Limb.* Bowers, W. H., ed. London: Churchill Livingstone, 1987, pp. 21–54.

This chapter has attempted to establish the fundamental issues relative to the biomechanics of the human hand. The theo-

retical and experimental topics covered should raise as many questions as they answer. This should stimulate the practicing clinician to attempt to answer some of these questions through observation of patients or the researcher to attempt to answer other questions via further refinement of the information and measurement techniques presented here. In this way, new insights may be shared by all and the practice of medicine elevated.—Authors' Summary

Centers for Disease Control. Diagnosis and management of mycobacterial infection and disease in persons with human immunodeficiency virus infection. Ann. Intern. Med. **106** (1987) 254–256.

Mycobacterial disease is common among patients with the acquired immunodeficiency syndrome (AIDS) and human immunodeficiency virus (HIV) infection. Among all patients with AIDS, the most frequently isolated cause is *Mycobacterium avium*-complex; but in some groups, such as Haitians and intravenous drug users, *M. tuberculosis* is commoner. Extrapulmonary disease and noncavitary, nonapical pulmonary tuberculosis are frequently seen. It is recommended that initial treatment of tuberculosis in these patients include at least three standard antituberculosis drugs and that therapy be continued for a minimum of 9 months. Treatment of disseminated disease due to *M. avium*-complex with this regimen is unsatisfactory, and a four-drug regimen that includes two experimental drugs, rifabutine and clofazimine, is the recommended treatment. Patients with AIDS or HIV infection and pulmonary tuberculosis should be considered potentially infectious, and appropriate infection-control and contact-tracing procedures applied. Persons with HIV infection should be given a tuberculin skin test and, if the reaction is positive (10 mm or more in diameter), isoniazid preventive therapy.—Authors' Abstract

Eisenach, K. D., Crawford, J. T. and Bates, J. H. Genetic relatedness among strains of the *Mycobacterium tuberculosis* complex. Analysis of restriction fragment heterogeneity using cloned DNA probes. Am. Rev. Respir. Dis. **133** (1986) 1065–1068.

Chromosomal DNA isolated from 3 reference and 2 clinical isolates of *Mycobacterium tuberculosis,* 2 reference strains each of *M. bovis* and *M. bovis* BCG, and 1 reference strain of *M. kansasii,* was digested with 9 restriction endonucleases. The restriction fragments were analyzed by agarose gel electrophoresis. With each enzyme tested, the patterns of the strains of *M. tuberculosis, M. bovis,* and *M. bovis* BCG were indistinguishable but clearly distinct from those of *M. kansasii.* A library of *M. tuberculosis* H37Rv DNA was prepared by cloning Bam Hl digest fragments into lambda 1059. Eight randomly selected clones, having multiple Bam Hl sites, were used to probe restriction digests of DNA from the strains of the tuberculosis complex. Two of the cloned segments hybridized with homologous fragments in four different enzyme digests of all strains. With two clones, hybridization differed among the strains; however, some homologous sequences were detected. With four clones, efficient hybridization occurred only with *M. tuberculosis* H37Rv DNA. These hybridization results indicate that some regions of the chromosome are highly conserved among members of the tuberculosis complex, whereas others are diverged. Selected DNA probes were useful in detecting differences among strains of the tuberculosis complex.—(*From* Excerpta Medica)

Narayanan, S., Paramasivan, C. N., Prabhakar, R. and Narayanan, P. R. Effect of oral exposure of *Mycobacterium avium intracellulare* on the protective immunity induced by BCG. J. Biosci. **10** (1986) 453–460.

The relative protective efficacy of oral administration of mycobacteria as compared to the conventional intradermal route of vaccination has been assessed in guinea pigs. Skin test reactivity to partially purified protein derivative and protective immunity to challenge with virulent *Mycobacterium tuberculosis* were used as parameters of protective immunity. Oral immunization of guinea pigs either with BCG or with *M. avium intracellulare* induces skin-test reactivity and protective immunity comparable to that induced by intradermal route of vaccination. Oral exposure of *M. avium intra-*

cellulare prior to oral or intradermal dose of BCG did not interfere with the protective immunity induced by BCG in guinea pigs challenged with *M. tuberculosis* H37Rv.—Authors' Abstract

Ottenhoff, T. H. M., Torres, P., Terencio de las Aguas, J., Fernandez, R., van Eden, W., de Vries, R. R. P. and Stanford, J. L. Evidence for an HLA-DR4-associated immune-response gene for *Mycobacterium tuberculosis*. Lancet **2** (1986) 310–312.

Antigens of *Mycobacterium tuberculosis, M. leprae, M. scrofulaceum,* and *M. vaccae* were injected intradermally in 86 caucasoid leprosy patients, and skin responses (measured in mm of induration at 72 hr) were analyzed in relation to HLA class II phenotypes. HLA-DR4 was associated with high responsiveness to antigens specific to *M. tuberculosis* but not to antigens shared with other mycobacteria ($p = 0.0005$). Because DR4 is associated with rheumatoid arthritis (RA) and because a role for *M. tuberculosis* antigens has been suggested both in experimentally induced autoimmune arthritis in rats and in RA, the DR4 associated regulation of the immune response to *M. tuberculosis* may be relevant to the pathogenesis of RA.—Authors' Summary

Patel, R., Kvach, J. T. and Mounts, P. Isolation and restriction endonuclease analysis of mycobacterial DNA. J. Gen. Microbiol. **132** (1986) 541–551.

A method for the isolation of DNA from mycobacteria propagated *in vitro* is described that utilizes organic solvents to extract lipoidal components from the outer membrane, and digestion with a protease (nagarse) and lysozyme to penetrate the cell wall. The mycobacterial cells were lysed by the addition of detergent, and the DNA was purified by digestion with pronase, sequential phenol and chloroform extractions, and digestion with RNase A. The isolated DNA, which was obtained in good yields, was of a relatively high M(r) and could be readily digested by restriction endonucleases. By this method, the genomes of *Mycobacterium avium, M. intracellulare, M. lepraemurium,* "*M. lufu,*" *M. marinum, M. phlei, M. scrofulaceum, M. smegmatis,* and *M. tuberculosis* were isolated and the restriction endonuclease digestion patterns analyzed. Each species could be distinguished by the digestion patterns, indicating that this approach can be used for identifying mycobacterial species. This approach is also sufficiently sensitive to differentiate strains since we were able to distinguish two independently isolated strains of *M. tuberculosis,* H37 and H4. In addition, no evidence was obtained for the presence of methylcytosine residues in the sequences 5′.CCGG.3′, 5′.CCCGGG.3′, 5′.CC(A/T)GG.3′ or for methyladenine at 5′.GATC.3′ in the DNA of the nine mycobacterial species examined using pairs of restriction enzymes that recognize and cleave at the same nucleotide sequence but differ in their sensitivity to 5-methylcytosine or ^{6}N-methyladenine.—(*From* Excerpta Medica)

Resnick, M., Bercovier, H., Aizer, F., Mor, N. and Levy, L. Death of *Mycobacterium lepraemurium* after multiplication in CBA, BALB/c and nude mice. Ann. Inst. Pasteur Microbiol. **138** (1987) 15–19.

The unexpected death of *Mycobacterium lepraemurium* in the course of systemic infection of mice, previously noted in the spleens of CBA mice, has been demonstrated in the spleens of BALB/c *nu/+* and BALB/c *nu/nu* mice and also in the livers and other organs of mice of all three strains. That the same phenomenon was observed in *nu/nu* mice indicates that the mechanism of bacterial death does not involve a T-lymphocyte-mediated cellular immune response on the part of the mice.—Authors' Summary

Srivastava, K. and Singh, N. B. Immunogenic behaviour of *Mycobacterium marinum* (SATO) in mice. Indian J. Med. Res. **84** (1986) 485–491.

Various species/strains of mycobacteria were screened with homologous soluble protein antigens for Listeria and Koch type responses. An atypical mycobacterium *M. marinum* (SATO) produced day 10 or Listeria-type response better than BCG (Phipps). In dose-dependent responses, this

strain showed superiority over BCG in all doses. Live as well as killed cultures of *M. marinum* revealed this response almost equally. This strain afforded protection in mice against virulent challenges with *M. tuberculosis* H37Rv, indicating its immunogenic characteristics.—Authors' Abstract

Vidhidharm, A., Matangkasombut, P. and Bovornkitti, S. T-cell subpopulations in tuberculosis and the effects of rifampicin. Asian Pac. J. Allergy Immunol. **3** (1985) 165–173.

T-cell subpopulations were evaluated by several recent methods in 38 tuberculosis patients (24 active and 10 quiescent cases of pulmonary tuberculosis; 2 of miliary and 2 of active extra-pulmonary tuberculosis) before and during rifampin (RMP) treatment. There was a significant reduction in the total number of T cells (E-RFC and OKT3+ cells) and of helper T cells (OKT4+) coinciding with an increase in the number of suppressor T cells when the 38 tuberculosis patients were compared with 21 healthy control subjects. When the changes of T-cell subpopulations in groups of subjects and patients with different clinical forms of the disease were analyzed, these changes could be clearly shown with both sets of assays (receptor assays and monoclonal antibody assays) among those with the active pulmonary form of tuberculosis, while similar changes could be demonstrated only by one or the other assay among those with the other forms of the disease. The effects of 1 month of RMP treatment on these parameters were much more obvious among the clinically active patients than the quiescent patients, i.e., a recovery of total T cells from a low pre-treatment to a near normal level accompanied by a significant reduction in the number of suppressor cells (OKT8+). In fact, among quiescent patients the number of suppressor cells (as TG) appeared to rise further with RMP treatment.—(*From* Excerpta Medica)

Wiker, H. G., Harboe, M., Nagai, S., Patarroyo, M. E., Ramirez, C. and Cruz, N. MPB59, a widely cross-reacting protein of *Mycobacterium bovis* BCG. Int. Arch. Allergy Appl. Immunol. **81** (1986) 307–314.

The MPB59 protein of *Mycobacterium bovis* BCG was purified to homogeneity from culture fluid of BCG substrain Tokyo, and characterized by biochemical and immunological techniques. The molecular weight was 28,000, determined by SDS-polyacrylamide gel electrophoresis, and the pI value was 5.3. The N-terminal amino-acid sequence was determined for 32 steps, and showed no significant homology with MPB64, MPB70, or MPB80. By crossed immunoelectrophoresis, MPB59 was found to belong to the BCG antigen 85 complex and identified as corresponding to the 85B component of this complex. The protein crossreacted extensively with other species of mycobacteria, and induced a marked humoral immune response in armadillos and monkeys during development of systemic mycobacterial infection after inoculation with *M. leprae.*—(AS/C. A. Brown, Trop. Dis. Bull)

Yoneda, T., Mikami, R., Sakaguchi, Y. and Shirai, F. The relationship between natural killer cell activity and delayed-type hypersensitivity reaction to 2,4-dinitrochlorbenzene in the spectrum of chronic, intractable pulmonary tuberculosis. Tubercle **64** (1987) 59–64.

We examined natural killer (NK) cell activity and delayed-type hypersensitivity reaction to 2,4-dinitrochlorbenzene (DNCB) in the spectrum of chronic, intractable pulmonary tuberculosis. A high-reactive group as defined by high NK cell activity and positive reaction to DNCB were characterized by stable clinical courses assessed by radiographical and clinical changes. A low-reactive group defined by nonaugmented NK cell activity and negative reaction to DNCB were characterized by progressive clinical courses. Although far advanced radiographic lesions were observed more frequently in the low-reactive group and moderately advanced lesions more frequently in the high-reactive group, there was not a significant statistical trend.—Authors' Summary