CURRENT LITERATURE

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

General and Historical

Pust, R. E. and Campos-Outcalt, D. Leprosy in the United States; risks, recognition, regimens, resources. Postgrad. Med. **5** (1985) 151–159.

Although leprosy is increasing in incidence in the United States, it is confined almost entirely to immigrants from developing countries and their close contacts. While the clinical disease has not changed, leprosy has diffused more widely throughout the United States as a result of migration. Primary care physicians should maintain a high index of suspicion in foreign-born individuals with skin or peripheral nerve problems.

Punch biopsy of skin lesions is the most practical diagnostic method for both the multibacillary and paucibacillary types of leprosy. Because of resistance to dapsone, multidrug treatment is now the rule; most patients are referred to or managed in consultation with a regional Hansen's disease clinic for long-term treatment. Consultation is available to any physician through the National Hansen's Disease Center in Carville, Louisiana, U.S.A.—(*From* the Summary)

Serrano-Espinosa, A. La lepra en Mexico. Revision bibliografica de 1941 a 1981. [Leprosy in Mexico. Revised bibliographic review from 1941 to 1981.] Dermatol. Rev. Mex. **28** (1984) 131–138. (in Spanish)

This paper deals with a bibliographic, critical review of 401 references on leprosy made by Mexican dermatoleprologists from 1941 to 1981. The preceding works on this subject of Gonzalez-Ureña, García de Leon, and Yolanda Ortiz are mentioned. Among Mexican authors who have more publications are: Saúl, Latapí, Rodriguez, Barba Rubio, and Novales. The preferred subjects of Mexican publications are on clinical, social, and general aspects of leprosy. The interest of Mexican dermatologists in training both medical staff and the general population about leprosy is stressed.—Author's English Summary

Siqueria, L. F. de G., Almeida, R. G. de and Belda, W. Métodos tintoriais utilizados na identificação do *Mycobacterium leprae*. Revisão histórica. [Staining methods used in the identification of *Mycobacterium leprae*. Historical review.] Rev. Saude Publica **18** (1984) 246–258. (in Portuguese)

A historical review of the staining methods utilized in the bacilloscopic identification of *Mycobacterium leprae* was made. Besides the description of each method and its variants, an extensive bibliographical review is given.—Authors' English Abstract

Stewart-Tull, D. E. S. Leprosy—in pursuit of a vaccine. Vaccine **2** (1984) 238–248.

Clinical leprosy is characterized by varying manifestations between tuberculoid and lepromatous leprosy. In the former state the patient is able to elicit a cell-mediated immune response whereas in the latter, there is usually a humoral response. An understanding of this immunological balance is crucial in the search for a vaccine which will control the disease. The development of a possible anti-*Mycobacterium leprae* vaccine has been advanced by the isolation of organisms from the infected tissues of the nine-banded armadillo, *Dasypus novemcinctus*; 125,000 doses can be prepared from one animal. In addition, recent studies on the biochemical activities of these organisms may provide the knowledge required to allow cultivation on a laboratory medium. Eventually it may be possible to pro-

duce a combined leprosy/tuberculosis vaccine for use in those parts of the world where both diseases are prevalent.—Author's Abstract

Woodward, W. Leprosy in New Zealand. NZ Nurs. J. **77** (1984) 8–9.

The responsibility for control of leprosy in New Zealand lies with the Department of Health. Leprosy is a notifiable disease, and on notification the close family members are listed and followed up. Any suspicious skin patches will be seen by the Medical Officer of Health. Regional leprologists (doctors who specialize in the study of leprosy) are available for consultation. Records are maintained in district health offices and are treated as confidential. Patients may be hospitalized until they are noninfectious (this may be after 2–3 weeks of multidrug therapy) and thereafter a medical examination and report will be filed annually.

There are more than 100 people in New Zealand currently receiving treatment for leprosy. The essential factors for limiting the incidence of this disease are careful following of all family contacts when a new case is notified, and thorough examination of these contacts at regular intervals so that new cases can be detected early and multidrug therapy initiated.—(*From* the Article)

Chemotherapy

Alvarenga, A., Leguizamón, O., Frutos, V. and von Ballestrem, W. Tratamiento combinado con rifampicina e Isoprodian en lepra, en Paraguay. [Combination treatment with rifampin and Isoprodian in leprosy in Paraguay.] Fontilles **15** (1985) 11–24. (in Spanish)

In the Paraguayan leprosy program a combined chemotherapy is being used, consisting of rifampin (RMP) plus a fixed combination of isoniazid 175 mg, prothionamide 175 mg, and dapsone 50 mg (Isoprodian, IPD). Medical specialists, a biochemist, and trained nurses are responsible for the supervision of patients. A program was initiated in 1974 with 21 lepromatous (L) cases. This was continued for a period of about 4 years and showed good acceptability, tolerance, and a high therapeutic effectiveness of RMP and IPD. In September 1979, the program was extended to include more patients with all forms of the disease. The Madrid classification was used because of its simplicity and reliability. Clinical, bacteriological (BI), and histopathological monitoring of patients was carried out at regular intervals. On 30 September 1983, we had 789 patients under RMP and IPD treatment.

During 4 years of observation, 25 cases abandoned the program for various reasons. Five patients died for reasons unrelated to the specific antileprosy therapy. The remaining 754 cases have the following classifications: L and B = 553 or 73.3%, T and I = 201 or 26.7%. Our evaluation revealed that 59.4% of patients were under post-chemotherapy observation and that 40.6% still took RMP and IPD. Some 24 L cases are 3–4 years and another 102 patients between 2–3 years on post-treatment observation. None of these so far has developed signs of relapse, either bacteriological positivity or clinical signs.

Side effects were observed in 14% of all cases, most of these (14%) had gastrointestinal complaints which were more frequent at the beginning of the treatment and generally could be normalized when the medication was taken after the principal meals and never on an empty stomach. Some 33% of the L and B forms had ENL-type reactions, of which 94% were of a passing nature and could be controlled with thalidomide. Hepatitis with jaundice was observed in 16 cases (2%). All of these improved completely when specific therapy was interrupted. In those cases where treatment had to be reinstated (high BI), the multidrug therapy was well tolerated without inconvenience until completion. In a single case, medication with RMP and IPD had to be suspended because of elevated enzymatic test values (GOT and GPT).

Clinical-bacteriological improvement was

particularly fast during the first 6 months of treatment. There was a 68% reduction, on the average, of the BI during this period. Between the subsequent 12–48 months, the decrease of the BI was slower (16%). A comparison between two groups of patients, one pretreated with dapsone monotherapy and the other without pretreatment, showed a somewhat slower BI reduction in the previously untreated group than in the pretreated patients. Following the first 12 months of multidrug therapy, the initially observed difference of BI reduction in these two groups disappeared and was very similar.—(*Adapted from* Authors' English Summary)

Arora, S. K., Singh, G. and Sen, P. S. Levamisole in cases of ENL. Indian J. Lepr. **57** (1985) 17–21.

Levamisole, the anthelmintic drug, was tried in erythema nodosum leprosum (ENL) cases using a double-blind control trial in a dosage of 150 mg/day on 3 consecutive days for 3 months. It was found that levamisole was not effective in ENL cases. No severe side effect was seen in these cases.—Authors' Abstract

Askew, A. D. Managerial implications of multidrug therapy. (Editorial) Lepr. Rev. **56** (1985) 89–97.

The managerial effects of multidrug therapy (MDT) are discussed, using 3 possible models of leprosy control programs, and tentative conclusions recorded. The 3 models may be modified according to local circumstances and local experience in particular control programs.—Author's Summary

Girdhar, B. K., Girdhar, A., Ramu, G. and Desikan, K. V. Short course treatment of tuberculoid cases—a feasibility study. Lepr. India **55** (1983) 719–724.

This important study, made by experienced leprosy workers from the Central JALMA Institute for Leprosy in India, was designed to investigate the value (if any) of additional periods of antileprosy drug treatment in nonlepromatous patients, after the point of inactivity. From an initial group of 100 patients 74 attended and took more than 75% of the prescribed dose of dapsone. Of these, treatment was given to 47 up to the point of inactivity and then stopped. In the remaining 27, an additional 18 months' maintenance therapy was given after this point. The patients were followed for 3 to 42 months in the group that received additional chemotherapy (control group), and from 5 to 47 months in the group in which chemotherapy was stopped at the point of inactivity (trial group). No significant difference in the relapse rate was noted. The authors conclude that a total period of 15 months may be adequate in paucibacillary cases. The efficacy of a shorter period of treatment is being studied.—A. C. McDougall (*From* Trop. Dis. Bull.)

Gupta, C. M., Bhate, R. D. and Singh, I. P. The dapsone syndrome. A case report. Indian J. Lepr. **57** (1985) 193–195.

The dapsone syndrome is a rare hypersensitivity reaction to dapsone. It is characterized by clinical features and laboratory abnormalities comprising high fever, generalized maculo-papular rash (Morbelliform), hepatitis, lymphadenopathy, hematological and biochemical abnormalities. A case of borderline tuberculoid (BT) leprosy who developed this syndrome following 4 weeks of dapsone therapy is reported and the subject is reviewed.—Authors' Abstract

Ji, B., *et al.* [Observation on bactericidal activity of several drugs against *M. leprae* by proportional bactericidal test.] Chin. J. Dermatol. **18** (1985) 28–31. (in Chinese)

The proportional bactericidal test (PBT) can be used to assess and compare the bactericidal activity of drugs against *Mycobacterium leprae* through the estimation of the most probable number (MPN) of viable bacilli and the number of acid-fast bacilli necessary to infect 50% of the mice, i.e., IDso. The bactericidal activity of two new rifamycin SV derivatives, i.e., isobutylpiperazinylrifamycin SV (R-76-1) and methyloxime of 3-formylrifamycin SV (AF-MO), was significantly superior to that of rifampin and dapsone by the PBT.—Authors' English Abstract

Kar, H. K., Balakrishnan, S., Kumar, G. V., Sirumban, P. and Roy, R. G. Hepatitis and multidrug therapy in leprosy with special reference to prothionamide. Indian J. Lepr. **57** (1985) 78–89.

Hepatotoxicity in 2 drug regimens was studied at the Central Leprosy Teaching and Research Institute, Chengalpattu (Tamil Nadu, India) during 1983–1984. In the "P" regimen, prothionamide 350 mg daily, dapsone 100 mg daily, and rifampin 600 mg at monthly intervals were given. In the "C" regimen, dapsone 100 mg daily, rifampin 600 mg once a month, and clofazimine 300 mg once a month and 100 mg on alternate days were given. The trial was started with 50 adult multibacillary leprosy patients in each group. Enzymatic hepatic dysfunction was noted in 52.58% of the cases even before the therapy was started.

In the "P" regimen, 4 cases of clinical jaundice and 6 cases of high bilirubinemia were noticed as against 2 cases each of clinical jaundice and high bilirubinemia in the "C" regimen. Of the 2 cases of clinical jaundice in the "C" regimen, 1 turned out to be a case of HBV infection. The study, which is in progress, indicated higher hepatotoxicity in the "P" regimen which is probably explained by the simultaneous use of 2 hepatotoxic drugs. Viral hepatitis is endemic in this area and might have aggravated the hepatotoxicity observed.—Authors' Abstract

Khalil, S. A. H., El-Khordagui, L. K. and El-Gholmy, Z. A. Effect of antacids on oral absorption of rifampicin. Int. J. Pharm. **20** (1984) 99–106.

A study has been carried out on the effect of concomitant administration of aluminium hydroxide gel (15 ml), magnesium trisilicate (2 g), and sodium bicarbonate (2 g) on the bioavailability of rifampin in healthy male volunteers. The antibiotic bioavailability, as measured by a urinary excretion method over a period of 24 hr, was significantly reduced by the 3 antacids following the sequence: magnesium trisilicate > aluminum hydroxide > sodium bicarbonate. Doubling the dose of either magnesium trisilicate or aluminum hydroxide gel produced no further reduction. The results obtained are interpreted in the light of the effect of gastric pH elevation on the solubility and dissolution rate of rifampin, chelation of the drug with aluminium ions and binding by magnesium trisilicate.—Authors' Summary

Kundu, S. K., Hazra, S. K., Chaudhury, S. K. and Chatterjee, B. Prothionamide and dapsone therapy in leprosy—a clinical study. Indian J. Lepr. **57** (1985) 90–96.

Combined therapy with prothionamide and dapsone was instituted in 15 active untreated lepromatous leprosy cases for a period of 18 months. Clinical improvement was good with attainment of zero morphological index in about 66% of the cases. Bacteriological improvement was rather unsatisfactory as 1 case only reached zero level. Side effects were observed in a few cases necessitating withdrawal of combined therapy and patients' prothionamide compliance was rather unimpressive.—Authors' Abstract

Lahuerta-Palacios, J. J., Gomez-Pedraja, J. F., Montalban, M. A., Shandas, G. J. and Gomez-Salazar, M. D. Proliferation of IgDx plasma cells after agranulocytosis induced by dapsone. Br. Med. J. **290** (1985) 282–283.

Although an excess of lymphoid, plasma, and stromal cells is a known characteristic of bone marrow in idiosyncratic neutropenia induced by drugs, proliferation of monoclonal plasma cells in this disorder has not previously been reported. We report on a patient in whom dapsone caused a reaction with these characteristics and the M component was IgDx.—(*From* the Article)

Levy, L. Chemotherapy of leprosy—a tool for leprosy control. (The Kellersberger Memorial Lecture, 1984.) Ethiop. Med. J. **23** (1985) 31–42.

The chemotherapy of leprosy may be seen to have two purposes: a) management of the individual patient and b) the control of leprosy. It is certainly important to treat the individual leprosy patient, arresting the progress of his disease process, limiting the severity of his deformities, and rendering

him a functioning member of society. But a more important purpose of chemotherapy may be to arrest the transmission of *Mycobacterium leprae* in the community. In leprosy-endemic developing countries, it may be necessary to make difficult choices. One may be required, by the lack of adequate resources, to choose between treating adequately a relatively small number of patients on the one hand and, on the other hand, deployment of the available drugs so as to have a maximal impact on the transmission of *M. leprae* within the community. The evidence that chemotherapy may both effect a cure of the patient and be employed to control leprosy was reviewed. The differences that characterize the chemotherapies used for these two purposes were examined and, finally, the possibility that one chemotherapy may accomplish both objectives was considered.—(*From* the Article)

Li, F. T. Experiences with phenazine dye, B628, in the treatment of leprosy in China. (Letter) Lepr. Rev. **55** (1984) 315–316.

The correspondent from the Shanghai Zun Yi Hospital, Shanghai, China, briefly reports the clinical use of B628, an analogue of clofazimine (B663), in 3 patients with lepromatous leprosy in southwest China and refers to a further 6 patients treated in Shanghai. B628 has previously been shown to be active against *Mycobacterium leprae* and *M. lepraemurium* in animal models. Treatment in the patients was given daily as monotherapy and resulted in "dramatic clinical improvement," but had to be replaced by dapsone therapy after 5 months because of the limited drug supply. Pigmentation in the patients treated with B628 was less serious than has been reported for B663. [B628 is 3-(*p*-chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-iminophenazine.]—C. A. Brown (Trop. Dis. Bull.)

Mathur, A., Venkatsan, K., Bharadwaj, V. P. and Ramu, G. Evaluation of effectiveness of clofazimine therapy. I. Monitoring of absorption of clofazimine from gastrointestinal tract. Indian J. Lepr. **57** (1985) 146–148.

The quantity of clofazimine absorbed from the gastrointestinal tract when administered to lepromatous leprosy patients at varying single doses of 600 mg, 400 mg, 300 mg, and 100 mg has been worked out by determining the amount of clofazimine present in total fecal excreta. Except in 100 mg doses where the percentage absorption was 62.5 ± 17, in all other cases the values were around 45%. The efficacy of daily administration of 100 mg clofazimine is discussed.—Authors' Abstract

Mester de Parajd, L., Rees, R. J. W., Ambrose, E. J. and Mester de Parajd, M. A series of new antileprosy compounds derived from serotonin. Acta Leprol. (Genève) **3** (1985) 21–28. (in English)

La desoxyfructo-sérotonine (DFS) inhibe l'incorporation du ^{3}H-DOPA par *M. leprae in vitro.* Par contre elle est inactive contre *M. tuberculosis* et *M. microti,* ce qui montre que l'activité antihansénienne de la DFS se manifeste au niveau de la DOPA-oxydase spécifique de *M. leprae.*

La dose active minimale de la DFS, établie par la technique conventionnelle sur les pattes de souris, est de 2 mg/kg, aussi bien pour les souches résistantes que les souches sensibles au dapsone.

Toujours sur les souris, l'activité de la DFS semble être plutôt bactériostatique que bactéricide. Mais tenant compte de récentes observations concernant le niveau de DFS dans le sang, il nous semble nécessaire de revoir cette question dans des conditions où un niveau constant de DFS est assuré dans le plasma. En effet, l'administration d'une unique dose de DFS n'augmente que pendant deux ou trois heures le niveau de DFS. C'est le cas notamment dans les expériences sur les pattes de souris. Déjà au cours des expériences cliniques à Bamako, où la dose quotidienne de DFS a été prise en deux portions étalées dans la journée, assurant un niveau élevé de DFS dans le sang pendant 4 à 6 heures, l'effet de la DFS semblait être plutôt bactéricide.

Afin d'assurer un niveau constant de DFS dans le sang, trois méthodes ont été examinées: 1) La DFS étant un métabolite humain, il existe peut-être des précurseurs actifs de la DFS, qui permettraient une prolongation de la durée du niveau élevé de DFS dans le sang. En effet, le desoxyfructo-5-hydroxytryptophane (DF-5HTP) et la de-

soxyfructo-5-methoxytryptamine (DF-5MT) montrent *in vitro* une activité semblable à celle de la DFS. 2) La préparation de dérivés lipophiliques de la DFS, soit par synthèse chimique, soit par complexion avec des substances lipidiques, pourrait donner également une solution à ce problème. En effet, ces dérivés lipophiliques montrent une pénétration plus lente et un niveau sanguin plus stable que la DFS, mais leur activité n'est pas toujours satisfaisante. 3) Finalement, la combinaison de l'administration orale de la DFS et d'une diète antilèpre (NAL), qui favorise la formation *in vivo* de la DFS, serait probablement la meilleure solution.—Authors' French Summary

Mittal, A., Seshadri, P. S., Conalty, M. L., O'Sullivan, J. F. and Nath, I. Rapid, radiometric *in vitro* assay for the evaluation of the anti-leprosy activity of clofazimine and its analogues. Lepr. Rev. **56** (1985) 99–108.

The effect of clofazimine and 6 analogues (B 3691, B 3713, B 3640, B 3648, B 720 and B 749) on the viability of *Mycobacterium leprae* was tested in a rapid, radiometric *in vitro* assay. Thirteen human and 1 armadillo-derived freshly extracted *M. leprae,* maintained in peritoneal macrophages, incorporated ^{3}H-thymidine to significant levels as compared to parallel cultures with heat-killed bacilli. Exposure of such cultures to clofazimine for 48 hr showed significant inhibition of the radiolabel uptake without any adverse effects on the host macrophages. A sharp linear increase in inhibition was observable at concentrations from 1 to 10 ng/ml, with a plateau up to 40 ng/ml. Further increases of drug concentration up to 100 ng/ml showed marginal increase in the percentage inhibition of ^{3}H-thymidine incorporation. The analogues tested showed levels of inhibition similar to that of clofazimine when left for 72 hr and 15 days in *M. leprae* macrophage cultures. However, they were less effective than clofazimine when tested for the shorter duration of 48 hr at the lower concentration of 5 ng/ml.—Authors' Summary

Nair, I. and Mahadevan, P. R. An *in vitro* test using cholesterol metabolism of macrophages to determine drug sensitivity and resistance of *Mycobacterium leprae.* J. Biosci. **6** (1984) 221–231.

Macrophages that have ingested live *Mycobacterium leprae* show a preferential accumulation of cholesterol ester. Such an accumulation is not seen on the ingestion of dead bacteria. Among the macrophages that ingest live *M. leprae,* the presence of dapsone or rifampin prevents largely the alteration in the anticipated increase in the cholesterol ester, indicating the sensitivity of the bacteria to the drug. In the small number of relapsed patients, the presence of dapsone did not reduce the cholesterol ester increase, suggesting that the *M. leprae* present are either resistant or escaped detection. The method provides a rapid drug-screening system for anti-*M. leprae* activity of known and unknown compounds.—Authors' Abstract

Nilakanta Rao, M. S. and Yellapurkar, M. V. Multidrug therapy for multibacillary cases in Wardha District, Maharashtra, India. Indian J. Lepr. **57** (1985) 159–163.

A total of 2786 infectious patients of Wardha District are now receiving multidrug therapy; 67% of those who have received 2 years' treatment have become bacteriologically negative. Side effects due to the drugs have been comparatively few. In the majority of patients who had such side effects, they have been mild.—Authors' Abstract

Pandian, T. D., Sithambaram, M., Bharathi, R. and Ramu, G. A study of relapse in nonlepromatous and intermediate groups of leprosy. Indian J. Lepr. **57** (1985) 149–158.

Dapsone has been used as a monotherapy, and in well-organized control units the prevalence of leprosy has come down. The relapse rates presented in this communication are quite low compared with those reported by various authors quoted in this paper. Relapse rates appear to remain steady at about 5/1000 for each year following release from control (RFC) for 7 years. This relapse rate does not appear to be related to regularity of treatment. From the relapse rates, it appears that the longer the duration of treatment, the earlier the relapse due to

the severity of the disease of those who had longer treatment. Health education for RFC cases on signs of relapse is a must before they are declared RFC. The levels of sulfone in the blood remain above the minimum inhibitory concentration (MIC) for as much as 10 days after the last dose and, therefore, dapsone allows self-administration. It is expected that with the introduction of bacteriocidal drugs in the treatment of paucibacillary leprosy, the leprosy rates will go down. As observed from a study conducted in Jalma, maintenance treatment as advocated by the NLCP (1964) and WHO (1970) does not seem to be necessary. The necessity of such maintenance treatment may be obviated with the use of multidrug regimens in paucibacillary leprosy. This would enable a large number of cases to be released from control, thereby reducing patient load considerably and making the supervised treatment of multibacillary cases more easy.—Authors' Abstract

Puavilai, S., Chutha, S., Polnikorn, N., Timpatanapong, P., Tasanapradit, P., Charuwichitratana, S., Boonthanom, A. and Wongwaisayawan, H. Incidence of anemia in leprosy patients tested with dapsone. J. Med. Assoc. Thailand **67** (1984) 404–407.

Fifty-seven patients with different types of leprosy treated with dapsone 50–100 mg per day were evaluated in regard to the frequency, onset and etiology of anemia. Fifty-one patients had normal G-6-PD; 6 patients had complete deficiency of G-6-PD. Significant anemia developed in 40 cases out of 51 patients with normal G-6-PD ($p < 0.05$). The onset of significant anemia in this group was 1 month after dapsone administration and continued throughout the course of treatment for 1 year. The methemoglobin level rose significantly ($p < 0.01$) in correlation with the dose of dapsone administered. Seventeen patients had hemolysis, 13 had Heinz bodies, and 1 had positive direct Coombs' test. Hemolytic anemia developed in 5 out of 6 patients with complete deficiency of G-6-PD, but not severe enough to necessitate discontinuation of treatment.—Authors' Summary

Schröder, J. M. and Matthiesen, T. Experimental thalidomide neuropathy: the morphological correlate of reduced conduction velocity. Acta Neuropathol. (Berl.) **65** (1985) 285–292.

Morphological studies of experimental thalidomide neuropathy have thus far failed to show any significant structural changes. The present investigation was performed on sural nerves of female New Zealand white rabbits showing a reduction of sensory conduction velocity after oral treatment with thalidomide (100 mg/kg b.wt. per day) for a period of 33 weeks. Rabbits of the same strain and equal sex, weight, and number served as controls. Very few nerve fibers were undergoing Wallerian degeneration in both groups, experimental animals and controls. Morphometry, however, revealed a statistically significant reduction of the mean myelin thickness of sural nerve fibers in the thalidomide group of rabbits as compared to controls. The mean myelin thickness of the largest nerve fibers was also significantly smaller than in the control group. On the other hand, axonal diameters were not significantly altered. The association between the decrease of the sensory conduction velocity, the reduction of the myelin sheath thickness, and the chronic thalidomide application is discussed.—Authors' Summary

Sharma, L., Thalliath, G. H., Girgia, H. S. and Sen, P. C. A comparative evaluation of levamisole in leprosy. Indian J. Lepr. **57** (1985) 11–16.

Ninety adult leprosy patients attending the skin outpatient department of Institute Hospital of Varanasi, India, were selected for the study. A group of 30 patients, including 10 cases of lepromatous, 10 of borderline, and 10 cases in reaction of which 5 were of type 1 and 5 were of type 2 reaction, were treated with levamisole and dapsone (DDS). Levamisole was given in the dose of 150 mg daily for 3 consecutive days which was repeated after every 12 days. The effects were compared with 2 similar groups receiving clofazimine plus DDS and DDS alone for 6 months. It was observed that levamisole was useful in bringing down both types of reactions in a shorter period than that required by clofazimine. Clinical improvement in nonreaction cases was found to be similar in all 3 groups. Minor side effects were also seen with levamisole and

clofazimine in some cases.—Authors' Abstract

Zhou, Y. Application of a simple method for the determination of 4,4′-diamino-diphenyl-sulfone (DDS) in urine. Chin. J. Clin. Dermatol. **14** (1985) 130–132. (in Chinese)

The paper reports a simple method for the determination of 4,4′-diamino-diphenyl-sulfone (DDS) in urine, a modification of (Bratton-Marshall's) paper spot test. In the method α-naphthylamine is used as a substitute for N-1-naphthyl-ethylene-diamine dihydrochloride, and plastic plates (or test tubes) for filter paper. The method has no false-negative reaction for Bratton-Marshall's method, and no false-positive reaction for p-dimethyl-amino-benzaldehyde reaction. The method is sensitive, reliable, simple and convenient, and does not require special instruments and reagents.—Author's English Abstract

Clinical Sciences

Agrawal, B. R. and Agrawal, R. I. Arteriography in leprosy. Indian J. Lepr. **57** (1985) 138–145.

The arterial patterns of hands and feet-vessels were studied in 26 patients of leprosy by percutaneous arteriography, and in these cases muscle biopsy was also done. The vessels in leprosy were showing smooth tapering, absence of collaterals, occlusion of the artery and rippling (tortuosity) of vessels. Such abnormal pictures were seen in all of the arteriograms studied, and in 82% of the cases moderate-to-severe vascular changes were seen. Decreased blood flow through the arteries in the distal part of the limb leading to ischemia is probably the cause for deformity and/or ulceration in leprosy. The presence of vascular thickening, perivascular granuloma and lymphocytic infiltration in the muscle may be possible explanations for the angiographic findings and are felt to be related to trophic ulceration.—Authors' Abstract

Arora, S. K., Singh, G. and Sen, P. C. Immunostimulatory effect of levamisole in borderline leprosy cases. Indian J. Lepr. **57** (1985) 22–26.

Eleven cases of borderline-borderline leprosy were subjected to levamisole therapy for 3 months. Levamisole was given 150 mg/day on 3 consecutive days every fortnight along with dapsone (DDS). Immunostimulation was assessed by the lepromin test using lepromin A supplied by WHO containing 4×10^2 bacilli/ml. It was found that there was statistically significant change in the lepromin reaction after levamisole.—Authors' Abstract

Bulakh, P. M., Ranade, S. M., Kowale, C. N., Chandorkar, A. G. and Burte, N. P. LDH isoenzymes subunit ratio in leprosy and the effect of clofazimine treatment on LDH isoenzymes subunit. Indian J. Lepr. **57** (1985) 115–123.

The clinical material for our studies of serum total LDH activity and LDH isoenzymes in leprosy included 255 patients consisting of tuberculoid (74), lepromatous leprosy (116), and lepromatous leprosy with lepra reaction (65); 20 patients with suspected dapsone (DDS) resistance and repeated attacks of lepra reactions were selected for clofazimine studies. All of the leprosy patients exhibited higher total LDH activity as compared to normals. The M/H ratio was significantly increased in patients with lepromatous leprosy and correlated closely with the clinical severity and advancement of the disease. Tuberculoid leprosy patients showed values close to normals. Hence M/H ratios could demarcate the 2 polar types of leprosy, i.e., tuberculoid and lepromatous leprosy.

Clofazimine treatment over a period of 1 year in patients with suspected DDS resistance and repeated attacks of lepra reaction decreased total LDH activity and the M/H ratio considerably. The fall in the M/H ratio during clofazimine treatment could be attributed to the clearance of "M" subunits

by the drug due to removal of blockade of the RES system produced by lepra bacilli.—Authors' Abstract

Faulstich, M. E. Behavioral analysis of sexual dysfunction in Hansen's disease. Percept. Motor Skill **60** (1985) 115–118.

Human sexual behavior is an interactive process including CNS, hormonal, and sex-gland activities. This process can be disrupted in males who have Hansen's disease if testicular atrophy occurs. Elevations of centrally mediated luteinizing hormone and deficient testosterone levels were found in a male with Hansen's disease whose insufficient erections were secondary to atrophic testes. Quasi-experimental (A-B) analysis provided evidence for the efficacy of testosterone treatment for such a condition.—Author's Summary

ffytche, T. J. and McDougall, A. C. Leprosy and the eye: A review. J. R. Soc. Med. **78** (1985) 397–400.

The eye may be involved in leprosy in a number of ways and these are dependent on the immune status of the patient. Direct invasion of the globe by *Mycobacterium leprae* occurs in the lepromatous form of the disease; the route of entry is believed to be chiefly bloodborne, although some local spread from adjacent structures may occur. Acute inflammatory reactions affecting the facial nerve, cornea, and iris are a feature of type 1 (reversal) and type 2 (erythema nodosum leprosum) reactions and may cause severe damage to the eye, either directly or indirectly. Ocular complications may also occur indirectly through impairment of lid closure (VII nerve) and corneal anesthesia (V nerve), and the combination of these two conditions is a potent cause of blindness in all forms of the disease. The eye may also be involved through damage to adnexal tissues, and secondary infection is always a risk in a chronically affected eye.

It is not surprising that with so many mechanisms of ocular involvement the clinical manifestations of ocular leprosy are diverse, and all forms of the disease may cause complications. Some, however, are more likely than others to give rise to serious visual symptoms, and a useful concept is the identification of "potentially sight-threatening" (PST) lesions as distinct from those considered only as "academic." The important PST lesions include lagophthalmos, corneal anesthesia, exposure keratitis, scleritis, staphyloma, acute and chronic iridocyclitis, low intraocular pressure, and cataract.—(*From* the Article)

Her Hsin Tsai and Suryawanshi, N. Ocular complications in patients with leprosy in Karigiri, South India. Lepr. Rev. **56** (1985) 135–141.

The examination of 143 leprosy patients revealed that 91 had ocular lesions attributable to leprosy and of these 13 were blind. The commonest lesion was madarosis and the commonest cause of blindness was chronic iritis (31% of all blind patients). Lagophthalmos remains an important condition and was found in 25% of patients, while corneal lesions account for a large number of ocular problems in leprosy (exposure keratitis 31%, interstitial keratitis 8%). Most affected is the 40–60 year age group, and most of the affected patients had long-term disease. Lepromatous patients were also encountered more frequently (70%) among the involved patients. A large number of patients also suffered from gross deformities (53%) and even more so among the blind (77%). This study does point out the importance of careful and regular examination of the eyes of leprosy patients by all involved with their care.—Authors' Summary

Kiran, K. U., Stanley, J. N. A. and Pearson, J. M. H. The outpatient treatment of nerve damage in patients with borderline leprosy using a semi-standardized steroid regimen. Lepr. Rev. **56** (1985) 127–134.

Thirty-three patients with borderline leprosy who had developed recent (less than 6 months' duration) loss of nerve function were treated with a semi-standardized course of corticosteroids, the average initial dose was 25 mg prednisolone daily, and the average duration was 5 months. Treatment was unsupervised and no patient was ad-

mitted to the hospital. The results were assessed by tests of voluntary muscle power and of sensory function; of the 57 nerves studied, 38 showed marked improvement and none got worse. There were no serious side effects. Patients were taught exercises to prevent deformity, and residual muscle weakness did not progress to contractures. Corticosteroid treatment is safe enough and confers sufficient benefit to be used in standard dosage under field conditions.—Authors' Summary

Lamba, P. A. and Kumar, D. S. Ocular involvement from leprosy. Indian J. Ophthalmol. **32** (1985) 61–63.

The proposed classification of ocular disabilities in leprosy gives a better understanding of the progress, the prognosis and also signifies when the preventive measures are likely to be successful. Only up to Grade-III the progress of the condition could be arrested or prevented. The potentially sight-threatening lesions (STL) have been identified and classified according to their severity as well as chronological order of appearance.—Authors' Summary

Ohkawa, S., Ozaki, M. and Izumi, S. Lepromatous leprosy complicated with systemic lupus erythematosus. Dermatologia **170** (1985) 80–83.

This paper presents a case of lepromatous leprosy complicated with systemic lupus erythematosus (SLE) and diagnosed as having typical SLE from clinical features and results of laboratory tests. We discuss the appearance of autoantibodies in SLE as well as in leprosy.—Authors' Abstract

Raj, D. V., Ingty, K. and Devanandan, M. S. Weight appreciation in the hand in normal subjects and in patients with leprous neuropathy. Brain **108** (1985) 95–102.

The Weber fraction was used as an index of the sensitivity with which subjects appreciated weights ranging from 20 to 500 g suspended from the middle finger. Normal subjects were able to appreciate weight when it caused cutaneous compression alone. The sensitivity increased when subjects lifted weights by flexing the metacarpophalangeal joint. This increase was more marked for weights ranging from 20 to 100 g than for weights ranging from 200 to 500 g. When subjects lifted weights by flexing the elbow joint, the sensitivity with which they appreciated weight was comparable to that from cutaneous compression alone. Leprous subjects having glove anesthesia were unable to appreciate any weight up to 500 g when it caused cutaneous compression alone. However, they were able to appreciate weights above 200 g when they lifted weights by flexing the metacarpophalangeal or elbow joints.—Authors' Summary

Robertson, I., Weiner, J. M. and Finkelstein, E. Untreated Hansen's disease of the eye: A clinicopathological report. Aust. J. Ophthalmol. **12** (1984) 335–339.

A Maltese immigrant presented with intermittent bilateral anterior uveitis for which no cause could be found. The inflammation did not respond to topical treatment and ultimately the left eye developed a hypopyon and was enucleated. Histological examination revealed granulomatous inflammation and large numbers of *Mycobacterium leprae* throughout the anterior segment. Occasional foci of inflammation containing *M. leprae* were found in the vitreous extending to the retina at the posterior pole. These findings in the posterior segment have rarely been reported. Eye infection in Hansen's disease is frequent and delays in diagnosis are common.—Authors' Abstract

Ryzen, E. and Singer, F. R. Hypercalcemia in leprosy. Arch. Intern. Med. **145** (1985) 1305–1306.

We report a case of hypercalcemia in a patient with leprosy. Aminoterminal parathyroid hormone and 25-hydroxycholecalciferol concentrations were suppressed. Urinary hydroxyproline concentrations were elevated. There was no evidence of malig-

nancy. The hypercalcemia resolved with corticosteroid therapy.—Authors' Abstract

Saha, K., Rao, K. N., Sehgal, V. N., Gadi, S., Jain, V. K. and Chakrabarty, A. K. Radioimmunoassay of serum cortisol levels in leprosy patients with special reference to type I and type II reaction. Lepr. Rev. **56** (1985) 117–125.

In the present paper, an attempt has been made to assess the adrenal cortical functions in leprosy patients, especially in LL cases and also in patients with type I and type II lepra reaction, by estimating serum cortisol levels in them and comparing these results with those in healthy adults. Both the patient and control population belonged to a copper mining district in an eastern state of India.

Eighty leprosy patients including 23 LL, 17 BL, 3 BB, 7 BT, and 30 patients with type I (10 cases) and type II (20 cases) lepra reactions formed the basis of this study. Forty-one age-matched normal adults from similar socioeconomic status served as controls. The mean basal serum cortisol levels in 23 LL cases and 41 controls were 18.21 and 12.98 μg/dl, respectively. Diurnal variation of serum cortisol level was studied in 4 LL cases who showed normal circadian rhythm. Intramuscular injection of ACTH in another 4 patients (2 LL and 2 BL) showed an increase of mean serum cortisol level from 14.75 μg/dl to 21.75 μg/dl after 30 min of challenge. Most interestingly, it was found that at the onset of type II lepra reaction the mean serum cortisol level increased to 17.35 μg/dl and after remission of ENL its level decreased to 11.41 μg/dl. The difference was statistically significant ($p < 0.01$). On the contrary, no such variation was found in patients with type I reaction.—Authors' Summary

Saúl, A. Papel de los hospitales generales en el control de la lepra. [The role of general hospitals in leprosy control.] Dermatol. Rev. Mex. **28** (1984) 185–194. (in Spanish)

Los hospitales generales de las diversas instituciones de salud del país pueden tener un papel activo en la atención del enfermo de lepra. Son instituciones polivalentes muy completas y pueden brindar la atención integral del paciente dentro de las nuevamente actualizadas ideas de medicina familiar de primer nivel. A la vez que brindan atención al paciente externo lo pueden hospitalizar y resolver otros problemas que se la puedan presentar. Son instituciones acreditadas, conocidas y aceptadas por la población. Juegan un papel en la lucha contra el prejuicio porque permiten al paciente mezclarse con otros enfermos distintos y acostumbran al personal médico y paramédico a manejar a estos enfermos y a ver a la lepra como una enfermedad más en la patología de su país.—(*From* the Article)

Saúl, A. and Novales, J. ¿Existen los casos tuberculoides subpolares? [Do cases of subpolar tuberculoid leprosy exist?] Acta Leprol. (Genève) **3** (1985) 29–35. (in Spanish)

The idea of the existence of subpolar tuberculoid cases is supported on the spectral conception of Ridley and Jopling who included the subpolar lepromatous patients between LL and BL cases.

Similar to Languillon, we have studied 40 tuberculoid cases to find out the clinical, bacteriological, immunological, and histopathological parameters of subpolar T cases.

The subpolar T cases do exist; they are unstable cases which can slide through the immunological spectrum towards the L pole. These cases are characterized by numerous, symmetrical, dysesthetic tuberculoid lesions with an important neural involvement. Bacilli can be found in small numbers in the smears of nasal mucosae in some cases. The Mitsuda reaction is always positive, and histopathologically the granu-

lomas, lymphocytes, and epithelioid cells are in less number without the epidermotropism which is very often seen in polar cases.—(*From* Authors' English Summary)

Schirren, C. Das Gesicht des Kranken, Verlaufsbeobachtung bei einem Leprösen. [The face of the patient, follow up of a leprosy patient.] Z. Hautkr. **59** (1984) 1507–1512. (in German)

We report on a 20-year follow-up study dealing with a male patient suffering from leprosy. Observation of the facial features showed deterioration in his general condition; it was possible to fix the moment of the manifestation of the bronchial cancer the patient finally died from.—Author's English Summary

Thirugnanam, T. and Rajan, M. A. Borderline reactions treated with clofazimine and corticosteroids. Indian J. Lepr. **57** (1985) 164–171.

Thirty-four cases of borderline reaction were treated with clofazimine and corticosteroids. The clinical findings including sensory and motor assessments and skin smears were made before treatment and again when the reaction subsided, subject to a minimum period of 1 year. These findings are analyzed and discussed.—Authors' Abstract

Wright, S. Essential fatty acids in the plasma phospholipids of patients with leprosy. Br. J. Dermatol. **112** (1985) 673–677.

Plasma phospholipid essential fatty acids were investigated in 40 patients with leprosy and 40 controls. A significant reduction in linoleic acid was found in the leprosy patients, with an increase in its metabolite dihomo-γ-linolenic acid. No difference was found between patients with multibacillary and paucibacillary leprosy. Patients treated for less than 6 months were found to have low levels of linoleic acid and high levels of dihomo-γ-linolenic and arachidonic acid compared with patients treated for more than 6 months.—Author's Summary

Immuno-Pathology

Atlaw, T., Kozbor, D. and Roder, J. C. Human monoclonal antibodies against *Mycobacterium leprae.* Infect. Immun. **49** (1985) 104–110.

Human hybridomas were constructed which produce antibodies against 3 different extracts of *Mycobacterium leprae.* A thioguanine-resistant (Thg^r), ouabain-resistant (Oua^r), human lymphoblastoid cell line, KR-4, was hybridized with Epstein-Barr virus-transformed cell lines from lepromatous leprosy patients with fusion frequencies of $>10^{-5}$. Non-Epstein-Barr virus-transformed donor cells fused at much lower rates ($<2 \times 10^{-7}$). Hybrids were selected in medium containing hypoxanthine aminopterin thymidine and 10^{-5} M ouabain. An enzyme-linked immunosorbent assay was used to screen for antibodies against 3 crude extracts of armadillo-derived *M. leprae,* including a) a soluble sonic extract preparation, b) sodium dodecyl sulfate extract of insoluble sonicated *M. leprae,* and

c) a purified phenolic glycolipid antigen. Of a total of 2200 final clones screened, 359 were found to secrete antibody which bound to soluble sonic extracts and the sodium dodecyl sulfate extract (6.7 and 9.6%, respectively), whereas 12.5% (21 out of 168) showed positivity to the glycolipid antigen. Four selected hybridomas also reacted with the deacylated derivative of *M. leprae* phenolic-glycolipid antigen. The specificity of these monoclonal antibodies was partially determined by screening on a panel of crude extracts from 4 other mycobacteria. Nine clones of 122 showed reactivity to *M. leprae* only. The predominant immunoglobulin was immunoglobulin M, and quantities up to 10 μg/ml were produced. Antibody production by hybrid clones was stable in more than 75% of the clones grown in continuous culture. By comparison, 10,000 Epstein-Barr virus-transformed lymphocyte clones from lepromatous leprosy patients were screened for anti-*M. leprae* antibody production, and all of the 42 clones that were initially positive in the enzyme-linked immunosorbent assay lost their antibody-producing capabilities within 6 weeks in culture. These results suggest that a combination of Epstein-Barr virus transformation and hybridization may be an optimal method in producing human monoclonal antibodies from leprosy patients.—Authors' Abstract

Bloom, B. R. and Mehra, V. Immunological unresponsiveness in leprosy. Immunol. Rev. **80** (1984) 5–28.

The various forms of leprosy form a clinical and immunological spectrum which offers extraordinary possibilities for insight into immunoregulatory mechanisms in man. At one pole, tuberculoid leprosy, patients develop high levels of cell-mediated immunity which ultimately results in killing of bacilli in the tissues, albeit often with damage to nerves. At the lepromatous pole, patients exhibit selective immunological unresponsiveness to antigens of *Mycobacterium leprae*. Even though all currently known protein species of *M. leprae* and BCG are crossreactive, lepromatous patients unreactive to *M. leprae* antigens frequently respond strongly to tuberculin. *In vitro* experiments suggest the existence of lepromin-induced suppressor activity, mediated by both monocytes and T cells. The T-suppressor (Ts) cells have the T8 phenotype of which 50% express the activation markers, Ia and FcR. The one unique species of antigen of the leprosy bacillus is a phenolic glycolipid, and it appears that the Ts cells largely recognize the terminal trisaccharide of this unique antigen. Depletion of the Ts cells restores *in vitro* reactivity of lymphocytes to lepromin in a portion of lepromatous patients, and addition of interleukin-2 (IL-2)-containing supernatants partially restores responsiveness of *M. leprae* antigens. Vaccination of lepromatous patients with a mixture of *M. leprae* and live BCG restores cell-mediated immunity in the majority of lepromatous patients, and concomitantly reduces the *in vitro* suppressor activity and number of activated T8 cells.

These experiments suggest the existence of stage-of-disease related suppressor cells in leprosy which appear to block the responsiveness of T-helper cells capable of responding to either specific or crossreactive mycobacterial antigens. The mode of action of these Ts appears to be the inhibition of production of IL-2 and other lymphokines. Successful immunotherapeutic vaccination appears to overcome this block in the majority of patients.—Authors' Summary

Collings, L. A., Tidman, N. and Poulter, L. W. Quantitation of HLA-DR expression by cells involved in the skin lesions of tuberculoid and lepromatous leprosy. Clin. Exp. Immunol. **61** (1985) 58–66.

A method is described which can be used to quantitate class II MHC antigens (HLA-DR) expressed by cells within tissue sections. A mouse anti-human HLA-DR monoclonal antibody is directly conjugated to the fungal enzyme glucose oxidase. The enzyme, in the presence of its substrate, can be used to reduce tetrazolium salts to insoluble colored formazans. The colored reaction product is proportional to the amount of antigen and can be eluted from cells and measured spectrophotometrically. The application of this technique to a study of the expression of HLA-DR antigens, functionally significant molecules, by mononuclear cells in the cutaneous lesions of leprosy, is described. When a quantitative measure of the HLA-DR expression was related to the

area of the granulomata, significant differences in the HLA-DR expression by cells in the infiltrates associated with lesions of tuberculoid and lepromatous leprosy were observed.—Authors' Summary

Dharma Rao, T., Lakshmana Rao, S. S., Rajan, R. and Rao, P. R. The effect of *Mycobacterium leprae* on PHA- and PPD-induced inhibition of leucocyte migration in leprosy patients. Lepr. Rev. **56** (1985) 109–116.

The effect of *Mycobacterium leprae* was studied on mitogen, PHA and antigen, PPD-induced leukocyte migration inhibition in 44 leprosy patients and 13 healthy controls using the leukocyte migration inhibition test. While *M. leprae* decreased the PHA-generated inhibition of migration of leukocytes in tuberculoid patients and healthy individuals, it enhanced the inhibitory effect on the leukocyte migration in lepromatous patients. However, a uniform decrease by *M. leprae* was observed on PPD-induced leukocyte migration inhibition in both groups of leprosy patients and healthy controls.—Authors' Summary

Gillis, T. P., Miller, R. A., Young, D. B., Khanolkar, S. R. and Buchanan, T. M. Immunochemical characterization of a protein associated with *Mycobacterium leprae* cell wall. Infect. Immun. **49** (1985) 371–377.

A panel of 9 monoclonal antibodies to *Mycobacterium leprae* were used to characterize a protein antigen of the bacillus. Two monoclonal antibodies (IVD8 and IIIE9) were specific for *M. leprae* and reacted with an epitope (CWP^a) present on a protein molecule associated with the cell wall fraction of *M. leprae*. This protein, designated cell-wall-associated protein (CWP), lost its immunoreactivity upon treatment with trypsin and had an apparent molecular weight of 65,000, although additional lower molecular weight forms of the protein were observed by immunoblotting. Four other crossreactive epitopes (CWP^b, CWP^c, CWP^d, and CWP^e) were defined on the same molecule using 7 independent monoclonal antibodies. Therefore, *M. leprae* possesses a trypsin-sensitive, heat-stable protein associated with the cell wall which contains at least 1 species-specific and 4 crossreactive antigenic determinants.—Authors' Abstract

Hoffenbach, A., Lagrange, P. H. and Bach, M.-A. Influence of dose and route of *Mycobacterium lepraemurium* inoculation on the production of interleukin-1 and interleukin-2 in C57BL/6 mice. Infect. Immun. **44** (1984) 665–671.

Groups of C57BL/6 mice were infected either intravenously or subcutaneously with 10^5 or 10^8 *Mycobacterium lepraemurium* cells, and the ability of their splenic macrophages and T cells to produce, respectively, interleukin-1 on lipopolysaccharide stimulation and interleukin-2 on concanavalin A (conA) stimulation was assessed during the course of infection. In all groups of infected mice, interleukin-1 production remained unaffected during the entire observation period; whereas interleukin-2 activity decreased as the infection progressed. Heavily infected mice (10^8 *M. lepraemurium* cells) showed an earlier and stronger deficient interleukin-2 production by conA-stimulated spleen cells than did mice infected with a lower dose (10^5 bacilli), without detectable influence by the route of inoculation. In mice receiving 10^5 bacilli, minor differences were seen according to the route of infection, with a slight delay in interleukin-2 decrease in mice injected intravenously. In subcutaneously inoculated mice, the failure of spleen cells to produce interleukin-2 after conA stimulation did not correlate with the number of bacilli developing in the spleen, suggesting the existence of suppressor mechanisms acting at a distance from the site of inoculation.—A. S. (*From* Trop. Dis. Bull.)

Kundu, S. K., Hazra, S. K., Chaudhuri, S. K. and Nandy, A. Immunomodulation with corticosteroids and levamisole in leprosy as gauged by *in vivo* lepromin and *in vitro* CMI responses. Indian J. Lepr. **57** (1985) 37–57.

Corticosteroids and levamisole are known to be immunosuppressive and immunostimulating agents, respectively. Their effects on polar types of leprosy, tuberculoid

and lepromatous, have been studied using *in vivo* lepromin and *in vitro* lymphocyte count, rosette formation, L.T.T. and L.M.I.T. parameters. The immunosuppressive effect of corticosteroids on tuberculoid leprosy is marked with reduced and negative lepromin sensitivity but this does not hold true with other *in vitro* CMI tests. Similar results are obtained with levamisole exhibiting its ineffectiveness in lepromin conversion in lepromatous cases although some improvement is observed in other *in vitro* CMI tests. Evaluation of the results showed: a) lack of correlation between *in vivo* lepromin and *in vitro* other CMI parameters with corticosteroids and levamisole, b) lepromin sensitivity has some unknown influence other than thymic factors, c) prolonged corticosteroid therapy may produce permanent immunosuppression in tuberculoid cases making them more vulnerable toward the lepromatous pole and d) lepromin sensitivity is more reliable, stable and easy to perform.—Authors' Abstract

Mehra, V., Brennan, P. J., Rada, E., Convit, J. and Bloom, B. R. Lymphocyte suppression in leprosy induced by unique *M. leprae* glycolipid. Nature **308** (1984) 194–196.

Leprosy remains a significant medical and social problem in many developing countries. The varied forms of the disease form a spectrum. At one pole, tuberculoid leprosy, patients develop high levels of cell-mediated immunity which results in the killing and clearing of bacilli in the tissues. At the lepromatous pole, patients exhibit a selective immunological unresponsiveness to antigens of *Mycobacterium leprae* so that the organisms inexorably multiply in the skin. We have suggested that in lepromatous leprosy one or a small number of unique antigenic determinants present on *M. leprae* might induce specific suppressor cells that inhibit the reactivity of helper T-cell clones capable of recognizing other specific or crossreactive determinants. Although unique epitopes have been identified by monoclonal antibodies on a small number of *M. leprae* proteins, the only unique species of antigen present in *M. leprae*, and not on any other species of mycobacteria so far examined, is a phenolic glycolipid (PGL-I). We show here that this unique antigen of *M. leprae* is capable of inducing suppression of mitogenic responses of lepromatous patients' lymphocytes *in vitro* and provide evidence that the suppressor T cells recognize the specific terminal trisaccharide moiety.—Authors' Abstract

Mistry, N. F., Birdi, T. J., Uplekar, M. and Antia, N. H. A sequential histopathological study of the lepromin reaction in leprosy patients. IRCS Med. J. **12** (1984) 287–288.

An immunoperoxidase study was made of sequential biopsies of lepromin reactions (Dharmendra antigen). Of 8 immunological markers tested, 3 served to differentiate reactors and nonreactors; at 21 days BCG antigen and lysozyme were detected in nonreactors only; and Ia antigen was expressed on macrophages only in 1 polar tuberculoid case. Helper and suppressor T lymphocytes, immunoglobulin, and C3 did not differ significantly between reactors and nonreactors.—D. S. Ridley (*From* Trop. Dis. Bull.)

Mohagheghpour, N., Gelber, R. H., Larrick, J. W., Sasaki, D. T., Brennan, P. J. and Engleman, E. G. Defective cell-mediated immunity in leprosy: failure of T cells from lepromatous leprosy patients to respond to *Mycobacterium leprae* is associated with defective expression of interleukin-2 receptors and is not reconstituted by interleukin-2. J. Immunol. **135** (1985) 1443–1449.

Patients with lepromatous leprosy (LL) but not borderline tuberculoid leprosy (BT) have defective cell-mediated immune responses to *Mycobacterium leprae*, despite normal responses to other stimuli, as judged by *in vivo* skin testing and *in vitro* lymphocyte transformation. To investigate the basis of the immune defect in LL patients, we studied the ability of patient mononuclear leukocytes to produce interleukin-1 (IL-1) and interleukin-2 (IL-2) upon stimulation with *M. leprae*, and determined the ability of exogenous IL-1 and IL-2 to reconstitute the LL patient response to this antigen *in vitro*. Equal numbers of adherent non-T cells from LL and BT patients produced similar amounts of IL-1 upon challenge with *M.*

leprae, and addition of IL-1 to the culture medium failed to reconstitute the response of lymphocytes from LL patients to *M. leprae.* On the other hand, T cells of LL patients failed to express receptors for IL-2 or to produce IL-2 in response to *M. leprae,* whereas similarly treated T cells of BT patients both expressed IL-2 receptors and produced IL-2. Finally, recombinant human IL-2 purified to homogeneity as well as crude supernatants of mitogen-activated lymphocytes failed to reconstitute the response of LL patients to *M. leprae.* These results suggest that T cells of LL patients fail to respond to *M. leprae* despite an ability to produce IL-1 and that their failure to express receptors for IL-2 may explain both defective proliferation and the failure of exogenous IL-2 to reconstitute the response.—Authors' Abstract

Ohkawa, S. Activation of human monocytes in leprosy. Microbiol. Immunol. **29** (1985) 265–274.

In leprosy, the common etiologic agent is the same *Mycobacterium leprae,* but the clinical manifestations are various, including the tuberculoid and lepromatous types. In tuberculoid type leprosy, macrophages in the granuloma differentiate into epithelioid cells; in the lepromatous type, in contrast, they differentiate into lepra cells containing multiple *M. leprae.* Thus host factors, which regulate macrophage activities, determine the type of leprosy.

To understand such regulation of macrophage activities, we assayed superoxide production, hydrogen peroxide production, and glucose consumption in monocytes *in vitro.* Glucose consumption spontaneously increased, with lymphokine enhancing the consumption rate. Superoxide production increased spontaneously and decreased from the 4th day; lymphokine added on the 4th day suppressed the decrease of superoxide production. Hydrogen peroxide production increased until the 3rd day of culture. Twenty-four hour incubation with lymphokine, from day 0 to the 1st day, had no effect on hydrogen peroxide production, while from the 2nd to 3rd day it enhanced such production. Supernatants of lymphocytes incubated with *M. leprae* were prepared from tuberculoid and lepromatous patients. Tuberculoid supernatant enhanced reactive oxygen production and glucose consumption, while that from lepromatous patients had no remarkable effect on glucose consumption or reactive oxygen production. The range of spontaneous increase and decrease of reactive oxygen production was greater than the regulatory effect of lymphokine on these activities.

These data show that rapid provision of new monocytes to the granuloma is one of the important factors in the defense mechanism, that lymphocytes separated from lepromatous patients are not activated in response to *M. leprae* antigen, and that they do not secrete corresponding lymphokines.—Author's Abstract

Praputpittaya, K. and Ivanyi, J. Detection of an antigen (MY4) common to *M. tuberculosis* and *M. leprae* by "tandem" immunoassay. J. Immunol. Methods **79** (1985) 149–157.

A novel "tandem" immunoassay for the detection of mycobacterial antigen was devised using a monoclonal antibody (ML34) both as solid phase "capture" and as the ^{125}I- or enzyme-labeled "tracer" antibody. This antibody binds to the repeating epitopes (MY4b) of a water-soluble, protease-resistant antigen from *Mycobacterium tuberculosis, M. leprae,* and some other species of mycobacteria. Optimal binding results could be obtained within 4 hr by the consecutive incubation of ML34-coated microtiter plates with antigen followed by the labeled ML34 antibody. The binding of intact bacilli was positive for *M. tuberculosis* but not for *M. leprae.* These results suggested that the MY4 antigen is expressed on the surface of *M. tuberculosis* and internally within *M. leprae.* Analysis of subcellular fractions suggested that this antigen is a constituent of cell walls.—Authors' Abstract

Ramanathan, V. D., Sharma, P., Ramu, G. and Sengupta, U. Reduced complement-mediated immune complex solubilization in leprosy patients. Clin. Exp. Immunol. **60** (1985) 553–558.

The ability of sera from leprosy patients to solubilize immune precipitates *in vitro* through the complement system was stud-

ied. The solubilizing capacity of sera from patients who did not have any reactions during 2 years or more after starting chemotherapy was comparable with that of normal laboratory volunteers. On the other hand, sera from borderline tuberculoid and lepromatous leprosy patients in reaction had markedly decreased levels of solubilization. Their total and the alternative pathway hemolytic levels did not show a corresponding decrease. Although the circulating immune complexes and serum C3d of these patients came down after the subsidence of reaction, their solubilization remained consistently low during a 3 month follow-up period.—Authors' Summary

Reichart, P. A., Metah, D. and Althoff, J. Ultrastructural aspects of oral and facial lepromatous lesions. Int. J. Oral Surg. **14** (1985) 55–60.

The ultrastructure of 10 lepromatous lesions in the face and the palatal mucosa after different duration and length of treatment was studied. Biopsies taken from patients who showed erythema nodosum leprosum (ENL) revealed different ultrastructural characteristics from those taken from "burnt out" and nonmedicated cases. Multiple secondary lysosomes were rarely seen in non-ENL cases. The presence of *Mycobacterium leprae* and an increased lysosomal activity in ENL reactive cases is interpreted as a reaction to the lysis of the cytoplasmic matrix of *M. leprae*; however, drug-specific (diaminodiphenylsulfone) reactions must also be considered.—Authors' Abstract

Results of a World Health Organization-sponsored workshop on monoclonal antibodies to *Mycobacterium leprae*. (Letter) Infect. Immun. **48** (1985) 603–605.

A total of 22 monoclonal antibodies generated in 6 different laboratories was submitted to the IMMLEP monoclonal antibody bank. Samples of these monoclonal antibodies were coded and sent to 7 laboratories for independent analysis by a variety of methods, including enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), electrophoretic or immunoblotting techniques, crossed immunoelectrophoresis, and indirect immunofluorescence assay (IFA). A summary of the results obtained by the 7 laboratories showed that 10 of 22 monoclonal antibodies tested were found to be specific for *Mycobacterium leprae*, with another 10 antibodies crossreactive for 1 or more of the 25 mycobacterial species tested. The remaining 2 monoclonal antibodies were found to be specific for a 95-kilodalton (kD) protein present in normal armadillo liver homogenates.

Three broad antigen classes were recognized by the monoclonal antibodies tested: a) Several protein antigens of different molecular weights were defined by electrophoretic immunoblotting techniques and sensitivity to proteolytic enzyme treatment. The possibility that 1 or more of the lower molecular weight species represents proteolytic breakdown products of a higher molecular weight antigen(s) remains to be determined. Cross-competition experiments are in progress to determine the minimal number of unique epitopes recognized by monoclonal antibodies reacting with polypeptides of the same molecular weight. b) *M. leprae*-specific phenolic glycolipids were defined by a solid-phase ELISA assay, using the deacylated form of the antigen. c) Carbohydrate or lipid class antigens or both were defined by resistance to proteolytic enzyme treatment.—(*From* the Letter)

Schroh, R. G., Ravettini, B. A., Magnin, P. H. and Casas, J. G. Inmunomarcacion de lisozima en granulomas hansenianos. [Immunoreactive lysozyme in leprosy granulomas.] Rev. Argent. Dermatol. **66** (1985) 56–65. (in Spanish)

The immunoperoxidase technique was used to assess the quantity and location of lysozyme (muramidase) in 37 skin biopsies which represented the leprosy spectrum from TT to LL. The quantities of lysozyme in the lesion produced peaks at TT-BT and LL, with a dip in the BB region.

With the PAP method to localize lysozyme in leprous tissues, two distinct staining patterns were found, granular and saccular. The granular pattern of lysozymal staining was found in epithelioid cells and in giant cells. A saccular pattern of lysozymal staining was found in lepromatous histiocytes. These two patterns of staining

probably represent different functional responses of monocyte derived granuloma cells.

The predominantly synthesizing and secretory nature of epithelioid cells suggests that lysozyme giving a granular staining pattern represents newly synthesized lysozyme in the endoplasmic reticulum.

An intense staining reaction for lysozyme in a monocytic derivative has been suggested as a sign of cellular activation. The purely granular pattern was observed in those patients, TT and BT, in whom cell mediated inmune responses against antigens of *Mycobacterium leprae* are demonstrable. In granular pattern in leprosy an intense staining for lysozyme is consistent with activation of epithelioid and giant cells by specifically sensitized T lymphocytes.

This association of resistance and activation was not observed in patients having a saccular pattern of lysozymal staining. Large numbers of leprosy bacilli, isolated and in aggregates (globi), were often found within these lysozyme positive saccules, leading us to suppose that these saccules might represent dilated secondary lysosomes or phagocytic vacuoles.

Histoid leproma was associated with a fall in tissue lysozyme and similar patterns have been found in dermatofibroma.

The results suggest the possibility that there is a common defect from BT to LL in which the generation of immunological factors within the lesion is a secondary response to the antigenic load.—Authors' English Summary

Tausk, F., Hoffmann, T., Schreiber, R. and Gigli, I. Leprosy: altered complement receptors in disseminated disease. J. Invest. Dermatol. **85** Suppl. (1985) 58s–61s.

We have studied the expression of the C3b receptor (CR1) on erythrocytes of 55 patients with Hansen's disease. We developed a radioimmunoassay utilizing a monoclonal antibody that recognized an epitope different from the C3b binding site, which therefore enabled us to measure total number of CR1 regardless of receptor occupancy. We observed that patients in the lepromatous pole of the disease had a mean of 310 CR1/erythrocyte, whereas the ones in the tuberculoid pole showed a mean of 577 CR1/erythrocyte; 77 normal controls had a mean of 512 CR1/erythrocyte. The number of C3b receptors on the cells of lepromatous patients was significantly decreased ($p < 0.001$) when compared to the normal population or tuberculoid patients. The presence of receptors for the C3b fragment of complement (CR1) on the surface of human erythrocytes enables these cells to participate in a number of immune functions including the clearance of circulating immune complexes. These findings could bear importance in the ability of the host to clear immune complexes from the circulation in patients with lepromatous leprosy.—Authors' Abstract

Teuscher, C., Yanagihara, D., Brennan, P. J., Koster, F. T. and Tung, K. S. K. Antibody response to phenolic glycolipid I in inbred mice immunized with *Mycobacterium leprae*. Infect. Immun. **48** (1985) 474–479.

The level of circulating antibody to phenolic glycolipid-I of *Mycobacterium leprae* was determined in 18 inbred strains of mice after immunization with *M. leprae* organisms. By using a solid-phase radioimmunoassay with phenolic glycolipid-I as test antigen, a continuous distribution of antibody levels ranging from high to low was observed. The level was found to be controlled by multiple genes, including both H-2 complex- and Igh allotype complex-linked genes. Low antibody response to phenolic glycolipid-I was shown to be inherited as a dominant trait in 3 combinations of high × low responder F_1 progeny.—Authors' Abstract

Verma, R. N., Tutakne, M. A. and Sharma, P. K. Histological changes in the apparently normal skin at the periphery of leprosy lesions. Indian J. Dermatol. Venereol. Leprol. **50** (1984) 211–212.

Apparently normal skin at the periphery of the lesions of various types of leprosy has been studied for histological confinement of the disease. TT cases showed good confinement without any histological change in the normal skin; while 21.4% of BT cases, 42.8% of BB cases, 66.6% of BL cases, and 80% of LL cases showed some of the changes, i.e.,

epidermal atrophy, subepidermal clear zone, acid-fast bacilli, attempt at granuloma formation, diffuse lymphocytic infiltration and nerve involvement. Loss of confinement was directly proportional to the expected deficiency in the immune status of the patients from BT to LL.—Authors' Abstract

Wear, D. J., Hadfield, T. L., Connor, D. H., Neafie, R. C., Banks, I. S., Meyers, W. M. and Binford, C. H. Periodic acid-Schiff reaction stains *Mycobacterium tuberculosis, Mycobacterium leprae, Mycobacterium ulcerans, Mycobacterium chelonei* (*abscessus*), and *Mycobacterium kansasii.* (Letter) Arch. Pathol. Lab. Med. **109** (1985) 701–702.

Formaldehyde-fixed, paraffin-embedded tissue sections from patients with anergic tuberculosis, lepromatous leprosy, Buruli ulcer (*Mycobacterium ulcerans*), and other mycobacterial infections (*M. chelonei* [*abscessus*], *M. kansasii,* and *M. avium-intracellulare*) were selected. All tissue sections were stained by the PAS method with light-green counterstain. *M. tuberculosis, M. leprae, M. ulcerans, M. chelonei* (*abscessus*), and *M. kansasii,* as well as *M. avium-intracellulare,* are PAS-positive.

Periodic acid-Schiff staining is conspicuous when *M. leprae* are packed within histiocytes in lepromatous leprosy or *M. avium-intracellulare* in patients with acquired immunodeficiency syndrome. The PAS-stained mycobacteria are easily missed on the standard PAS stain when the mycobacteria are few, extracellular, and centered in a background of PAS positivity such as necrotic debris. The PAS-stained mycobacteria are better seen against a light-green background.

Although PAS staining does not differentiate among the 6 medically relevant mycobacteria, PAS staining does alert the examiner to intracellular bacteria that might otherwise be missed on routine stains.—(*From* the Letter)

Young, D. B., Fohn, M. J. and Buchanan, T. M. Use of a polysulfone membrane support for immunochemical analysis of a glycolipid from *Mycobacterium leprae.* J. Immunol. Methods **79** (1985) 205–211.

Polysulfone membranes have been used as a solid support for chromatography and immunoblotting of phenolic glycolipid-I from *Mycobacterium leprae.* These membranes have an advantage over other supports such as nitrocellulose and silica gel in that very little nonspecific background binding of antibodies occurs and assays can readily be carried out with IgM antibodies from human sera. An example of use of the polysulfone chromatography system for detection of phenolic glycolipid-I in sera from leprosy patients is described.—Authors' Abstract

Young, D. B., Fohn, M. J., Khanolkar, S. R. and Buchanan, T. M. Monoclonal antibodies to a 28,000 mol. wt protein antigen of *Mycobacterium leprae.* Clin. Exp. Immunol. **60** (1985) 546–552.

Monoclonal antibodies (MoAb) have been used to analyze a protein antigen from *Mycobacterium leprae* with a subunit mol. wt of 28,000 daltons. Three different patterns of species specificity were observed with 2 antibodies being specific for *M. leprae,* 2 partially specific, and 1 broadly crossreactive among all mycobacteria. Competitive binding and sandwich assays demonstrated that the specific and partially specific antibodies recognized closely related regions of the molecule, while the crossreactive antibody recognized a spatially separate epitope on the same polypeptide chain. Identification of specific and crossreactive epitopes on a single antigenic molecule may be of considerable importance for understanding the functioning of the cell-mediated immune system during leprosy infection and the use of MoAb for such analyses is discussed.—Authors' Summary

Microbiology

Allen, B. W. Fixation of *Mycobacterium leprae* in unstained smears prior to shipment or storage: evaluation of formaldehyde using *Mycobacterium tuberculosis* as indicator of efficacy. Med. Lab. Sci. **42** (1985) 53–55.

In the absence of an *in vitro* culture system for *Mycobacterium leprae* the value of exposing smears to formaldehyde vapor was assessed using *M. tuberculosis.* Of 98 sputum specimens positive on slide culture, 21 yielded growth after exposure for 10 min at room temperature. However, exposure of smears at 37°C resulted in negative cultures from all smears. It is concluded that controlled conditions are necessary to ensure that smears are safe for further handling.—Author's Abstract

Brennan, P. J. *Mycobacterium leprae*—the outer lipoidal surface. J. Biosci. **6** (1984) 685–689.

There is now a considerable body of evidence to suggest that the phthiocerol-containing lipids, including the phenolic glycolipids, comprise the so-called "peribacillary substance," "spherical droplets," "foamy structures," and "capsular materials" of *Mycobacterium leprae.* Thus, the phthiocerol-containing lipid capsule may be directly responsible for the intracellular survival of *M. leprae.*—Author's Abstract

Burchard, G.-D. and Bierther, M. Study on the micromorphology of *Mycobacterium leprae.* Arch. Dermatol. Res. **277** (1985) 220–224.

The micromorphology of *Mycobacterium leprae* is described. After fixation with osmium tetroxide supplemented with calcium ions, the cell wall was seen to be composed of three layers; the cytoplasmic membrane exhibited the architecture of an elementary membrane. The mesosomes were best visualized after fixation with glutaraldehyde; they were sometimes in contact with the nuclear equivalent. Only one sort of phosphate body was found. The nucleoid was best visualized after fixation with osmium tetroxide.—Authors' Summary

Chatterjee, B. R. Growth of *Mycobacterium leprae* in a redox system. Lepr. India **55** (1983) 426–449.

The author claims to have grown *Mycobacterium leprae* from human lepromatous nodules in a culture system that ensured a low oxygen tension to begin with and a gradually increasing oxygen tension as growth occurred. A tissue culture medium, Watson and Reid's medium, and trypticase soy broth were combined to make the basal medium. To this were added sheep or human serum, a mixture of cholesterol and lecithin in the form of liposomes, n-tetradecane, Tween 80 and menadione, a redox–amine mixture and, finally, gelatin. The reduced oxygen tension was achieved by using alkaline pyrogallol in the side arm of the culture vessel and a partial vacuum.

The initial growth occurred in 1–2 weeks and consisted mainly of non-acid-fast rods and cocci, but after 3 months acid-fast bacilli were abundant. Neither the rods nor the cocci could be grown on conventional media, but the cocci grew readily on a special enrichment medium.

The evidence for calling the organisms isolated *M. leprae* appears to be a positive DOPA oxidase test and negative results for catalase, niacin production, nitrate reduction, and Tween hydrolysis. However, the DOPA oxidase test was positive only when sonicated suspensions of the organism were tested. There is no mention of checking the identity of the isolates by mouse foot pad inoculation.—P. A. Jenkins (*From* Trop. Dis. Bull.)

David, H. L. and Rastogi, N. Antibacterial action of colistin (polymyxin E) against *Mycobacterium aurum.* Antimicrob. Agents Chemother. **27** (1985) 701–707.

Mycobacterium aurum was susceptible to the antibiotic colistin (polymyxin E), which had an MIC of 5 μg/ml and an apparent bactericidal effect at concentrations above 50 μg/ml. Treatment of actively growing cells with sublethal concentrations of colistin (15 μg/ml) resulted in synchronized cell division once the antibiotic was removed. Un-

der conditions of synchronized cell growth, one cycle of DNA replication lasted 120 min and one cycle of cell division lasted about 180 min. Although the antibiotic treatment during synchronization experiments did not produce apparent changes in the bacterial envelope, it was accompanied by the accumulation of a polysaccharide-like substance in the bacterial cytoplasm which gradually decreased after the removal of antibiotic and by an increase in the number of mesosomes at 3 hr after antibiotic removal. This step was closely linked to the doubling time of bacteria. Lethal concentrations of colistin of 50 and 100 μg/ml, which caused about 90 and 99% cell death, respectively, produced significant cytoplasmic membrane injuries, patchy appearance of the cell wall outer polysaccharide layer, and little cell lysis. These data indicate that the cytoplasmic membrane is a site of action of colistin and raise a question as to whether an outer bilayer exists in mycobacteria, at least functionally.—Authors' Abstract

Kato, L. Absence of mycobactin in *Mycobacterium leprae*; probably a microbe dependent microorganism. Implications. Indian J. Lepr. **57** (1985) 58–70.

Ferric mycobactins were prepared from *Mycobacterium phlei, Mycobacterium avium-intracellulare* A and H, isolated respectively from armadillo and human leprosy specimens. Attempts were made to extract mycobactin from host-grown *M. leprae* cells. The crude ferric mycobactin extracts were tested for growth-supporting effect on the mycobactin-dependent *M. paratuberculosis* strain ATCC 19698. Mycobactins prepared from *M. phlei* and the 2 *M. avium-intracellulare* strains had growth-promoting effects on *M. paratuberculosis*. The same test organism did not grow in media supplemented with the extract prepared from *M. leprae*. Results indicate the absence of mycobactin from host-grown *M. leprae*. Since *M. leprae* cells contain cytochrome c and since mycobactin is essential to the growth of all mycobacteria, *M. leprae* might be considered as a microbe-dependent microbe. It is proposed that secondary mycobacteria present in *M. leprae*-infected humans and armadillos might provide mycobactin for *in vivo* multiplication of *M. leprae*.—Author's Abstract.

Katoch, V. M., Sharma, V. D., Datta, A. K., Shivannavar, C. T., Katoch, K., Kannan, K. B. and Bharadwaj, V. P. Isoenzymes of mycobacteria. II. Relevance of LDH zymograms in taxonomy and identification. Indian J. Lepr. **57** (1985) 107–114.

Cell-free extracts of mycobacteria, namely, *Mycobacterium kansasii, M. avium, M. tuberculosis,* BCG (Glaxo), *M. gastri, M. phlei, M. smegmatis, M. vaccae, M. w., M. scrofulaceum, M. gordonae, M. nonchromogenicum, Escherichia coli, Staphylococcus aureus,* and *M. leprae* infected skin have been electrophoresed and stained for LDH activity. Normal skin tissue was also taken as a control. It was found that all the organisms tested showed distinct species-specific LDH isoenzyme patterns. There was no extra band but an aberrant zone of LDH activity was seen in *M. leprae*-infected human skin in comparison to LDH isoenzymes from normal skin. No strain variations were found among the different strains of species investigated. Results described in the present paper indicate that LDH isoenzyme patterns of mycobacteria could be of identification value at the species level.—Authors' Abstract

Minnikin, D. E., Dobson, G. and Draper, P. The free lipids of *Mycobacterium leprae* harvested from experimentally infected nine-banded armadillos. J. Gen. Microbiol. **131** (1985) 2007–2011.

The free lipids of a sample of *Mycobacterium leprae* were extracted by a procedure designed to produce separate non-polar and polar fractions. The composition of these lipids was analyzed semi-quantitatively by 5 special thin-layer chromatographic systems covering the total range of mycobacterial lipid polarities. In order of increasing polarity, the major lipids were dimycocerosates of phthiocerol A, phthiocerol B and phthiodiolone A, glycosyl phenolphthiocerol dimycocerosates and phospholipids, including monoacylphosphatidylinositol di- and pentamannosides. The diacylated forms of these latter lipids, found in most myco-

bacteria, were not present. The composition of the free lipids of the leprosy bacillus, surveyed over the total polarity range for the first time, showed that the patterns were particularly related to those of *M. bovis, M. kansasii,* and *M. marinum.*—Authors' Abstract

Minnikin, D. E., Dobson, G., Goodfellow, M., Draper, P. and Magnusson, M. Quantitative comparison of the mycolic and fatty acid compositions of *Mycobacterium leprae* and *Mycobacterium gordonae.* J. Gen. Microbiol. **131** (1985) 2013–2021.

The mycolic and fatty acids of 3 samples each of *Mycobacterium leprae* and *M. gordonae* were compared. Acids released by whole-organism alkaline hydrolysis were converted to 4-nitrobenzyl esters and mycolic acids were further derivatized to *t*-butyldimethylsilyl ethers. Thin-layer chromatography of the derivatized long-chain extracts showed that all 3 *M. leprae* preparations contained so-called α-mycolates and ketomycolates but that the *M. gordonae* samples had a methoxymycolate in addition to the above types. Silica gel normal-phase high-performance liquid chromatography of the total mycolic acid derivatives confirmed the lack of detectable amounts of methoxymycolates in *M. leprae* and reverse-phase chromatography of the individual mycolate types demonstrated the homogeneity of the chain lengths of the mycolic acids in each species. Non-hydroxylated fatty acid 4-nitrobenzyl esters were transformed to methyl esters and examined by gas chromatography. Tuberculostearic (10-methyloctadecanoic) acid was a major component of the lipids of all 3 *M. leprae* preparations but it was absent in 1 *M. gordonae* strain and a very minor component in the other representatives of this latter species. On the basis of fatty and mycolic acid compositions, therefore, a previously suggested close relationship between *M. leprae* and *M. gordonae* was not supported.—Authors' Abstract

Mukherjee, R. and Antia, N. H. Intracellular multiplication of leprosy-derived mycobacteria in Schwann cells of dorsal root ganglion cultures. J. Clin. Microbiol. **21** (1985) 808–814.

Organized nerve cultures of dorsal root ganglia from neonatal mice were infected with *Mycobacterium leprae,* the causative agent of leprosy. A significant multiplication of the acid-fast bacilli was observed within the Schwann cell component of the culture. The growth of these bacilli was sensitive to antileprosy drugs and was not observed directly in bacteriological media. These organisms were brightly stained with the monoclonal antibody to phenolic glycolipid-I, a *M. leprae*-specific marker. The antigenic, pathogenic, and biochemical characteristics of this mycobacterium are under investigation.—Authors' Abstract

Nakamura, M. and Dhople, A. M. Intrinsic energy potential of *Mycobacterium lepraemurium* essential for growth in NC-5 medium. Microbios **42** (1985) 97–102.

The intrinsic energy potential of the fastidious mycobacterium, *Mycobacterium lepraemurium,* essential for growth in axenic liquid medium was determined by ultraviolet irradiation of the inoculum. The growth potential in NC-5 medium of irradiated inocula was measured by an ultrasensitive method of adenosine triphosphate (ATP) using a luciferin-luciferase acceptor system. The results indicate that growth of bacilli ceased when more than one third of the ATP pool in 7×10^6 cells was lost. The residual level of ATP is roughly equivalent to that found in 10^5 non-irradiated cells.—Authors' Abstract

Portaels, F., De Ridder, K. and Pattyn, S. R. Cultivable mycobacteria isolated from organs of armadillos uninoculated and inoculated with *Mycobacterium leprae.* Ann. Immunol. (Paris) **136A** (1985) 181–190.

Mycobacteria were cultivated from 16 out of 32 samples of tissues from armadillos inoculated with *Mycobacterium leprae.* Three out of 7 samples from noninoculated armadillos held in captivity were also positive for cultivable mycobacteria. Some isolated strains belonged to the *M. avium-intracellulare-scrofulaceum* complex, while

others were identified as *M. gordonae* and *M. terrae*. Unclassified mycobacteria were isolated from *M. leprae*-inoculated armadillos only. Taxonomic studies confirmed that these new armadillo-derived mycobacteria (ADM) are different from all presently known species of mycobacteria, including *M. leprae*. The same new species were isolated from different armadillo colonies and were never found in noninoculated animals.

Different factors influence the isolation of these ADM in primary culture. Analysis of some specific markers of these ADM has been suggested in order to quantitatively determine the proportion of these ADM to *M. leprae* in armadillo tissues.

No mycobacteria were cultivated from nude mouse foot pads infected with *M. leprae*.—Authors' Summary

Prabhakaran, K. and Harris, E. B. Temperature-induced changes in viability, diphenoloxidase and permeability of *Mycobacterium leprae*. Microbios **43** (1985) 7–15.

Among mycobacteria secretion of the enzyme diphenoloxidase has been established as a property of *Mycobacterium leprae*. The antileprosy drug dapsone (DDS), which completely inhibits the enzyme from plant and mammalian sources, does not readily penetrate intact *M. leprae*. When the drug is complexed with polylysine, it easily permeates the bacteria and produces 100% inhibition of its diphenoloxidase, suggesting a permeability barrier of the cytoplasmic membrane of *M. leprae* to dapsone. In this study: 1) when the organisms, purified from fresh tissues of experimentally infected armadillos, were treated with dilute alkali or exposed to warmer temperatures, DDS penetrated the bacteria and inhibited the diphenoloxidase. Washing with trypsin had no effect. Dapsone easily permeated the bacilli, purified from tissues stored at 0°C or at −80°C. 2) Diphenoloxidase of freshly prepared *M. leprae* was stimulated when the bacteria were exposed to 50°C for 10 min; at 60°C the activity decreased, and at 100°C the enzyme was completely inactivated. When the enzyme was assayed at temperatures below 37°C, the activity was considerably lower, indicating that *M. leprae* may not be a psychrophilic organism in this respect. 3) The bacteria exposed to 50°C failed to multiply in mouse foot pads. *M. leprae* remained viable in tissues stored at 0°C or −80°C but when the bacteria purified from these tissues were frozen, they lost their viability. On the other hand, the organisms separated from fresh tissues remained viable when frozen at −80°C. The inhibition of diphenoloxidase of *M. leprae* by dapsone could serve as an indirect method to assess the integrity of the bacterial cell membrane and to predict whether the bacteria would retain their viability on freezing.—Authors' Abstract

Prasad, H. K. and Hastings, R. C. Alternate radiolabeled markers for detecting metabolic activity of *Mycobacterium leprae* residing in murine macrophages. J. Clin. Microbiol. **21** (1985) 861–864.

This study demonstrated the utility of using 4% NaOH as a murine macrophage cell-solubilizing agent to discriminate between host macrophage metabolism and that of intracellular *Mycobacterium leprae*. A 4% concentration of NaOH had no deleterious effect on labeled mycobacteria. Thereby, alternate radiolabeled indicators of the metabolic activity of intracellular *M. leprae* could be experimented with. Significant incorporation of ^{14}C-amino acid mixture, [^{14}C]leucine, [^{14}C]uridine, and carrier-free ^{32}P was observed in cultures containing freshly extracted ("live") strains of *M. leprae* as compared with control cultures containing autoclaved bacilli.—Authors' Abstract

Sharma, V. D., Katoch, V. M., Datta, A. K., Shivannavar, C. T., Kannan, K. B., and Bharadwaj, V. P. Isoenzyme patterns of mycobacteria. I. Factors influencing LDH isoenzymes of mycobacteria. Indian J. Lepr. **57** (1985) 97–106.

Cell-free extracts of a fast-growing mycobacterium (*Mycobacterium phlei*) and a slow-growing mycobacterium (*M. tuberculosis* H37Ra) were analyzed for lactate dehydrogenase (LDH) isoenzymes under different experimental conditions. It was observed that growth of *M. phlei* when taken from Löwenstein-Jensen (LJ) as well as Sauton's medium showed identical bands

but for *M. tuberculosis* H37Ra the number of bands observed was less when grown on LJ medium. There was no difference in LDH isoenzyme patterns when the mycobacteria were incubated at 30°C and 37°C and under different pH conditions (6.2–8.2). Actively growing cultures of both the species showed distinct LDH isoenzyme patterns, whereas the activity and bands became indistinct in old cultures. The LDH bands from lyophilized growth studied resembled those of fresh growth. The treatment of growth with 1 M NaOH for 1 hr resulted in marked diminution of LDH activity. Sonication with wet growth weight of 0.5 g per ml of distilled water was found to give clearer bands as compared to phosphate buffer. No loss of LDH isoenzyme activity was noticed after storing the extracts at −80°C for 1 month, treating to 58°C for 1 hr or freezing and thawing for 2 times, whereas these isoenzymes were quite unstable at other storage temperatures. Increasing the staining time was found helpful in getting clearer bands when activity was low. It is concluded that the factors studied have an important bearing on LDH isoenzyme patterns of mycobacteria and must be kept in mind while studying the LDH zymograms for any taxonomic identification of mycobacteria or for studying their metabolic role. These are important both for the sensitivity and reproducibility of the LDH zymograms.—Authors' Abstract

Wang, H., *et al.* [Study on viability of *Mycobacterium leprae*: effect of various temperatures.] Chin. J. Dermatol. **18** (1985) 25–27. (in Chinese)

The viability of *Mycobacterium leprae* in normal saline was measured by the mouse foot pad technique. The results were as follows: a) The viability of *M. leprae* obviously decreased when they were kept in a refrigerator (4°C) for 8 days. After 20 days, the viability was lost. b) At an average room temperature of 21°C (14–24°C) for 3 days, the viability of *M. leprae* decreased slightly. After 6–10 days, multiplication in the mouse foot pad was delayed. After 2 weeks, the viability of *M. leprae* was lost. c) At 45°C (in a water bath) from 5–30 min, their viability showed no significant change. When *M. leprae* were kept at 60°C for 5 min, the viability was lost rapidly. At 100°C for 5 min, no multiplication of *M. leprae* was observed in the mouse foot pads.

Our observations lead us to conclude that the viability of *M. leprae* was related to the temperature and time stored.—(*From* Authors' English Abstract)

Experimental Infections

Baliña, L. M., Valdez, R. P., de Herrera, M., Costa Cordova, H., Bellocq, J. and Garcia, N. Reproduccion experimental de lepra en *Dasypus hybridus.* [Experimental reproduction of leprosy in *Dasypus hybridus.*] Rev. Argent. Dermatol. **66** (1985) 7–12. (in Spanish)

Three armadillos native to Argentina (*Dasypus hybridus*—7-banded armadillos) inoculated with human *Mycobacterium leprae* are reported. The inoculum consisted of 1.32×10^8 bacilli for each animal. The bacilli were from a previously untreated lepromatous leprosy patient. The animals all developed disseminated lepromatous leprosy 15.3 months as an average after inoculation. The disease affected mainly the skin, nerves, liver, spleen, lymph nodes, striated muscle, lungs, and meninges.

The acid-fast bacilli harvested from these animals were identified as *M. leprae* by means of a) the failure to grow on common mycobacterial culture media, b) extraction of their acid-fastness with pyridine, c) characteristic multiplication of the bacilli in the foot pads of mice, and d) characteristic histopathological features. Bacterial loads in the livers were 2.1×10^8 bacilli per gram of tissue; in the spleens, 1.3×10^8 bacilli per gram of tissue; in the lymph nodes, 6.9×10^8 per gram of tissue.—(*From* Authors' English Summary)

Ganguly, N. K., Kumar, B., Kaur, S., Vaishnavi, C. and Chakravarti, R. N. Influence

of levamisole on lymphocytes and *M. leprae* in mice. Indian J. Lepr. **57** (1985) 27–36.

Normal uninfected (N) and *Mycobacterium leprae*-infected mice (NI) were given levamisole in the dose of 2.5 mg/kg body weight. The animals were observed over a period of 9 months for bacillary load, T and B cell counts and blast transformation with PHA. A significant increase in B cell counts was observed in the levamisole treated normal (NL) compared to normal control (N) group. T cell counts and blast transformation, however, remained unaffected. However, T cell counts and blast transformation improved significantly in the infected and levamisole treated (NIL) as compared to the infected group (NI) not given levamisole. Bacillary loads remained unaltered in both the infected (NI) and levamisole treated (NIL) groups.—Authors' Abstract

Job, C. K., Sanchez, R. M., McCormick, G. T. and Hastings, R. C. First lesion in experimental armadillo leprosy. Indian J. Lepr. **57** (1985) 71–77.

Eighteen armadillos were infected intravenously with 10^8 *Mycobacterium leprae* and 10 intracutaneously with 10^7 *M. leprae*. Among those which developed disseminated disease, a nodule at the site of inoculation was the first lesion noticed in 14 of the 16 infected intravenously and 4 of the 4 infected intradermally. It is possible that in human leprosy the first sign of infection is localized proliferation of *M. leprae* at the site of entry, and even nodule formation in lepromatous patients. It may be important to search for asymptomatic swelling or keloid-like lesions in skin or in nasal mucosa while screening a population for early leprosy.—Authors' Abstract

Lefford, M. J. *Mycobacterium lepraemurium* infection of nude athymic (*nu/nu*) mice. Infect. Immun. **49** (1985) 190–196.

Nude athymic (*nu/nu*) mice on a BALB/c background and their heterozygous euthymic litter mates (*nu/+*) were infected with either 10^8 or 10^6 *Mycobacterium lepraemurium* organisms intravenously or in the left hind foot pad (LHF). After LHF infection with 10^8 *M. lepraemurium* organisms, *nu/+* mice slowly developed a response that consisted of LHF swelling and local resistance to *Listeria monocytogenes*. The lower inoculum induced a proportionately lower response in *nu/+* mice, but the *nu/nu* mice developed neither LHF swelling nor resistance to *L. monocytogenes* in response to either dose of *M. lepraemurium*. Counts of *M. lepraemurium* in the LHF revealed no difference between the *nu/+* and *nu/nu* mice. After intravenous infection the *nu/+* mice developed splenomegaly, but did not otherwise differ from *nu/nu* mice with respect to resistance to intravenous challenge with *L. monocytogenes* or growth of *M. lepraemurium* in the spleen. In light of the poor responsiveness of *nu/+* mice in this experiment, they were then compared with CB6 and B6D2 mice, which are genetically susceptible and resistant to *M. lepraemurium*, respectively. These mice were infected with either 10^8 or 10^6 *M. lepraemurium* cells or 10^6 *M. bovis* BCG cells in the LHF. Once again the *nu/+* mice responded poorly to *M. lepraemurium*, the CB6 mice responded very strongly, and the B6D2 mice gave an intermediate response with respect to LHF swelling and resistance to *L. monocytogenes*. However, *M. lepraemurium* grew to higher numbers in the LHF of *nu/+* and CB6 mice than in B6D2 mice, revealing, in CB6 mice, a dissociation between resistance to *L. monocytogenes* and *M. lepraemurium*. All three mouse strains responded strongly to *M. bovis* BCG, but there was a suggestion that *nu/+* mice might be more susceptible to this agent than the other two strains. I concluded that the failure of *nu/+* mice to restrict the growth of *M. lepraemurium* more than *nu/nu* mice was due to the intrinsic genetic susceptibility of both types of mice. In effect, the *nu/+* mice behaved like *nu/nu* mice, as if they too were deficient in T lymphocytes that were responsive to *M. lepraemurium*.—Author's Abstract

Levy, L., Aizer, F., Bejar, C., Lutsky, I. and Mor, N. Experimental mycobacterial infections of CBA/N mice. Israel J. Med. Sci. **20** (1984) 598–602.

In an effort to assess the contribution of B-lymphocyte-mediated mechanisms to the immune responses to several mycobacteria,

responses to these intracellular pathogens were compared in immunologically normal CBA/CaHN mice and in histocompatible CBA/N (*Xid*) mice, which exhibit abnormalities of B-lymphocyte function. Swelling in response to local inoculation with *Mycobacterium marinum* was significantly greater in the hind feet of CBA/CaHN mice than in those of CBA/N mice, but the difference was very small. Survival of mice of both strains after i.v. challenge with *M. marinum* or i.p. challenge with *M. lepraemurium* did not differ significantly. Finally, multiplication of *M. leprae* in the foot pads of mice of both strains did not differ significantly. Thus, B-lymphocyte-mediated mechanisms do not appear to be important in the immune responses of mice in experimental infections with these mycobacterial species.—Authors' Abstract

Rojas-Espinosa, O., Quesada-Pascual, F., Estrada-Parra, S. and Ramirez-Almaraz, J. A. An attempt to infect turtles (*Kinosternon leucostomum*) with *Mycobacterium leprae* and *M. lepraemurium*. Dev. Comp. Immunol. **9** (1985) 147–150.

Seven turtles received *Mycobacterium lepraemurium* intraperitoneally and 5 turtles were given *M. leprae* intravenously. Three armadillos were also inoculated with the human *M. leprae* used to inoculate the turtles, and 2 of the armadillos developed a massive systemic infection 17 months after inoculation. Periodic harvesting of the turtles showed limited multiplication of *M. lepraemurium* up to 9 months after inoculation. The degree of infection was considerably less than that occurring in mice inoculated simultaneously. *M. leprae*-inoculated turtles showed bacilli persisting up to 23 months post-inoculation. There was no clear evidence of multiplication of *M. leprae,* but the bacilli appeared viable as measured by the fluorescein diacetate-ethidium bromide staining technique.—(*From* the Article)

Epidemiology and Prevention

Assefi, V. [Clinical and epidemiological importance of leprosy in children in some regions of Iran.] Acta Med. Iranica **24** (1982) 99–106. (in French)

Writing from the Clinical Research Service of the Pasteur Institute in Teheran, the author describes an epidemiological survey in the regions of Khorassan in the northeast and Guilan in the north, Behkadeh Radji, and 2 leprosaria, during which 6250 children between the ages of 0 and 15 years were examined. Clinical examination was supplemented in suspicious cases by slit-skin smears and biopsy. Forty-two children with leprosy were discovered, and the source of infection (in mother or father, or both) was identified in no fewer than 31 cases. Attention is drawn to the fact that leprosy in children is not exceptional in Iran, and that the early signs may easily be confused with a number of simple dermatological conditions.—A. C. McDougall (*From* Trop. Dis. Bull.)

Ferreira, J., Bernardi, C. D. V. and Gerbase, A. C. An analysis of leprosy incidence by patient age and the clinical form of the disease. PAHO Bull. **18** (1984) 400–403.

The proportion of individuals at risk of contracting a multibacillary form of leprosy is roughly the same in any age group, since the proportion of those unable to develop cellular immunity remains constant. However, the proportion of individuals at risk of contracting a paucibacillary form diminishes with age, since the proportion of Mitsuda-positive individuals (those who have developed cellular immunity) increases with age. This explains why the proportion of paucibacillary forms is greater among children and young adults than it is among older adults.

Since the number of contagious patients (and, consequently, the "supply" of bacilli capable of causing infections) is greater in high-prevalence areas, the probability that

an individual will receive a bacillary dose sufficient to induce leprosy at an early age is much greater in high-prevalence areas than in low-prevalence areas. Consequently, one would logically expect to find a much larger proportion of young patients in high-prevalence regions and since paucibacillary forms predominate among such patients, one would also expect paucibacillary forms to predominate in such regions. Conversely, in low-prevalence areas it is less likely that people will be exposed to an infectious dose of *Mycobacterium leprae* early in life and so the people contracting the disease will tend to be older, and there will tend to be a higher proportion of multibacillary forms.—(*From* the Article)

Jesudasan, K. and Christian, M. Surveillance in leprosy. Indian J. Lepr. **57** (1985) 132–137.

Surveillance in leprosy forms an integral part of leprosy control activities. Usually contact and school surveys are done annually and general population surveys are done once every 3–5 years. Data is presented which suggest that frequently done surveys may not be cost effective as a means of case detection. Carefully done general surveys once every 3 years, covering contacts as well as school children, may be adequate and more cost effective in endemic areas; whereas contact surveys, surveys of high risk groups and contact tracing may be more relevant in low-endemic areas.—Authors' Abstract

Khotko, N. I. [On the epidemiological characteristics of Angola.] Zh. Mikrobiol. Epidemiol. Immunobiol. **2** (1985) 81–88. (in Russian)

The epidemiological analysis of infectious morbidity for recent years has been made and the main nosological forms existing in Angola (malaria, tuberculosis, leprosy, African trypanosomiasis, plague, intestinal diseases, etc.) have been briefly characterized on the basis of primary medical reports and the data provided by the literature and experimental work. This analysis creates the necessary prerequisites which enable the local public health organs to determine the regularities of the epidemic process, thus making it possible to take rational prophylactic measures and to organize proper epidemiological supervision.—Author's English Abstract

Ramanan, C., Manglani, P. R. and Chandrakar, K. P. A retrospective study of leprosy in an industrial hospital. J. Commun. Dis. **16** (1984) 169–175.

A retrospective study of leprosy patients registered between January 1980 to December 1982 with the Skin Department of the Main Hospital, Bhilai Steel Plant, was carried out in order to ascertain the magnitude of the leprosy problem among the employees and their dependents and to plan preventive control measures based on the findings. The attendance and the number of leprosy patients registered showed an increase during the period 1980–1982. The commonest clinical type of leprosy in adults and children was the tuberculoid variety (44.41% and 49.43%, respectively). The maximum number of cases was found in the age group 31–45 years. There was high childhood leprosy. Progressive forms of leprosy in children were encountered in 17%. There were only 4 cases of lepromatous leprosy in children. The maximum (40.6%) were from within the sectors of the township, while 36% were from Khursipara and a labor camp area on the outskirts. The largest number (43.7%) of the cases of lepromatous leprosy was from the villages and mines around Bhilai, followed by the Khursipara and labor camp areas (30.5%). The significance of these findings is discussed and the need for a leprosy control program is stressed.—Authors' Abstract

Schlagel, C. J., Hadfield, T. L. and Meyers, W. M. Leprosy in the Armed Forces of the United States: newly reported cases from 1970 to 1983. Milit. Med. **150** (1985) 427–430.

Despite recruitment of U.S. military personnel from areas where leprosy is endemic, and despite assignment of personnel to endemic areas, the prevalence of leprosy among active duty personnel and dependents has never been reported. During the period 1970–1983, leprosy was diagnosed in 89 active duty U.S. military personnel

and 54 dependents at U.S. military hospitals and clinics. The incidence of leprosy in the military was low, but greater than that in the general U.S. population. The military medical community must be cognizant of the clinical presentation of leprosy because of continued recruitment from and assignment of personnel to leprosy-endemic areas.—Authors' Abstract

Tiwari, V. D. and Tutakne, M. A. Epidemiological and clinical aspects of leprosy in Indian Armed Forces. Indian J. Lepr. **57** (1985) 124–131.

Epidemiological and clinical aspects of leprosy in 1911 cases of the disease in armed forces personnel were studied. Typewise distribution of cases was: tuberculoid 53.53%, lepromatous 20.57%, indeterminate 8.74%, borderline 11.67%, and polyneuritic 5.49%. The maximum leprosy cases occurred in those belonging to Uttar Pradesh, 17.11%. The maximum number of cases (88.55%) were detected in the age group 20–30 years. The incidence of leprosy increased with increased years of service; 11.82% of the patients were illiterate; 89.85% of the patients earned Rs. 200–499 per month; 56.08% of the patients had no landed property; the houses of 47.29% of the cases were located in congested areas; and 68.23% of the patients had to support larger families. In 95.94% of the cases no family members were examined for leprosy. Diet, smoking, and alcohol appear to have had no relation to the disease in the cases studied. The clinical presentation of the cases was classical and type specific. Skin eruption and loss of sensation were the most common symptoms. Leprosy lesions were detected on almost all parts of the body. Thermal sensation was the most common modality lost. The ulnar, lateral popliteal, and greater auricular were the nerves frequently affected. Among complications, paralytic deformities were common (16.09%); 545 complications were detected in 1911 cases. While 84.29% of the patients had put in more than 4 years of service, the source of infection was known in only 0.57% positively (intrafamilial).—Authors' Abstract

Zahaf, A. and Abbes, S. La lèpre dans le Sud tunisien. Etude préliminaire. [Leprosy in southern Tunisia. A preliminary report.] Acta Leprol. (Genève) **3** (1985) 83–84. (in French)

One hundred-nine leprosy cases have been recorded by the service of dermatology in Sfax (the only specialized center in the south of Tunisia) during the last decade. These cases have essentially come from two regions: Sfax and Malloulech. The delay in diagnosis and the failure to discover additional cases are due to a number of factors.—(*From* Authors' English Summary)

Zahaf, A., Abbes, S. and Yengui, M. Quatre nouveaux cas de lèpre dépistés dans une localité du Sud tunisien. [Four new cases of leprosy tracked down in one locality in southern Tunisia.] Acta Leprol. (Genève) **3** (1985) 79–82. (in French)

Having found a new case of leprosy from Beni Khadech, the Sfax service of dermatology decided to investigate two places in this region: Elmenzla and Gattar. Beni Khadech, an endemic region, had 30 cases of leprosy in 1978 compared with 164 known cases in Tunisia as a whole. Of 1688 systematically examined subjects, 4 new cases were discovered, 3 of whom were contagious.—(*From* Authors' English Summary)

Rehabilitation

Beine, A. Extensor pollicis brevis deviation graft operation in case of Z-deformity of the thumb in ulnar palsy—a preliminary report. Indian J. Lepr. **57** (1985) 196–199.

This new procedure is described as an analogous operation to the extensor diversion graft operation for correction of claw fingers. The procedure is found useful to stabilize the metacarpophalangeal joint of

the thumb preventing or correcting Z-deformity and weakness of the pinch showing many advantages compared to other procedures. A single case is shown as a preliminary report.—Author's Abstract

Chaise, F. and Roger, B. Neurolysis of the common peroneal nerve in leprosy. A report on 22 patients. J. Bone Joint Surg. [Br.] **67** (1985) 426–429.

Thirty-two operations on the common peroneal nerve for leprous neuritis are reported. A combined medical and surgical approach to treatment is recommended, and the technique of operation is described. Recovery of motor power was satisfactory but depended on many factors, including the duration of the neuritis, the extent of the compression, the immunopathological status of the patient, and the efficacy of medical treatment. The main indication for neurolysis is hyperalgesic neuritis. The only contraindication is painless long-standing paralysis; in this condition the degree of neural fibrosis prevents any hope of improvement.—Authors' Abstract

Chaise, F. and Sedel, L. Évaluation des pressions canalaires et sous-épineurales dans les syndromes de souffrance du nerf médian lépreaux au poignet. [Measurement of carpal tunnel and intraneural pressures in leprous neuropathies of the median nerve in the wrist.] Ann. Chir. Main **3** (1984) 271–274. (in French)

The authors measured the carpal tunnel and intraneural pressures with a soft catheter in 15 cases of leprous neuritis of the median nerve at the wrist. In the carpal tunnel, the pressure was about 40 mm of mercury, while within the nerve, the pressure was about 25 mm of mercury. In 5 healthy male volunteers, the same pressures were measured but the results were much lower (2 to 4 mm of mercury). The authors call attention to the high pressures found in acute leprous neuritis. They conclude that the sooner a surgical procedure (careful neurolysis) is performed, the better the results, before irreparable nerve damage occurs.—Authors' English Summary

Kaur, P. and Singh, G. Deformities in leprosy patients attending urban leprosy clinic at Varanasi. Indian J. Lepr. **57** (1985) 178–182.

An analysis of the records of the urban leprosy clinic of the University Hospital, Varanasi, India, for deformities was done for the years 1979 to 1983. The gross deformity rate was only 3.73%. The majority of the cases of deformity were in the borderline type of leprosy. The most common deformity was claw hand. It is discussed that dapsone treatment does not increase the chance of deformity. The rate, however, increased with age.—Authors' Abstract

Kumar, K., Kant, M. and Belsare, R. K. Neuropathic plantar ulceration. Indian J. Lepr. **57** (1985) 172–177.

The management of 111 trophic plantar ulcers existing in 100 patients with leprosy has been reviewed. Male preponderance with 4th and 5th decade of life predilection were observed. Most of the patients were of the lepromatous type. Ulcers were grouped into superficial and deep. They were treated by various methods, i.e., plaster cast alone and in combination with curettage, posterior tibial neurovascular decompression and metatarsectomy. It was observed that ulcers heal faster if conservative plaster cast treatment is combined with surgical treatment of the ulcer. Indications for various surgical methods are defined. The role of proper footwear in the management of planter ulcers has been emphasized.—Authors' Abstract

Lang-Stevenson, A. I., Sharrard, W. J. W., Betts, R. P. and Duckworth, T. Neuropathic ulcers of the foot. J. Bone Joint Surg. [Br.] **67** (1985) 438–442.

We report a prospective study of the causes and treatment of 26 long-standing neuropathic ulcers of the foot in 21 patients. The most important casual factor, well illustrated by pressure studies, was the presence of a dynamic or static deformity leading to local areas of peak pressure on insensitive skin. All but one of the 26 ulcers had healed after an average of 10 weeks of treatment in a light, skin-tight plaster cast, with the prohibition of weight bearing.

Recurrent ulceration was prevented in all but one foot by early operation to correct

the causative deformity; this was performed after the ulcer had healed and before allowing weight bearing on the limb. Pressure studies after operation confirmed that pressure points had been relieved.—Authors' Abstract

Other Mycobacterial Diseases and Related Entities

Badukshanova, N. M., Fadeeva, N. I., Dykhno, M. M. and Gerasina, S. F. [Characteristic of protein complexes in clinical strains of mycobacteria.] Zh. Mikrobiol. Epidemiol. Immunobiol. **5** (1985) 46–49. (in Russian)

The protein composition of *Mycobacterium tuberculosis* and the molecular weight of proteins contained in these organisms have been determined by the method of electrophoresis in the porosity gradient of polyacrylamide gel. Close similarity between the electrophoretograms of *M. tuberculosis* clinical and laboratory strains has been revealed. The study of 18 strains of different groups of mycobacteria has shown that *M. tuberculosis* are essentially different from opportunistic mycobacteria and acid-resistant saprophytes. These data may be important for the identification and taxonomy of mycobacteria.—Authors' English Abstract

Delaha, E. C. and Garagusi, V. F. Inhibition of mycobacteria by garlic extract (*Allium sativum*). Antimicrob. Agents Chemother. **27** (1985) 485–486.

Thirty strains of mycobacteria, consisting of 17 species, were inhibited by various concentrations of garlic extract incorporated in Middlebrook 7H10 agar. The concentration required ranged from a low of 1.34 mg/ml to a high of 3.35 mg/ml of media. When there were multiple strains of a species, a mean inhibitory concentration was determined for that species. Six strains of *Mycobacterium tuberculosis* required a mean inhibitory concentration of 1.67 mg/ml of media.—Authors' Abstract

Dickinson, J. M. and Mitchison, D. A. Activity of the combination of fludalanine and cycloserine against mycobacteria *in vitro*. Tubercle **66** (1985) 109–115.

The initial steps in the incorporation of alanine into the bacterial cell wall include the conversion of natural to D-alanine by a racemase followed by the coupling of 2 D-alanine molecules by a synthetase to yield a dipeptide. A combination of fludalanine (3-fluoro-2-deutero-D-alanine), an analogue of D-alanine that irreversibly inactivates the racemase, and cycloserine, which inhibits the synthetase, has been found to be more active against a wide range of non-mycobacterial organisms than either fludalanine or cycloserine alone. When tested against 16 strains of slowly growing mycobacteria including *Mycobacterium tuberculosis,* the combination was no more active than cycloserine alone. However, the cycloserine minimal inhibitory concentration (MIC) of the combination against the rapidly growing species *M. phlei* and *M. fortuitum* was much lower than the MIC of cycloserine alone, particularly with low ratios of fludalanine to cycloserine, and was within the range attainable by therapeutic cycloserine plasma concentrations in man, suggesting its possible use in the treatment of disease due to *M. fortuitum.*—Authors' Summary

Hardas, U. D. and Jayaraman, V. S. Pattern of drug resistance in tuberculosis. Indian J. Tuberc. **31** (1984) 168–170.

Examination in Nagpur, India, of 255 isolates of *Mycobacterium tuberculosis* from previously untreated patients and 500 isolates from patients who had been treated for more than 6 months and still had active disease showed that the prevalence of initial drug resistance (16.9%) and of acquired drug resistance (71.1%) for isoniazid and streptomycin (singly or combined) had not changed from that reported from the same laboratory 15 years previously in 1966.—C. A. Brown (Trop. Dis. Bull.)

Hopewell, P. C., Sanchez-Hernandez, M., Baron, R. B. and Ganter, B. Operational evaluation of treatment for tuberculosis: results of a "standard" 12-month regimen in Peru. Am. Rev. Respir. Dis. **129** (1984) 439–443.

This study of patient records in Peru reveals a disappointing and disturbingly high level of failure in tuberculosis chemotherapy with a 12-month regimen of isoniazid, streptomycin and thiacetazone. Among the major problems were: inadequate record keeping, high treatment abandonment rates (76% of selected group within 6 months), lack of any bacteriological evaluation of treatment, an estimated high proportion (up to 25% of patients treated) with organisms resistant to isoniazid or streptomycin. Introduction of new shorter treatment regimens would require a tighter program control and, as rifampicin and pyrazinamide would be used, would cost 5–8 times more—gloomy prospects for a developing country.—M. Hooper (Trop. Dis. Bull.)

Kürkçüoğlu, N., Atakan, N. and Eksioğlu, M. Thalidomide in the treatment of recurrent necrotic mucocutaneous aphthae. (Letter) Br. J. Dermatol. **112** (1985) 632.

Thalidomide seems to have beneficial effect in the treatment of recurrent mucocutaneous aphthae. We treated 5 patients with thalidomide, all of whom had suffered from recurrent, painful, deep necrotic mucocutaneous aphthae for the last 2 years. Several treatments including topical tetracycline, topical corticosteroids, levamisole, and colchicine had been employed with only temporary beneficial effect. The dosage of thalidomide was 400 mg/day for the first 5 days, followed by 200 mg/day thereafter in 2 divided doses for 30 days. The oral ulcerations improved and healed completely within 7 days. We did not seen any recurrence during the treatment period. Only 1 patient experienced a recurrence after the cessation of thalidomide. However, the new lesion was much smaller and disappeared without any medication within a couple of days.—(*From* the Letter)

Martin, W. J., II, and Kachel, D. L. Reduction of neutrophil-mediated injury to pulmonary endothelial cells by dapsone. Am. Rev. Respir. Dis. **131** (1985) 544–547.

The presence of activated neutrophils in the alveolar structures is thought to contribute to parenchymal cell injury in various acute and chronic lung disorders. This study indicates that dapsone (30 μg/ml), an agent with anti-inflammatory properties, can significantly reduce neutrophil-mediated injury to ^{51}Cr-labeled bovine pulmonary artery endothelial (BPAE) cells with a reduction in the injury (expressed as a cytotoxic index) from 65 ± 3 to 33 ± 3 ($p < 0.001$). Dapsone was unable to protect ^{51}Cr-labeled BPAE cells injured by the chemical generation of superoxide, hydrogen peroxide, or neutrophil-derived, myeloperoxidase-dependent hypohalite ion. In contrast, dapsone significantly inhibited the respiratory burst of the neutrophil, with a reduction in the generation of superoxide, hydrogen peroxide, and conversion of nitroblue tetrazolium to formazan ($p < 0.01$, all comparisons). Thus, dapsone appears to protect lung parenchymal cells such as endothelial cells from neutrophil-mediated injury by directly inhibiting the respiratory burst of the neutrophil, with a consequent diminution in the generation of toxic, oxygen-derived radicals.—Authors' Summary

Mizuguchi, Y., Ogawa, M. and Udou, T. Morphological changes induced by β-lactam antibiotics in *Mycobacterium avium-intracellulare* complex. Antimicrob. Agents Chemother. **27** (1985) 541–547.

In vitro activity of 7 β-lactam antibiotics against strains of *Mycobacterium avium-intracellulare* was evaluated by the agar dilution method. The activity was influenced by the presence or absence of Tween 80 in Dubos medium, and cephazolin and cefotaxime were effective against most strains in the presence of Tween 80. β-Lactam antibiotics at low concentrations induced long filamentous cells with branching. In contrast to the filaments induced by ampicillin, in which septation was rarely observed, filaments induced by cephazolin had many septa, suggesting that the mechanisms of filament induction were different from the

drugs used. At high concentrations, ampicillin and cephazolin induced osmotically sensitive cells with bulging at the polar end of the cells. Analysis of penicillin-binding proteins (PBPs) of the organism showed that there were at least 9 PBPs with molecular weights between 32,000 and 94,000 in the cytoplasmic membrane. Ampicillin showed the highest affinity for PBPs 1a or 1b, or both, and also PBPs 3a or 3b, or both. In contrast, there was very little specificity of binding of cephazolin for any of the PBPs.—Authors' Abstract

Mookerjee, B. K. and Pauly, J. L. Human recombinant interleukin-2 is mitogenic to human lymphocytes. J. Leukocyte Biol. **38** (1985) 553–556.

Reported are the results of studies demonstrating that purified recombinant human interleukin-2 (hrIL-2) is a potent mitogen for lymphocytes of healthy human donors. The specificity of the hrIL-2-induced response was defined in experiments in which mitogenicity of this T cell growth-promoting lymphokine was completely abrogated by blocking the T cell membrane receptor for IL-2 with the anti-Tac monoclonal antibody. Depletion of adherent mononuclear leukocytes markedly reduced lymphocyte reactivity to hrIL-2, but the response could be fully recovered by the addition of interleukin-1.

Increased proliferative responses were observed using a combination of hrIL-2 and a monoclonal antibody OKT3 that defines a T cell membrane antigen. These studies demonstrate that hrIL-2, as with antigens and phytomitogens, may serve as the first signal of T cell proliferation.—Authors' Abstract

Mukerjee, C. M. and McKenzie, D. K. Safety of thrice-weekly rifampicin for tuberculosis in Southeast Asian refugees. Aust. NZ J. Med. **15** (1985) 226–229.

The incidence and types of adverse reactions to rifampin (in combination with isoniazid, pyrazinamide, and ethambutol) have been studied in 86 Southeast Asian refugees treated for tuberculosis in Australia. Most patients received daily therapy initially (mean 3.5 months) followed by supervised thrice-weekly treatment (mean 4.6 months). Minor adverse reactions occurred with similar frequencies during daily (5%) and intermittent (5%) treatment but in no case was modification of rifampin dosage required. Withdrawal of pyrazinamide was necessary in 2 patients (2.3%) with clinical hepatitis.—Authors' Abstract

Murray, R. I. and Daya, H. Intra-ocular tuberculosis associated with a penetrating injury. A case report. S. Afr. Med. J. **67** (1985) 603–604.

A case of histologically proven intra-ocular tuberculosis is described. The condition was preceded by penetrating trauma, and no evidence of systemic tuberculosis other than a positive Mantoux test was found. Numerous acid-fast bacilli were seen in pathological sections of the eye. We suspect that a tuberculosis bacillemia from an undetected healed focus coincided with the trauma and thereby produced intra-ocular tuberculosis.—Authors' Summary

Orme, I. M. and Collins, F. M. Efficacy of *Mycobacterium bovis* BCG vaccination in mice undergoing prior pulmonary infection with atypical mycobacteria. Infect. Immun. **44** (1984) 28–32.

Interactions between the host response to atypical mycobacteria and to BCG vaccine have been suggested as a possible explanation for the failure of the BCG vaccination trial against tuberculosis in Madras. To test this hypothesis the authors infected mice aerogenically (10^4 viable organisms delivered to the lungs over 30 min) with 1 of 4 strains of atypical mycobacteria, immunized the mice intravenously 30 days later with 10^6 viable BCG (Trudeau), and then gave the mice an aerogenic challenge after a further 60 days with 10^4 *Mycobacterium tuberculosis* H37Rv. The course of the various infections was followed by counts of viable organisms in the tissues and by mouse survival.

Viable *M. scrofulaceum* became undetectable in the lungs within 30 days, numbers of *M. kansasii* and *M. simiae* declined slowly over 150 days, and *M. avium* showed a progressive increase in numbers for at least 120 days. BCG vaccination had no effect on

the course of infection with the last 3 organisms.

In the absence of BCG vaccination, *M. scrofulaceum* failed to protect against *M. tuberculosis* but a pulmonary infection with *M. kansasii, M. simiae* or *M. avium* prolonged the survival of mice challenged with *M. tuberculosis.* The extent of protection depended on the atypical mycobacterium tested, and *M. avium* appeared as effective as BCG in restricting growth in the lungs. Pulmonary infection with any of the 4 atypical mycobacteria had no effect on the protection provided by BCG against challenge with *M. tuberculosis* H37Rv.

The authors conclude that previous infection with atypical mycobacteria may mask the protective effect of BCG vaccination in that protection already exists but that even a pulmonary infection with a moderate dose of those organisms tested does not interfere with the efficacy of BCG vaccination in the conditions used.—Carolyn A. Brown (*From* Trop. Dis. Bull.)

Orme, I. and Collins, F. M. Prophylactic effect in mice of BCG vaccination against nontuberculous mycobacterial infections. Tubercle **66** (1985) 117–120.

The effect of prior vaccination of mice with *Mycobacterium bovis* BCG on the subsequent course of acute aerogenic infection with various environmental mycobacteria was tested. A protective effect was recorded against infection with *M. avium,* and *M. kansasii*; by contrast no effect was noted against *M. simiae* or *M. intracellulare.* The prophylactic effects of BCG were demonstrated regardless of whether vaccination was given by the intravenous or aerogenic routes. These findings support the hypothesis that BCG vaccination, if given prior to contact, can provide some degree of protection against certain nontuberculous mycobacterial infections.—Authors' Summary

Perkus, M. E., Piccini, A., Lipinskas, B. R. and Paoletti, E. Recombinant vaccinia virus: immunization against multiple pathogens. Science **229** (1985) 981–984.

The coding sequences for the hepatitis B virus surface antigen, the herpes simplex virus glycoprotein D, and the influenza virus hemagglutinin were inserted into a single vaccinia virus genome. Rabbits inoculated intravenously or intradermally with this polyvalent vaccinia virus recombinant produced antibodies reactive to all 3 authentic foreign antigens. In addition, the feasibility of multiple rounds of vaccination with recombinant vaccinia virus was demonstrated.—Authors' Abstract

Prioli, R. P., Tanna, A. and Brown, I. N. Rapid methods for counting mycobacteria—comparison of methods for extraction of mycobacterial adenosine triphosphate(ATP)determined by firefly luciferase assay. Tubercle **66** (1985) 99–108.

A comparison of 5 different methods of extraction of adenosine 5′-triphosphate (ATP) from mycobacterial cells was carried out using *Mycobacterium bovis,* BCG as a model. ATP was measured using the luciferin-luciferase bioluminescence reaction. Boiling buffer extraction was the best method. The amount of ATP extracted correlated with the number of colony-forming units over a wide range of counts. Although great sensitivity in terms of number of bacilli detectable was not achieved, the method was rapid and appears suitable for drug sensitivity testing of tubercle bacilli.—Authors' Summary

Shatrov, V. A., Kuznetsouva, L. V. and Belyanovskaya, T. I. [Evaluation of the peripheral blood monocyte function in patients with pulmonary tuberculosis.] Zh. Mikrobiol. Epidemiol. Immunobiol. **5** (1985) 76–78. (in Russian)

As the result of the study of the peripheral blood monocyte function in patients with pulmonary tuberculosis, the ingestive capacity of monocytes has been found to be suppressed, which indicates the pathological state of oxygen-dependent mechanisms governing the bactericidal activity of cells, the most pronounced disturbances of monocyte functions being observed in patients with fibrocavitary and disseminated tuberculosis.—Authors' English Abstract

Steffen, C. Eosinophilic pustular folliculitis (Ofuji's disease) with response to dapsone therapy. Arch. Dermatol. **121** (1985) 921–923.

A 50-year-old man had eosinophilic pustular folliculitis (Ofuji's disease) characterized by follicular pustular papules on the face, confluent vesicles on the fingers, and a papulopustular area on the upper portion of the back. Extensive examinations and cultures of both pustular material and tissue revealed no organisms, except *Staphylococcus epidermidis.* The disease responded to dapsone therapy.—Author's Abstract

van Tiel, F. H., Boere, W. A. M., Harmsen, T., Kraaijeveld, C. A. and Snippe, H. Determination of inhibitory concentrations of antiviral agents in cell culture by use of an enzyme immunoassay with virus-specific, peroxidase-labeled monoclonal antibodies. Antimicrob. Agents Chemother. **27** (1985) 802–805.

An enzyme immunoassay (EIA) to determine 50% inhibitory concentrations of drugs which suppress Semliki Forest virus replication is described. Inhibition of virus replication was measured in L cells, seeded as monolayers in 96-well plates by use of horseradish peroxidase-labeled monoclonal antibodies directed against the E1 glycoprotein of Semliki Forest virus. The antiviral agents tested were cycloheximide, tunicamycin, NH_4Cl, and disodium cromoglycate. The 50% inhibitory concentration of these antiviral agents was arbitrarily defined as the concentration of drug, in culture medium, associated with 50% reduction of the control absorbance value measured on Semliki Forest virus-infected cells without drug in the culture fluid. Twenty-two hours after infection the 50% inhibitory concentrations of the drugs were 0.2 μg/ml for cycloheximide, 0.8 μg/ml for tunicamycin, 0.3 mg/ml for NH_4Cl, and 4.9 mg/ml for disodium cromoglycate. These values are similar to those determined by others with conventional methods of virus quantification. This test is sensitive and easy to perform and therefore is suited for large-scale experiments.—Authors' Abstract

Young, R. A., Bloom, B. R., Grosskinsky, C. M., Ivanyi, J., Thomas, D. and Davis, R. W. Dissection of *Mycobacterium tuberculosis* antigens using recombinant DNA. Proc. Natl. Acad. Sci. U.S.A. **82** (1985) 2583–2587.

A recombinant DNA strategy has been used systematically to survey the *Mycobacterium tuberculosis* genome for sequences that encode specific antigens detected by monoclonal antibodies. *M. tuberculosis* genomic DNA fragments with randomly generated endpoints were used to construct a large λgt11 recombinant DNA expression library. Sufficient numbers of recombinants were produced to contain inserts whose endpoints occur at nearly every base pair in the pathogen genome. Protein antigens specified by linear segments of pathogen DNA and produced by the recombinant phage of *Escherichia coli* were screened with monoclonal antibody probes. This approach was coupled with an improved detection method for gene isolation using antibodies to clonally isolate DNA sequences that specify polypeptide components of *M. tuberculosis.* The methodology described here, which is applicable to other pathogens, offers possibilities for the development of more sensitive and specific immunodiagnostic and sero-epidemiological tests for tuberculosis and, ultimately, for the development of more effective vaccines.—Authors' Abstract