The 1984 JOURNAL—a Continuing Perspective

Nineteen hundred eighty-four marked the 52nd year of publication of the JOURNAL and witnessed the largest International Leprosy Congress to date in New Delhi with hundreds of stimulating presentations. It seems appropriate to once more review the considerable progress that has been made in our understanding of leprosy as reflected in the 1984 JOURNAL.

In the March issue, the original article by Pattyn, *et al.* (1–6)* found that 4.5% of patients treated with daily rifampin plus ethionamide/prothionamide developed hepatotoxicity with an overall mortality of 26% from this hepatitis. Shepard, *et al.* (7–9) were not able to show antibacterial activity by mouse foot pad testing with four 2-acetylpyridine thiosemicarbazones. Stenstrom (10–18) described an apparatus to measure the relative evaporation of moisture from the skin and applied these measurements to leprosy patients. Surprisingly, anesthetic areas showed substantial increases in humidity after sun exposure. Soaking was shown not to be necessary to provide hydration. Topical Vaseline or zinc tape application was quite effective alone. Douglas, *et al.* (19–25) developed an enzyme-linked immunosorbent assay (ELISA) for detection of antibodies in leprosy using whole mycobacterial cells as antigen. *Mycobacterium smegmatis* was the most reactive against lepromatous sera other than *M. leprae* itself. Douglas and Worth (26–33) applied the ELISA using *M. smegmatis* under field conditions and found that elevated antibody levels were detected up to two years prior to clinical onset of disease in 70% of new cases. Humber (34–40) described a stratified sample technique for counting bacilli which improves the accuracy of the count by partially compensating for the uneven distribution of bacilli across the customary counting area. Mori, *et al.* (41–43) described a Percoll gradient centrifugation method for separating leprosy bacilli from infected armadillo liver which is simple, effective, and avoids the use of enzymes. Saha, *et al.* (44–48) studied circulating immune complexes in erythema nodosum leprosum (ENL) patients after precipitation of the complexes with polyethylene glycol. These precipitates contained predominantly IgG, IgM and Clq, and had variable anti-complementary properties. Wheeler (49–54) detected superoxide dismutase in cell-free extracts of purified *M. leprae.* Martens and Klingmuller (55–60) studied the subepidermal grenz zone of lepromatous leprosy and found that this zone contained leprosy bacilli and was not necessarily characteristic of leprosy since it could be found in other diseases as well. Ridley, *et al.* (61–65) studied the ultrastructure of connective tissue ENL. In the ENL lesions of the type associated with severe damage to connective tissue, large quantities of bacterial debris were demonstrated intracellularly and extracellularly, particularly bound to collagen and elastic fibers. The suggestion was made that complexing of mycobacterial antigen to connective tissue elements may be the major factor in the connective tissue damage. Ferreira, *et al.* (66–73) outlined three criteria for "statistical discharge" of Hansen's disease patients from active records based on the patient's age, number of years out of control, and probability of being alive, calculated according to a recent mortality table.

In the Editorial section of the March issue, Shepard (74–77) outlined the two principal viewpoints regarding the immunology of leprosy. In the traditional leprologist's approach, Mitsuda reactivity is considered to be genetically controlled. In the so-called immunologist's view, the unresponsiveness in lepromatous leprosy is due to tolerance and is an accident of infection. Decisive evidence to select between these points of view does not seem to be presently available. Hastings (78–91) reviewed the contents of the 1983 JOURNAL.

In the Correspondence section of the March issue, Mathur, *et al.* (92–93) and Liu (93) discussed the role of epidermal Langerhans' cells in leprosy. Tanke, *et al.* (93–96) carefully examined the fluorescence mi-

* Numbers in parentheses refer to page numbers in the INTERNATIONAL JOURNAL OF LEPROSY, Volume 52, 1984.

croscopy involved in the fluorescent leprosy antibody absorption test. Furukawa, *et al.* (96–98) found a high proportion of borderline leprosy patients with circulating immune complexes and anti-nuclear antibodies. Kato (99–100) found that humic acid had a definite promoting effect on the growth of "*Mycobacterium X*" derived from *M. leprae*-infected tissues. Nine cases of rifampin-resistant leprosy were described by Guelpa-Lauras, *et al.* (101–102).

In the News and Notes section of the March issue, Baba Amte was honored as the 1983 Damien-Dutton Award winner (103–104).

In the Current Literature section of the March issue, Almeida, *et al.* (112) estimated that between 89 and 116 patients per 1000 dapsone-treated patients with LL and BL disease were estimated to harbor dapsone-resistant *M. leprae* according to results of mouse foot pad inoculations. Barnhill and McDougall (113) reviewed the use of thalidomide in reactional lepromatous leprosy and in various other conditions. Boddingius, *et al.* (113) were not able to find dapsone in peripheral nerve tissue from mice fed dapsone, supporting a previous hypothesis that dapsone may not sufficiently penetrate and/or be stored in sufficient quantities in peripheral nerves to be active against *M. leprae* in those locations. Cynamon and Palmer (115) reported that *M. fortuitum* was susceptible to amoxicillin or cephalothin, particularly in combination with clavulanic acid. Hastings and Jacobson (115) showed that ansamycin (LM-427) is active against *M. leprae* in mouse foot pad infections. Polasa and Krishnaswamy (116) reported that food significantly interfered with the bioavailability of oral rifampin. Girdhar, *et al.* (117) described a case of borderline lepromatous leprosy in a 19-month-old infant with onset at the age of 9–10 months. Katoch, *et al.* (118) found no relationship between leprosy and the prevalence of hepatitis-B surface antigen in blood. Mathur, *et al.* (118) reported regrowth of eyebrows in lepromatous leprosy patients being treated with zinc sulfate plus dapsone. In cases lacking eyebrows who received dapsone alone, no regrowth was observed. Pannikar, *et al.* (119) studied 17 patients with primary neuritic leprosy. During a follow-up period of two years, 4 of the 17 developed skin lesions; 7 of the 17 were classified as lepromatous and 10 as nonlepromatous. Piepkorn, *et al.* (119) reported improvement in a patient with Lucio's phenomenon after immunosuppressive therapy and plasmapheresis. Based on EKG changes, Zawar, *et al.* (120) suggested that the myocardium may be involved in patients with lepra reaction. Boddingius, *et al.* (120–121) found solid *M. leprae* inside a tibial nerve from an amputated extremity of a patient treated for 20 years with dapsone and 5 years with rifampin. Bullock, *et al.* (121) showed that lepromatous leprosy patients' peripheral blood mononuclear cells have abnormally high responses by B lymphocytes in secreting immunoglobulin in response to pokeweed mitogen. This abnormality is associated with a loss of regulatory function by suppressor T lymphocytes in lepromatous patients. Mshana, *et al.* (122–123) were not able to show *in vitro* lymphocyte stimulation to peripheral nerve myelin proteins in leprosy patients, suggesting that hypersensitivity to intraneurally located *M. leprae* antigens is the main mechanism whereby nerve damage is produced in leprosy. Olcén, *et al.* (123) found evidence of antigens of *M. leprae* and anti-*M. leprae* antibodies of the IgG class in the urine of newly diagnosed, untreated leprosy patients. Sengupta, *et al.* (123) studied lymphoproliferative responses in *vitro* to *M. leprae* in peripheral blood mononuclear cells from lepromatous leprosy patients and seven siblings of these patients who were HLA-D identical. These HLA-D identical siblings responded normally to *M. leprae* antigen, while the lepromatous leprosy patients showed no response. The specific unresponsiveness of lepromatous leprosy patients to *M. leprae* antigen does not, therefore, seem to result from an HLA-linked genetic defect. Cocito and Delville (124) reviewed the properties of leprosy-derived corynebacteria and concluded that these organisms represent an homogeneous and unique group of corynebacteria immunologically related to *M. leprae.* Dutta, *et al.* (124) found that some cultivable mycobacterium, notably *M. vaccae* and *M. phlei,* as well as *M. leprae*, lost their acid fastness when extracted with fresh pyridine for two hours at room temperature. Khanolkar and Wheeler (125) found that *M. leprae* incorporated purines more rapidly than py-

rimidines into nucleic acids *in vitro.* Purine synthesis *de novo* took place at a very slow rate, suggesting a preference of the organism for pre-formed purines. In murine and human macrophages *in vitro,* Mittal, *et al.* (125) showed that the uptake of ^{3}H-thymidine by *M. leprae* was significantly inhibited by concentrations of rifampin as low as 3 ng/ml. Wheeler and Bharadwaj (125) found that *M. leprae* has a NAD-dependent malate dehydrogenase, a FAD-dependent malate-vitamin K reductase, but does not possess malic enzyme. Hamilton (128) emphasized the importance of preventing deformity among leprosy patients by detecting and treating neuritis, particularly "silent neuritis." Kumar and Anbalgan (128) described socio-economic deterioration of significant proportion in leprosy patients following the diagnosis. Ridell (131) studied 141 strains of *Actinomycetales* and related organisms by immunodiffusion for the presence of antigens which crossreact with the antigens of *M. tuberculosis* H37Rv. More than 90% of these strains were shown to have one or more antigens in common with the tubercle bacillus and 77% had two or more antigens in common.

In the June issue's Original Articles, Miller, *et al.* (133–139) measured antibody to mycobacterial arabinomannan in the sera of leprosy patients using an enzyme-linked immunosorbent assay. The antibody levels were directly proportional to the quantity of *M. leprae* present in each patient. Stach, *et al.* (140–146) found that macrophages from lepromatous leprosy patients inhibited the *in vitro* incorporation of uracil into viable BCG to a greater extent than did cells from tuberculoid patients. Nelson, *et al.* (147–153) found that children from lepromatous families who were initially PPD negative showed significantly decreased PPD conversion rates after BCG vacination compared with children from tuberculoid families or normal families. Sarracent and Finlay (154–158) studied the macrophages of mice treated with clofazimine and found that the drug increased the activity of various lysosomal enzymes and increased the phagocytic capability of these cells. Sinha, *et al.* (159–162) found reduced blood ascorbic acid levels in both polar types of leprosy. Lactic and pyruvic acids were found to be significantly raised. After ascorbic acid supplementation, blood ascorbic acid levels increased and lactic and pyruvic acid levels fell to near normal values. Haimanot, *et al.* (163–170) reported findings in sural nerve biopsies from leprosy patients. Pathological changes which were greater in degree than those suggested by skin biopsy or clinical examination were frequently found. Gupta, *et al.* (171–175) presented details of laryngeal involvement in a series of ten lepromatous leprosy patients. Kvach, *et al.* (176–182) described a petroleum ether separation technique to purify *M. leprae* from armadillo liver tissue and improved methods for a fluorescent staining procedure designed to measure the viability of *M. leprae* based on the use of fluorescein diacetate and ethidium bromide. Dhople (183–188) measured ATP levels in *M. leprae* from leprosy patients under chemotherapy. There was an excellent correlation between ATP levels and viability by mouse foot pad inoculation. Sharp and Banerjee (189–197) utilized macrophages from nude mice, nude rats, and armadillos in searching for cells which would support the growth of *M. leprae in vitro.* No significant growth of *M. leprae* was obtained after over 200 days of culture, although *M. lepraemurium* were able to multiply in this system. Fukunishi, *et al.* (198–207) studied the ultrastructural features of lepromas from leprosy-infected monkeys and an armadillo by the freeze-etching technique. The ultrastructural features of the leprosy bacilli and the accumulations of small spherical droplets around the leprosy bacilli were similar in all tissues examined.

In the Editorial section of the June issue we were fortunate to have an authoritative review of the biochemistry of *M. leprae* by Wheeler (208–230). In a prize-winning essay, Pallen reviewed the immunological and epidemiological significance of environmental mycobacteria in relation to the control of leprosy and tuberculosis (231–245).

The death of Dr. Jose Tolentino was noted with sadness (246–248).

In the Correspondence section of the June issue, Shetty and Antia (249–251) demonstrated a high incidence of sciatic nerve fibers with myelination around multiple axons in mice inoculated with *M. leprae* into the foot pad and treated with oral dapsone. The occurrence of multiple axonal myelination in this model may represent a mis-

guided regenerative response following partial denervation of unmyelinated fiber groups and thus may imply a defective Schwann cell axon interaction under these conditions. Dang Duc Trach, *et al.* (251–253) showed that lepromatous patients without ENL had significant reductions in theophylline-resistant E-rosette-forming cells in their peripheral blood (a population enriched in helper T lymphocytes) and significantly increased percentages of theophylline-sensitive E-rosetting cells (a population enriched for suppressor T lymphocytes). Amezcua, *et al.* (254–255) presented evidence of naturally acquired leprosy in a Mexican armadillo. Gupta, *et al.* (255) found anti-spermatozoal antibodies in 8 out of 24 male tuberculoid leprosy patients. Testicular biopsies from these patients showed atrophic changes, suggesting that the testes may be damaged by delayed hypersensitivity mechanisms in tuberculoid disease which then results in the production of anti-spermatozoal antibodies.

In the News and Notes section of the June issue, the well-deserved recognition of Dr. Ma Haide in honor of his 50th anniversary of work in China was noted (257).

In the Current Literature section of the June issue, Kumar and Balakrishnan (267) reported an operational study to monitor the regularity of dapsone intake by leprosy outpatients. Only 36% of patients were absolutely regular in attending the clinics. Drug-induced hepatitis was noted in 22–56% of leprosy patients treated with combined therapy by the Leprosarium of Hai-an County, China (267). Mathur, *et al.* (267) found oral zinc sulfate to be useful in the management of recurrent ENL reactions. Moore (268) reviewed the side effects of clofazimine. Valdes-Portela, *et al.* (268) reported that clofazimine treatment of mice increased their antibody-forming cell response after 14 and 21 days of treatment. Green, *et al.* (269) quantitatively estimated the number of leprosy bacilli in exhaled nasal breath and found that 3.8×10^4 bacilli were excreted per breath in nontreated borderline and lepromatous patients. Lamba, *et al.* (270) estimated that approximately 1.5 million leprosy patients are expected to be affected with potentially sight-threatening lesions. Moll (270) found that 14.8% of leprosy patients had diabetes mellitus compared with 6.6% of age-matched controls. Prabhakar, *et al.* (270) suggested that local treatment of the nose of lepromatous leprosy patients with a bactericidal agent might make them noninfectious more rapidly than with systemic treatment alone, and thereby help control the transmission of the disease. Sengupta, *et al.* (271) reported an interesting case of foot drop caused by a Baker's cyst of the knee joint which mimicked neuritic manifestations of leprosy. Venkatesan, *et al.* (271) found elevated levels of serum copper and decreased levels of serum zinc in active lepromatous and reactional BL/LL patients compared to controls and BT patients. A case of Lucio's phenomenon was reported from China by Yuan (272). Bjune, *et al.* (272–273) suggested that research in leprosy should focus more on subclinical and indeterminate leprosy in order to learn more about protective mechanisms which could be non-immunological in nature as well as immunological. Curtis and Turk (273) pointed out the possible dissociations between allergy and effective cell-mediated immunity in clinical leprosy. Holla, *et al.* (274) described a leproma occurring in a lepromatous leprosy patient at autopsy which was attached to the atrial surface of the lateral leaflet of the mitral valve. Kumar, *et al.* (274) studied the effects of dapsone on T cells and their *in vitro* responsiveness to PHA and lepromin in polar tuberculoid leprosy patients. Patients who had taken dapsone, 50 mg daily, for 12–14 months showed a significant decrease in the number of T lymphocytes and significant decreases in their response to PHA and lepromin compared to untreated patients. Mor (275) studied the location of *M. leprae* in normal and immune-deficient mice and concluded that *M. leprae* multiply free in the cytoplasm of the foot pad macrophages of infected mice, whereas the *M. leprae* cells found within the phagosomes of the macrophages are dead. Mshana, *et al.* (275–276) stained peripheral nerve biopsies from leprosy patients with anti-BCG antibody and demonstrated intraneural mycobacterial antigens in a peroxidase-antiperoxidase system. Rook (277) reviewed the immunology of leprosy. In tuberculoid leprosy, the cell-mediated immune response could be directed toward antigenic components which are not released by live organisms so that

the immunologic attack occurs in the wrong places, around dead or "leaking" bacilli. Sathish, *et al.* (277) found that peripheral blood monocytes from polar lepromatous patients were unable to support *M. leprae*-induced lymphoproliferation by HLA-D matched T cells from tuberculoid leprosy patients. Saha, *et al.* (280) described a rapid method for the demonstration of *M. leprae* in the sera of lepromatous leprosy patients by precipitating the sera with polyethylene glycol and examining the precipitates for acid-fast bacilli. Silva and Macedo (280) studied the membranes of *M. leprae* at an ultrastructural level and showed that it was 7.04–7.08 nm thick and has PAS-positive components in both layers, while the membranes of other mycobacterial species studied were 6.36–6.37 nm thick and have PAS-positive components only in the outer layer. Vithala, *et al.* (281) described a technique to measure the sensitivity of *M. leprae* to drugs. The technique is based on the uptake of ^{14}C-acetate into lipids by *M. leprae*-infected tissues from lepromatous leprosy patients in the presence of drug *in vitro*. Baliña, *et al.* (281) described disseminated leprosy in seven-banded armadillos (*Dasypus hybridus*) after inoculation with *M. leprae*. Chehl, *et al.* (282) described the patterns of growth of *M. leprae* in nude mice. Job, *et al.* (282–283) found *M. leprae* in large numbers in liver tissue from an experimentally infected nude mouse. The bacilli were present in large numbers in Kupffer cells, macrophages, endothelial cells, and liver parenchymal cells. Meier, *et al.* (283) studied the ultrastructure of the mycobacteria and host response in the liver and spleen of Texas armadillos with naturally occurring leprosy. Evidence for acid-fast bacillary proliferation in these organs and penetration of hepatocytes was presented. Folse and Smith (283) surveyed wild armadillos from the Texas Gulf Coast and demonstrated naturally occurring leprosy in 4.66% of the animals. Gardner, *et al.* (288–289) described interesting immunologic studies in a patient with long-standing *M. fortuitum* infection. *M. fortuitum* antigen-activated suppressor cells contributed to a lack of bactericidal activity against the organism *in vitro*. The activity of these suppressor cells could be eliminated by the *in vitro* treatment of blood mononuclear cells with a combination of a cholinergic agonist and indomethacin, but not with either drug alone. Administration of the two drugs to the patient resulted in a reversal of the bactericidal defect and dramatic improvement. Radin, *et al.* (291) found that patients with active tuberculosis clearly had higher levels of IgG antibody activity to PPD antigen than did healthy individuals as measured by an enzyme-linked immunosorbent assay.

The Original Articles in the September issue began with the work of Pattyn, *et al.* (297–303) who studied active untreated multibacillary patients receiving six months' combination chemotherapy with rifampin and dapsone and/or rifampin, prothionamide and dapsone, followed by six months' dapsone alone (12 months total) and then discontinuing treatment. No relapses have been observed 4½–5 years after therapy has been discontinued. Jesudasan, *et al.* (304–310) studied relapse rates among indeterminate, tuberculoid (TT), and BT patients whose dapsone monotherapy was discontinued after a minimum period of treatment of 4½ years (two years after the disease had become inactive). Overall, 3% of the patients relapsed for an overall rate of 9.7/1000 person years at risk. Rea, *et al.* (311–317) studied peripheral blood T lymphocyte subsets and found that active, nonreactional lepromatous leprosy patients had a significant lymphopenia but no alteration in the relative proportions of helper and suppressor cells. Nonreactional BL patients had a significant selective deficiency in helper/inducer cells. Active ENL, long-term treated LL, and BT patients showed no abnormalities. Wallach, *et al.* (318–326) analyzed T cell subsets in the dermal granulomas of leprosy patients and found predominantly helper T cells in tuberculoid granulomas with suppressor T cells located at the periphery of well-circumscribed granulomas. In untreated lepromatous lesions, total T cells and helper cells were reduced with twice as many suppressor T cells as helper T cells. In ENL lesions, total T cells increased and there were increased helper and reduced suppressor T cells. Mathur, *et al.* (327–330) found reduced serum zinc levels in leprosy patients in the borderline to lepromatous end of the spectrum. Mathur, *et al.* (331–338) used supplemental oral zinc as an immunostimulant in addition to conventional

antileprosy drugs. Patients treated with zinc showed faster clinical improvement, falls in bacterial index, lymphocyte influx and neovascularization of the granulomas, and more upgrading reactions compared to controls. Soni and Chatterji (339–342) found that 40% of leprosy patients showed some degree of impairment of the sense of smell and suggested that the olfactory nerve may be directly involved in the disease process. Abe, *et al.* (343–350) studied *M. leprae*-specific antibody in saliva of leprosy patients. Salivary IgA, but not IgG or IgM, antibodies were frequently found and were more frequently found in tuberculoid or borderline patients compared to lepromatous cases. Lovik, *et al.* (351–361) succeeded in obtaining partial resistance in genetically highly susceptible C3H mice to *M. lepraemurium* by immunizing with water-soluble antigens of ultrasonicated *M. lepraemurium* in Freund's incomplete adjuvant. Job, *et al.* (362–364) suggested that nine-banded armadillos could be bred in captivity if housed in open-air pens. Sebille, *et al.* (365–370) described a model of *M. leprae*-induced neuropathy using Wistar rats inoculated with *M. leprae* in the foot pad and assessed repeatedly by electrophysiologic methods. After 21 months, bilaterally decreased motor conduction velocities in the sciatic nerves, at the leg, and reduced sensory conduction velocities on the inoculated side were seen. Wheeler, *et al.* (371–376) detected lactate dehydrogenase (LDH) in extracts of *M. leprae* from armadillo liver and showed that the enzyme originated from the bacillus and not the host tissue. Liu and Qiu (377–383) presented pathological findings in 103 autopsies, 210 tuberculoid peripheral nerve biopsies, and 106 inguinal lymph nodes from leprosy patients. Ridley and Ridley (383–394) described an entity termed exacerbation reactions, which are acute reactions occurring locally in highly active lepromatous lesions with heavy bacterial loads.

In the Editorial section of the September issue, Ellard (395–401) reviewed the rationale for the WHO multidrug regimens for the chemotherapy of leprosy for control programs. Srinivasan (402–413) suggested a new model for leprosy based on the "cusp" model in catastrophe theory which can be used in explaining how continuous causes can produce sudden or discontinuous changes.

The deaths of Dr. A. Felix Contreras Duenas of Spain (414–415), Dr. C. G. S. Iyer of India (416–417), and Dr. Pierre Richet of France (418) were noted with sadness.

The Correspondence section of the September issue contained very interesting letters from Ottenhoff, *et al.* (419–422) and Mustafa, *et al.* (422–424). Ottenhoff, *et al.* found that interleukin 2 (IL2) reversed the *in vitro* unresponsiveness of peripheral blood mononuclear cells from 2 BL patients to *M. leprae* antigens, but in 4 other BL patients and in 6 LL patients no such responses were seen. Mustafa, *et al.*, defining responses on the basis of an increase of 5000 or more counts per minute in the cultures, and also studying patients drawn from the same population as Ottenhoff, *et al.*, found that only 2 of 8 patients responded to T-cell conditioned medium as a source of interleukin 2. Both groups conclude that lepromatous leprosy patients (including apparently BL as well as LL patients) are heterogeneous with regard to responses to IL2 under these conditions. Mukherjee and Girdhar (424–425) made the interesting observation of BT-BB lesions in the intima just beneath the endothelial layer of a vein in 1 of 24 nonlepromatous patients biopsied.

In the Current Literature section of the September issue, Ferracci, *et al.* (433) biopsied four patients who had been treated with deoxyfructo-serotonin for one year. Bacilli from the biopsies multiplied in mouse foot pads in each case. Goloschchapov, *et al.* (433) treated 121 leprosy patients with a new original antileprosy preparation called diucifon with good results. Interestingly, in ten lepromatous patients the disease was transformed into the tuberculoid type. Li, *et al.* (433–444) reported beneficial results in treating LL and BL patients with isobutylpiperazinyl rifamycin (R761). Sheskin, *et al.* (435) found that supidimide, a non-teratogenic analog of thalidomide, was not effective in the treatment of leprosy reactions in the dosages utilized. Waldinger, *et al.* (435) reported a severe motor and a minor sensory neuropathy developing in a man being treated with dapsone for dermatitis herpetiformis. The patient had received

dapsone for 16 years before neurotoxicity became evident; the daily dosage of dapsone was 100 mg prior to the development of the neuropathy. Ramanujam, *et al.* (438–439) reported a case of "neural histoid" nodules occurring in the peripheral and cutaneous nerves in an otherwise inactive lepromatous leprosy patient. Zawar, *et al.* (439) studied systolic time intervals in reactional lepromatous leprosy patients and found abnormalities characteristic of left ventricular dysfunction. Apte, *et al.* (440) demonstrated regional lymph node involvement in approximately three-fourths of tuberculoid leprosy patients. Gupta, *et al.* (441) found varied histopathology from biopsies of different lesions and even from multiple biopsies of the same lesion in nonreactional borderline patients. Kaufmann (442) developed a murine T-cell clone with reactivity to *M. leprae.* The clone also responded to BCG *in vitro,* indicating crossreactivity between BCG and *M. leprae* at the clonal level. Krahenbuhl and Humphres (442–443) found no evidence in mice that the synthetic adjuvant, muramyl dipeptide (or three of its analogs) enhanced resistance to infection with *M. leprae.* Local, but not systemic treatment with *Proprionibacterium acnes* did increase resistance to the growth of *M. leprae* in the mouse foot pad infection. Lele, *et al.* (443) studied skin biopsies and lymph node biopsies from lepromatous leprosy patients undergoing treatment and found the lymph node pathology to be more consistently positive than that in the skin. Reitan, *et al.* (445) studied the response of leprosy patients to a cell wall antigen fraction, MLW1, prepared from *M. leprae.* MLW1 compared favorably with standard lepromin in skin tests and may be an alternative to lepromin with regard to the early (48 hour) skin test reaction. Shannon, *et al.* (445) reported studies in armadillos suggesting that *in vitro M. leprae*-induced suppression of a ConA response is associated with resistance in the animals to infection with *M. leprae.* Young, *et al.* (447) characterized nine monoclonal antibodies to the phenolic glycolipid of *M. leprae* and found that those antibodies directed at the terminal saccharide of glycolipid showed the greatest specificity for *M. leprae* in enzyme-linked immunoassays. In indirect immunofluorescence experiments, the phenolic glycolipid was demonstrated to be on the surface of *M. leprae.* David, *et al.* (447–448) studied 36 slow-growing mycobacteria isolated from the tissues of leprosy patients and found that the strains formed five clusters but that none of the strains were identical to the leprosy bacillus. Fujiwara, *et al.* (448) chemically synthesized antigens of *M. leprae* based on the native phenolic glycolipid. Only those phenolic glycolipids containing the 3,6-di-*O*-methyl-β-D-glucopyranosyl at the nonreducing terminus were efficient in binding specific antibodies, indicating that this was the primary antigenic determinant in phenolic glycolipid I of *M. leprae.* Bovine serum albumin containing reductively aminated 3,6-di-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3-di-*O*-methyl-L-rhamnose was prepared and shown to be highly active as a synthetic antigen in the serodiagnosis of leprosy. Wheeler (450) demonstrated that all the enzymes of the tricarboxylic acid cycle are present in *M. leprae.* Mathew, *et al.* (451) showed no apparent correlation between sensitized Lyt 1 cells capable of antigen-induced T lymphocyte proliferation and protective immunity in BALB/c and C57BL/6 mice infected with *M. lepraemurium* intravenously. Samuel, *et al.* (452) showed that athymic nude mice could be housed in a "clean room" environment and survive adequately for widespread dissemination of *M. leprae* infections. Turcotte and Ishaque (452) found that long-term *in vitro* cultivation of *M. lepraemurium* on Ogawa egg yolk medium resulted in a significant decrease in virulence as measured by pathogenicity in C3H and C57BL mice. Lumpkin, *et al.* (454) reported five leprosy patients from Texas, each of whom had had extensive and chronic contact with armadillos. The authors believe that these patients may have contracted leprosy from naturally infected armadillos. Neelan, *et al.* (455) reported a controlled study of the prophylactic value of acedapsone against leprosy among disease-free children who were household contacts of active multibacillary patients on treatment. Among the children receiving acedapsone prophylaxis, 6.3% developed the disease compared to approximately 12% among the control group receiving placebo injections. Mitchell (459)

studied murine cutaneous leishmaniasis in reconstituted nude mice and found both resistance-promoting and disease-promoting Ly 1+2− T cells in mice of various genotypes.

In the Original Articles of the December issue, Miller and Buchanan (461–467) described the development of an IgM monoclonal antibody specific for mycobacterial arabinomannan. The monoclonal antibody recognized the arabinomannans from all mycobacteria tested, including *M. leprae*. Almeida, *et al.* (468–470) described a method to estimate the proportion of drug-resistant *M. leprae* in a sample using the mouse foot pad test which they termed the "drug-resistant proportion test." Gelber (471–474) tested 54 multibacillary untreated leprosy patients for primary dapsone resistance by mouse foot pad testing and found only one patient with bacilli resistant to the lowest dietary level of dapsone, 0.0001%. Magna and Beiguelman (475–481) studied NADH-methemoglobin reductase activity, hemoglobin, and methemoglobin levels in adult leprosy patients receiving 100 mg of dapsone daily. The mean value of NADH-methemoglobin reductase activity did not differ between the leprosy patients and healthy individuals. On the other hand, leprosy patients showed significant variation and the proportion of individuals showing a partial deficiency of NADH-methemoglobin reductase was significantly higher among the leprosy patients than among healthy individuals. Koticha, *et al.* (482–487) analyzed the attendance of 8574 leprosy patients at leprosy clinics in Bombay. Younger patients were more regular than older patients. Students, white collar, mill and factory workers were more regular than others. Borderline patients were more regular than lepromatous or BT cases. Deformity was associated with regularity of attendance. Koticha, *et al.* (487–495) presented an enormous amount of data on leprosy in Bombay. Case detection rates among industrial workers surveyed for leprosy (17 per 1000) were second only to contacts (31.7 per 1000) in yielding new cases. Sarno, *et al.* (496–500) studied T lymphocyte subsets in the cutaneous lesions of leprosy patients prior to and after chemotherapy. The low ratio of helper T cells (OKT4) to suppressor cells (OKT8) persisted in LL patients after treatment in spite of reductions in bacillary loads. Pannikar, *et al.* (501–505) studied the value of medial epicondylectomy and external decompression in leprosy patients with early ulnar neuritis and were unable to demonstrate any added benefit with surgical intervention as compared to steroid treatment alone. Kamala, *et al.* (506–514) studied the effects of mouse foot pad in infections with various mycobacteria on the sciatic nerves. Among the mycobacteria tested, only three strains of mycobacteria cultivated from biopsies of leprosy patients, namely FMR strains No. 51 and 75 and ICRC, showed sciatic nerve changes of segmental demyelination typical of those produced by *M. leprae*. David, *et al.* (515–523) demonstrated that the mycobacteriophage D_{29} absorbed on *M. leprae* and injected its DNA into *M. leprae*. Liu, *et al.* (524–526) studied Langerhans' cells in leprosy lesions, using the monoclonal antibody OKT6. In most of the cases, the processes of the Langerhans' cells were reduced or diminished and some were disintegrated.

In the Editorial section of the December issue, Draper (527–532) elegantly reviewed the cell wall biosynthesis pathways in *M. leprae* and outlined possible sites of action for new antimycobacterial drugs.

The death of Dr. Victor P. Das was noted with sadness (533).

In the Correspondence section of the December issue, Pearson, *et al.* (534–535) identified 6 tuberculoid patients from a population of about 1000 who had clinical evidence of primary dapsone-resistant disease, suggesting that the prevalence of such cases in the area of Hyderabad, India, is less than 1%. Gelber (536–538) reported that cycloserine was active in mouse foot pad infections with *M. leprae* at dietary concentrations of 0.5% and 2%. Kato (538–541) reported that *Mycobacterium X*, which had been cultivated from *M. leprae*-infected tissues, had been identified as *M. intracellulare*. Rastogi and Rastogi (541–543) reviewed references to leprosy in ancient Indian writings. Sehgal, *et al.* (543–544) described ENL occurring in a patient with histoid leprosy.

In the Current Literature section of the December issue, Clemmensen, *et al.* (565) found evidence of a peripheral neuropathy in 5 of 6 patients treated with thalidomide

for either prurigo nodularis or discoid lupus erythematosus. Jenner, *et al.* (565) studied the pharmacokinetics of thiacetazone and found that drug concentrations capable of inhibiting the multiplication of *M. leprae* would only be maintained for about three days if patients discontinued the drug. Pattyn (567) studied the incubation period of 21 relapses after stopping dapsone monotherapy in paucibacillary leprosy patients and found that 50% of the relapses occurred during the first 2–3 years. Warndorff van Diepen, *et al.* (567) put 212 lepromatous patients with suspected dapsone-resistant leprosy on a trial of full-dosage dapsone monotherapy. On clinical grounds, 55% of these patients proved to be dapsone resistant but the remaining 45% continued to respond to full dapsone doses after 4.5–9 years. Dapsone sensitivity studies in mice indicated that 86% of these patients harbored dapsone-resistant bacilli. The relevance of partial dapsone resistance, diagnosed in mouse foot pad tests, is challenged. Arruda, *et al.* (567–568) pointed out the value of the Rubino reaction as one of the criteria for determining cure in lepromatous patients. Brinton, *et al.* (568) evaluated cancer risks in 1678 leprosy patients admitted to Carville between 1939 and 1977. Overall, no increased cancer mortality was observed. Duncan and Pearson (568–569) studied women with lepromatous leprosy during pregnancy and found that 10 out of 45 BL patients (22%) and 20 out of 34 LL patients (59%) developed ENL. Ersek and Lorio (569) successfully treated indolent cutaneous ulcers in leprosy patients with porcine xenografts. Gourie-Devi (569) found abnormal greater auricular sensory nerve conduction in 16 of 24 nerves examined in leprosy patients. Joffrion and Brand (570) outlined the pathophysiology, diagnosis, and management of the main features of ocular leprosy. Kannan and Vijaya (570–571) studied endocrine testicular functions in leprosy patients and found that the hypogonadism in leprosy results from primary testicular failure. Similar results were reached by Kou, *et al.* (571). Rheumatic manifestations in leprosy mimicking rheumatoid arthritis were emphasized by Morley, *et al.* (572) and Nsibambi (572). Singh, *et al.* (573) found impaired hearing in 52% of lepromatous cases. Conductive deafness was due to eustachian tube obstruction secondary to atrophic rhinitis associated with the disease. Sritharan, *et al.* (573) found significantly raised high-density lipoprotein cholesterol to total cholesterol ratios in lepromatous leprosy patients. This may be an explanation for the low risk of myocardial infarction due to atherosclerosis among lepromatous leprosy patients. Birdi, *et al.* (574) found that peripheral blood-derived macrophages from lepromatous leprosy patients were unable to interact with lymphocytes in the presence of *M. leprae*, and (574) that this defect could be overcome *in vitro* with levamisole. Birdi, *et al.* (574–575) found that conditioned medium of *M. leprae*-infected macrophage cultures of lepromatous leprosy patients contained a factor which inhibited lymphocyte transformation to *M. leprae* in leukocyte cultures from normal individuals. Generation of the factor was inhibited by indomethacin, suggesting that the factor may be a prostaglandin. Klatser, *et al.* (576–577) studied antigens of *M. leprae* by SDS-polyacrylamide gel electrophoresis and immunoperoxidase using patients' sera. A 33 kD antigen seemed to be a common mycobacterial antigen with one or more *M. leprae*-specific determinants. Antigens of 12, 22, 28, 36, 41, and 86 kD were heat stable and seemed to be *M. leprae* specific. Mshana, *et al.* (577) demonstrated cellular infiltration and axonal degeneration in rabbits that had been sensitized with *M. leprae* and then had *M. leprae* sonicate injected into the sciatic nerves at the peak of hypersensitivity. Thus, specific cell-mediated hypersensitivity to *M. leprae* antigens seemed to be involved in the pathogenesis of major nerve trunk damage in the tuberculoid end of the leprosy spectrum, especially during acute reversal reactions. Reitan and Closs (577) showed that the intensity of responses to an antigen fraction from *M. leprae* called MLW1 in the lymphocyte stimulation test increases with the closeness of contact in healthy individuals exposed to leprosy patients. Ridley, *et al.* (578) demonstrated C-reactive protein of host origin in association with *M. leprae in vivo* in humans. Sinha, *et al.* (579) described a novel serological assay for leprosy based on inhibition by test serum of the binding of radio-labeled MLO4 monoclonal antibody to *M. leprae* sonicate-coated microtiter plates. van Eden, *et al.* (579–580)

showed that HLA-DR3 was associated with skin test responsiveness to mycobacterial antigens. Van Hale, *et al.* (580) examined the ultrastructure of dermal inflammatory responses in leprosy patients and found occasional intraendothelial bacilli, especially in patients with Lucio's phenomenon. Young, *et al.* (580) showed that humans respond predominantly with IgM class immunoglobulin to the species-specific glycolipid of *M. leprae,* and that most of the human IgM antibody response to the surface of the bacilli was directed against the phenolic glycolipid antigen. Zhu, *et al.* (581) found *M. leprae* in the capillary endothelium in biopsies from 49 of 50 leprosy patients studied. Brett, *et al.* (581) found significant serologic crossreactions between the major phenolic glycolipid purified from *M. leprae* and glycolipids from *M. bovis* and *M. kansasii.* The crossreactivity which was observed seems to be associated with the phenol ring of the antigen and the disaccharide seems to be the unique antigenic determinant of *M. leprae.* Klebanoff and Shepard (582) showed that *M. leprae* could be killed by a myeloperoxidase or eosinophil peroxidase, H_2O_2, and a halide. Almeida, *et al.* (583) found that growth of *M. leprae* in dapsone-treated mice could be observed if as few as 1 in 1000 of the *M. leprae* tested were dapsone resistant. The mouse foot pad test therefore appears to be sensitive to minute portions of drug-resistant bacilli in the samples being tested. Hunter and Thomas (586–587) suggest that there is some cross-interference between tuberculosis and the milder, paucibacillary forms of leprosy and a negative correlation between leprosy and urbanization.

The December issue contained the abstracts of the U.S.-Japan Leprosy Conference held in Tokyo. Nomaguchi, *et al.* (601) confirmed the loss of pathogenicity of *in vitro*-cultivated *M. lepraemurium* as smooth colonies, and demonstrated that these smooth colonies could regain pathogenicity upon passage in tissue culture cells or *in vivo* in immunodeficient hosts. Mori, *et al.* (601–602) did not detect catalase activity in cell-free extracts of purified *M. leprae.* Malate and succinate dehydrogenases which are contained in the tricarboxylic acid cycle were detected but glyceraldehyde-3-phosphate dehydrogenase and α-glycerophosphate dehydrogenase which are part of the glycolysis enzyme system were not detected. Matsuo, *et al.* (602) described an improved staining method for determining the viability of mycobacterial cells using fluorescein diacetate-ethidium bromide. Kusaka and Mori (602–603) analyzed the mycolic acids of *M. leprae* from nude mouse foot pads. Fukunishi (603) studied two lipids isolated from lepromas and suggested that the phenolic glycolipid and phthiocerol dimycocerosate are included in the small spherical droplets around *M. leprae* in tissues. Franzblau, *et al.* (603–604) outlined an approach aimed at producing an *in vitro* growth-competent variant of *M. leprae* using mycobacterial plasmids. Matsuoka, *et al.* (604–605) infected nude mice with *M. leprae* intravenously, subcutaneously, and intracutaneously. After intravenous inoculation there were pronounced differences in the multiplication of the bacilli among the various organs examined. Nakamura and Yogi (605–606) compared the susceptibilities of various strains of nude rats and nude mice to *M. leprae.* The SHR nude rat gave excellent results, and in both rats and mice the genetic background of the athymic animals influenced the development of the infection. Gormus, *et al.* (606–608) reviewed their progress in experimental leprosy in monkeys. The sooty mangabey monkey is a good model for the transmission and study of leprosy, and both African green and rhesus monkeys show promise of becoming alternative, more available species that may be susceptible to the disease. Kohsaka, *et al.* (608) showed that thymus transplantation inhibited the growth of *M. leprae* in nude mice. Hastings and Chehl (608–609) found that large inocula of *M. leprae* from a patient treated with dapsone multiplied in nude mice fed the drug, but that the same strain was inhibited by the same concentration of dietary dapsone in immunocompetent BALB/c mice given conventional inocula. Ito, *et al.* (609) found no inhibition of growth of *M. leprae* in nude mice fed isoniazid. Gidoh and Tsutsumi (609–610) analyzed hydrophilic dapsone metabolites by high-pressure liquid chromatography. Tsutsumi and Gidoh (610) studied immunostimulators, immunopotentiators, and immunomodulators in a variety of immune systems. Collins and Orme (610–611) studied the

immunocompetence of mice heavily infected with mycobacteria. Spleen cells from these anergic animals showed an enhanced capacity to remove IL2 from cultures. Lefford (611–612) found that the growth of *M. lepraemurium* was the same in nude mice and immunocompetent nu/+ heterozygote mice, both of a BALB/c background. Ohkawa (612) found that lymphocytes from lepromatous leprosy patients do not secrete lymphokine in response to *M. leprae in vitro*. Rea, *et al.* (612–614) studied the number and distribution of anti-IL2 and anti-Tac positive cells in the tissues of BT, LL, and ENL patients. The number of cells with receptors for IL2 (Tac positive) were similar in LL and BT patients, but IL2-producing cells were significantly reduced in lepromatous as compared with BT patients. These findings suggest that the specific defect in cell-mediated immunity against *M. leprae* in lepromatous patients is not a failure of antigen presentation or an event pre-emptive of antigen recognition but, rather, is secondary to reduced, probably inhibited, IL2 production, In ENL the number of IL2-producing cells is similar to that in BT lesions, suggesting that ENL is a cell-mediated immune response and that de-inhibition of IL2 production may be involved in its pathogenesis. Minauchi, *et al.* (614) found low levels of antibodies to the peripheral nervous system in sera from patients with lepromatous leprosy, but the existence of these antibodies did not seem to correlate with clinical findings. Matsuo, *et al.* (614) suggested that the β-glucuronidase of *M. leprae* might be important in allowing the bacilli to penetrate from the blood stream to the endoneurium to initiate leprous neuropathy. Levis, *et al.* (614–615) found that serum IgM antibodies to the phenolic glycolipid antigen of *M. leprae* increased in relation to a rising bacterial index, and that patients with ENL have lower antibody levels than do patients without this complication. Izumi, *et al.* (615–616) described a complement fixation test to detect antibodies to the phenolic glycolipid I (PGI) antigen of *M. leprae*. Abe, *et al.* (616) found that Mitsuda lepromin skin testing increased the level of anti-*M. leprae* antibodies as measured by the fluorescent leprosy antibody absorption test. Hirata, *et al.* (616) found that one third to one half of lepromatous leprosy patients had teichoic acid precipitins in their sera. Kikuchi, *et al.* (617) presented evidence suggesting that an HLA-linked gene controlled the clinical manifestation of leprosy. Mohagheghpour, *et al.* (617–618) found that recombinant IL2 augmented the proliferative activity of peripheral blood lymphocytes *in vitro* from all types of leprosy patients regardless of the presence or absence of antigenic stimuli. Thus recombinant IL2 did not specifically restore the response of lepromatous leprosy patients to *M. leprae* antigens. Brennan, *et al.* (618–620) described the synthesis of sensitive and specific antigen probes for the serodiagnosis of leprosy based on the terminal sugar of the PGI antigen, the 3,6-di-*O*-methyl-β-D-glucopyranosyl epitope. Chemical and immunologic procedures for the quantitation of PGI antigen from biologic materials were described. Fujiwara, *et al.* (620) synthesized a number of derivatives of the disaccharides of the PGI antigen of *M. leprae* and compared their serologic activities by an ELISA inhibition assay. Douglas and Steven (620–621) compared the seroreactivities of *M. leprae*—the PGI antigen of *M. leprae*, and a synthetic antigen in which the terminal sugar of the PGI antigen was conjugated to bovine gamma globulin. The synthetic antigen was found useful in detecting clinical leprosy and may provide a valuable replacement for the natural antigen. Young, *et al.* (621–622) characterized over 100 monoclonal antibodies to *M. leprae*. Monoclonal antibodies are available to at least eight different molecules of the leprosy bacillus. In addition to those directed against the PGI antigen, a number of the monoclonal antibodies directed against proteins seem to be specific for *M. leprae*.

A great deal of progress has been made in our understanding of leprosy in 1984. From a personal perspective a number of developments seem particularly relevant.

In the field of chemotherapy, it has been confirmed that the hepatotoxicity of daily rifampin and daily ethionamide or prothionamide is unacceptable. Nine additional cases of rifampin-resistant leprosy have been described. Supplemental zinc may be helpful in leprosy chemotherapy. Cycloserine has shown anti-*M. leprae* activity in the mouse foot pad model. Emphasis has been

placed on relapse rates among patients discontinuing dapsone or other chemotherapy. It may well be that dapsone does not penetrate into peripheral nerves, and this could account for persisting organisms in that location after years of dapsone monotherapy. Controversy has continued regarding the degrees of correlation between drug sensitivity testing by mouse foot pad and clinical responses to dapsone monotherapy. It seems that mouse foot pad drug resistance does not always correlate with clinical dapsone resistance, and this may well be because the mouse foot pad test is very sensitive in detecting small proportions of drug-resistant organisms. Fortunately, the problem of clinically relevant dapsone resistance in parts of South India seems to be less than 1%.

In clinical studies, it has been shown that soaking is apparently not necessary for skin hydration in denervated leprosy skin. A leproma has been described attached to the lateral leaflet of the mitral valve of the heart, and reactional lepromatous leprosy may affect cardiac function. Lepromatous patients have increased, high-density lipoprotein cholesterol to cholesterol ratios, and this may be related to a relative lack of atherosclerotic coronary artery disease among lepromatous patients. Almost 40,000 *M. leprae* are in each exhaled breath of untreated, active borderline or lepromatous leprosy patients. Antecdotal evidence has been presented for humans contracting leprosy from naturally infected armadillos in Texas.

In immunology work continues to advance rapidly. Anergy in lepromatous leprosy could be genetic or on the basis of acquired tolerance, and it seems impossible to determine which is more important based on present knowledge. Loss of the regulatory function of suppressor T cells in lepromatous leprosy seems to be responsible for the nonspecific increases in immunoglobulin in the circulation. Antigens of *M. leprae* and IgG class anti-*M. leprae* antibodies have been detected in the urine of leprosy patients. Macrophages of lepromatous leprosy patients seem to release an inhibitor of lymphoproliferation, possibly a prostaglandin. Lepromatous macrophages will apparently not support lymphocyte blast transformation by T cells from HLA-D-matched tuberculoid lymphocytes. There have been elegant studies of T lymphocyte subsets in leprosy granulomas. These have suggested that lepromatous patients have reduced IL2 production but that antigen has been recognized. Additional studies on the effects of IL2 *in vitro* have shown that it seems not to be capable of augmenting lymphoproliferative responses of cells from polar lepromatous patients in any *M. leprae*-antigen-specific fashion. There seems to be a heterogeneity among lepromatous leprosy patients in this regard. Viable *M. leprae* may predominantly multiply in the cytoplasm of macrophages, with only dead bacilli being found in phagosomes. The terminal sugar of the phenolic glycolipid I antigen of *M. leprae* is its primary antigenic determinant. The characteristic antibody response to this antigen is of the IgM class. A number of monoclonal antibodies have been developed and described with specificity toward antigens which seem to be specific for *M. leprae*. Exacerbation reactions have been described in highly active lepromatous lesions.

In microbiology major advances have been made in our understanding of the biochemistry of *M. leprae*. Of particular interest, *M. leprae* apparently preferentially utilize pre-formed purines in synthesizing nucleic acids rather than undertaking *de novo* purine synthesis. The biosynthesis of cell walls may be a site for development of antileprosy drugs. The mycobacteriophage D_{29} was reported to absorb on *M. leprae* and inject its DNA into the bacillus.

In the field of experimental infections, evidence for naturally acquired leprosy in an armadillo from Mexico has been presented. Almost 5% of armadillos along the Texas Gulf Coast have been shown to have natural infections with *M. leprae*. A model of *M. leprae*-induced neuropathy has been produced in rats. Experimental work continues with non-human primate models of leprosy.

As seen from the perspective of the 1984 JOURNAL, great strides are being made in many areas of leprosy. In other areas progress seems exasperatingly slow. Overall, it seems clear that the groundwork has been laid for major advances in our understanding of the disease. I look forward with impatient optimism to the further unraveling of the leprosy puzzle in 1985.—RCH