

ABSTRACTS

NINTH JOINT LEPROSY RESEARCH CONFERENCE

Kyoto Kaikan, Kyoto, Japan
October 16-18, 1974

U.S.-Japan Cooperative Medical Science Program

INTRODUCTORY REMARKS

Ladies and gentlemen, allow me to speak a few words of welcome and inauguration. It is a pleasure and privilege to welcome all of you to Kyoto and to inaugurate this Ninth Joint Conference.

This city was the former capital of Japan from A.D. 794 to 1868, and it has 1,200 years of history and tradition in Japanese civilization. It is significant for us to have a scientific conference here in the quiet circumstances of the old capital, reviewing our works, discussing and exchanging our experiences. Research should not be a mere seeker of truth; it should be good for the happiness and welfare of people as well.

Even though the relief of patients now suffering from the disease is urgent, control of it must be even more important. Even if the segregation of infectious cases of the disease may be necessary and stands to reason scientifically, it is impossible socially in many areas. Accordingly, the improvement of practical control measures is urgently needed together with relief by treatment. Recently there has been progress in research, and owing to your efforts in every field, bright prospects are expected.

Next I wish to express my appreciation for the kind cooperation and advice of Dr. Shepard, Dr. Horton and Dr. Okada in the preparation for this congress.

Lastly, I beg your forbearance in that the malignant inflation under which we are laboring may cause our precious guests some inconveniences.

Now I declare the conference open with my best wishes for a successful and fruitful outcome.

Thank you.

MASASHI NAMBA, *Chairman*
Japan Leprosy Panel

Program of the Ninth Joint Leprosy Research Conference

Contents

16 October 1974, Wednesday

Opening and Introductory Remarks: Dr. M. Namba, Chairman, Japan Leprosy Panel

Morning Session: Chairmen: Dr. J. H. Peters
Dr. M. Namba

- Takizawa, H., Nishiura, M. and Okada, S.** Bacterial clearance time of lepromatous leprosy during chemotherapy in Japan.
- U.S. Leprosy Panel.** Recent advances in the chemotherapy of leprosy. A review of U.S. Leprosy Panel activities. (Presented by L. Levy)
- Tsutsumi, Sadae, Sakamoto, Yoshiki, Gidoh, Saiichi and Nakagawa, Hideo.** Several findings in the metabolic fate of RFP.
- Gordon, G. R., Ghoul, D. C., Murray, J. F., Jr., Peters, J. H. and Levy, L.** Plasma and tissue levels of sulfones in mice and rats receiving dietary dapsone.
- Peters, J. H., Gordon, G. R., Murray, J. F., Jr., Levy, L., Russell, D. A. and Shepard, C. C.** Acedapsone treatment of leprosy: metabolic disposition versus therapeutic response.

Afternoon Session: Chairmen: Dr. R. S. Weiser
Dr. M. Nakamura

- Hirata, Tsunehiko and Nakayama, Tetsu.** Cytomorphological studies on leprosy bacilli in the host cell.
- Endo, Hiroko and Nakayama, Tetsu.** Mycobacterium KNBE—its nutrition and elongation.
- Mori, Tatsuo.** Red pigment produced in medium by *M. lepraemurium*.
- Nakamura, Masahiro.** Essential component for the growth of *M. lepraemurium* in cell-free liquid medium and related problems.
- Matsuo, Yoshiyasu.** Pathogenicity of *Mycobacterium lepraemurium* maintained in mouse foot pad cell culture and association of mitosis in the cells with intracellular growth of bacilli.
- Ito, Tonetaro and Kohsaka, Kenji.** *M. leprae* in mouse macrophages *in vitro*.

17 October 1974, Thursday

Morning Session: Chairmen: Dr. W. E. Bullock
Dr. M. Abe

- Takizawa, H., Okada, S. and Nishiura, M.** The etiology of *erythema nodosum leprosum*: a clinical approach.
- Namba, Masashi, Sasaki, Norisuke and Ohira, Kaoru.** Report on a suspected case of bulbar palsy.
- Jacobson, R. R., Hastings, R. C., Morales, M. J. and Slack, C. E.** The treatment of leprosy with transfer factor.
- Abe, Masahide, Minagawa, Fumishige, Yoshino, Yuji and Itoh, Masayasu.** Immunoglobulin levels and antibody titers in nasal secretion versus serum of leprosy patient.

Afternoon Session: Chairmen: Dr. L. Levy
Dr. M. Nishiura

- Morrison, Norman E. and Collins, Frank M.** Thymosin reconstitution of antimycobacterial immunity in the T-cell depleted mouse.
- Bullock, W. E., Evans, P. D. and Wyatt, C. R.** Depletion of lymphocyte subpopulations in the spleen by murine leprosy.
- Shepard, Charles C.** Immunity to *M. leprae* infections in mice caused by GVH reactions and by injections of *M. leprae* and BCG.
- Matsuhashi, Tyoku and Usui, Mitsuko.** Specific suppression of antibody production to a given antigen by the injection of oil droplets enfolding a mixture of the antigen and an immunosuppressant.
- Joint Meeting of U.S.-Japan Leprosy Panel Members.**

18 October 1974, Friday

Morning Session: Chairmen: Dr. C. C. Shepard
Dr. T. Ito

- Nakamura, Kazunari, Yogi, Yasuko and Matsuoka, Masanori.** Studies on *Mycobacterium leprae* transmission into various animals—transmission into the foot pad of the bank vole (*Apodemus speciosus*) and field mouse (*Microtus montebelli*).
- Sasaki, Norisuke, Kawatsu, Kunio, Minagawa, Fumishige and Matsuoka, Masanori.** The effect of radioisotope ¹³¹I combined with immunosuppressive agents on experimental leprosy.
- Sushida, Kiyo.** Host-parasite relationship in the experimental transmission of leprosy bacilli in mice.
- Fieldsteel, A. Howard.** Detection of a few viable *M. leprae* in an inoculum of 10⁷ dead *M. leprae* by inoculation into foot pads of neonatally thymectomized rats.
- Evans, Michael J.** Mononuclear cell turnover in mouse foot pads infected with *Mycobacterium leprae*.
- Shepard, Charles C.,** Chairman, U.S. Leprosy Panel. Closing remarks.

PARTICIPANTS

U.S. Leprosy Panel

Shepard, Charles C. (*Chairman*), Chief, Leprosy & Rickettsia Branch, Virology Division, Center for Disease Control, Atlanta, Georgia 30333

Bullock, Ward E. Division of Infectious Diseases, Department of Medicine, University of Kentucky Medical School, Lexington, Kentucky 40506

Levy, Louis, Chief, Leprosy Research Unit, US PHS Hospital, 15th and Lake Street, San Francisco, California 94118

Peters, John H., Program Manager, Biomedical Research Department, Life Sciences Division, Stanford Research Institute, 333 Ravenswood Avenue, Menlo Park, California 94025

Weiser, Russell S., Department of Microbiology, University of Washington, School of Medicine, Seattle, Washington 98105

Japan Leprosy Panel

Namba, Masashi (*Chairman*), Director, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo

Nishiura, Mitsugu, Professor, Leprosy Research Laboratory, Kyoto University School of Medicine, Sakyo-ku, Kyoto

Abe, Masahide, Director, 2nd Research Department, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo

Nakamura, Masahiro, Professor of Bacteriology, Kurume University School of Medicine, Asahi-Cho, Kurume-shi, Fukuoka-ken

Ito, Tonetaro, Professor, Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Yamadakami, Suita, Osaka

* * * * *

Drutz, David J., Chief, Division of Infectious Diseases, Department of Medicine, University of Texas, Health Science Center, San Antonio, Texas 78284

Endo, Hiroko, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo

Evans, Michael J., Stanford Research Institute, 333 Ravenswood Avenue, Menlo Park, California 94025

Fieldsteel, Howard A., Stanford Research Institute, 333 Ravenswood Avenue, Menlo Park, California 94025

Gordon, G. Ross, Biomedical Research Department, Life Sciences Division, Stanford Research Institute, 333 Ravenswood Avenue, Menlo Park, California 94025

Hazama, Shogo, National Leprosarium Tama, Zensho-en, Higashimurayama-shi, Tokyo

Hirata, Tsunehiko, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo

Horton, Richard E., Geographic Medicine Branch, NIAID, NIH, Bldg. 31, Room 1B62, Bethesda, Maryland 20014

Jacobson, Robert R., Medical Department, US PHS Hospital, Carville, Louisiana 70721

Kawaguchi, Yoichiro, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo

Kohsaka, Kenji, Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Yamadakami, Suita, Osaka

Kusaka, Takashi, Kawasaki Medical College, Kurashiki-shi, Okayama-ken

Lew, Joon, Department of Microbiology, School of Medicine, Yonsei University, Seoul, Korea

Matsuhashi, Tyoku, Department of Allergology, Institute of Medical Science, University of Tokyo, Minatoku, Tokyo

Matsuo, Yoshiyasu, Department of Bacteriology, Hiroshima University, Kasumi-cho, Hiroshima-shi

Mayama, Akira, National Leprosarium, Nagashima Aisei-en, Oku-cho, Okayama-ken

Mifuchi, Ichiji, Department of Microbiology, Shizuoka College of Pharmacy, Kojika, Shizuoka-shi

- Mori, Ryoichi**, Department of Bacteriology, Kyushu University
- Mori, Tatsuo**, Department of Leprology Research Institute for Microbial Diseases, Osaka University, Yamadakami, Suita, Osaka
- Morrison, Norman E.**, Leprosy Research Laboratory, Johns Hopkins-Leonard Wood Memorial, 615 North Wolfe St., Baltimore, Maryland 21205
- Nakamura, Kazunari**, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo
- Nakayama, Tetsu**, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo
- Nomaguchi, Hiroko**, Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Yamadakami, Suita, Osaka
- Ogawa, Tatsuji**, Urawa City Hospital, Urawa-shi, Saitama-ken
- Oiwa, Koji**, Division of Bacteriology, Chest Disease Research Institute, Kyoto University, Sakyo-ku, Kyoto
- Okada, Seitaro**, Leprosy Research Laboratory, Kyoto University School of Medicine, Sakyo-ku, Kyoto
- Okamura, Kazuko**, National Leprosarium, Tama Zensho-en, Higashimurayama-shi, Tokyo
- Ozawa, Toshiharu**, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo
- Sansarricq, Hubert**, Leprosy Division of Communicable Diseases, World Health Organization, 1211 Geneva 27, Switzerland
- Sasaki, Norisuke**, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo
- Sushida, Kiyo**, Department of Microbiology, Tokyo Women's Medical College, Shinjuku-ku, Tokyo
- Takizawa, Hideo**, Leprosy Research Laboratory, Kyoto University School of Medicine, Sakyo-ku, Kyoto
- Tanaka, Yoshinori**, Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Yamadakami, Suita, Osaka
- Tsutsumi, Sadae**, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo
- Yamagami, Akira**, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo
- Yanagisawa, Kenichiro**, Ministry of Health and Welfare, Kasumigaseki, Chiyoda-ku, Tokyo
- Yasuhira, Kimio**, Chest Disease Research Institute, Kyoto University, Sakyo-ku, Kyoto
- Yoshie, Yoshio**, Former Director, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo

Program of Leprosy Conference

Takizawa, H., Nishiura, M. and Okada, S.

Bacterial clearance time of lepromatous leprosy during chemotherapy in Japan.

As many clinicians have previously observed, skin lesions in lepromatous leprosy during chemotherapy show distinct improvement several years before acid-fast bacilli disappear from the lesions. Consequently an indicator called Bacterial Clearance Time (BCT) was chosen to represent the clinical course and prognosis of each case of lepromatous leprosy. BCT is the period of time from the start of treatment until skin smears from the skin lesions become negative. Negativity of bacilli is defined here as negative skin smears over a twelve month period with no clinical evidence of activity.

This study concerns the 56 patients for whom a diagnosis of lepromatous leprosy was made in our clinic between 1952 and 1964, whose records were relatively complete, and who were followed for more than five years. In addition, 123 lepromatous patients admitted to Tama-Zenshoen Leprosarium in Tokyo between 1957 and 1963 were studied to learn whether different results in the two institutes were observed.

There was no significant difference in the BCT distribution of lepromatous cases in the two institutions. The cumulative ogive of BCT for all the cases showed a sigmoid curve. The BCT of 4.5 and 7.5 years can be roughly regarded as inflection points of this sigmoid curve. The following three groups were defined in relation to the above inflection points:

1. Rapid decrease group
(RA group) BCT \leq 4.5
2. Standard decrease group
(ST group) 4.5 < BCT \leq 7.5
3. Slow decrease group
(SL group) 7.5 < BCT

In most lepromatous leprosy in Japan, 4.5 to 7.5 years of chemotherapy was necessary to gain bacterial negativity. Our data showed that there was no geographical difference in the bacterial negativity of lepromatous patients in the world (Rodriguez 1970, Jacobson 1971). The cases with acute infiltration reaction (Tajiri) in their clinical course were in the RA group. Some other cases in this group had a history of previous treatment or

showed near lepromatous leprosy. The skin lesions in the RA group were localized and were not extensive. One of the main causes of the slow response in the SL group was irregular treatment due to ENL or other reasons.—[Leprosy Research Laboratory, Kyoto University School of Medicine]

U.S. Leprosy Panel. Recent advances in the chemotherapy of leprosy. A review of U.S. Leprosy Panel activities.

The nine years of existence of the U.S. Leprosy Panel have seen intense activity in leprosy chemotherapy. Clinical trials of several kinds have been conducted in four geographic areas. Short-term trials in lepromatous leprosy have been carried out in Cebu, the Philippines, and in the U.S. A long-term trial has been in progress for more than five years in the Karamui District of New Guinea, and another has recently started in Cebu. The trial in New Guinea has also included a large group of patients with paucibacillary leprosy, and a trial of several therapies in paucibacillary leprosy has recently been started in Cebu. Finally, a trial of chemoprophylaxis was undertaken among the Pingelapese population of Pingelap and Ponape.

Short-term trials of leprosy chemotherapy are intended to measure the efficacy of various treatment regimens in terms of the initial killing of *Mycobacterium leprae*. The rate of killing of the organisms is measured by means of mouse inoculation of organisms recovered from the lesions of patients at intervals during treatment. By means of the mouse foot pad technic, killing of the first 99% to 99.9% of the viable *M. leprae* (those infectious for the mouse) can be observed. At the time that viable organisms can no longer be detected, a large number of viable *M. leprae* may remain in the patient's body. Employing this technic, dapsone (DDS), acedapsone (DADDS), clofazimine (B663), and daily rifampin were shown to be efficacious in trials conducted in the U.S. More recently, the efficacy of several clofazimine regimens were compared, and the efficacy of daily rifampin was confirmed in short-term trials conducted in Cebu. In progress in Cebu at this time is a comparison of ace-

dapsone, acedapsone *plus* intermittently-administered rifampin, and daily rifampin. Finally, in a trial in the U.S., single doses of 600 mg to 1500 mg rifampin appear to produce as much killing of *M. leprae* as 100 days of daily dapsone therapy.

The rate at which *M. leprae* are killed by a single dose of rifampin is almost too rapid to measure by the mouse foot pad technic. And there is not available a more sensitive technic capable of detecting proportions of viable *M. leprae* much smaller than 1/1000, that would permit the study of short-term trial of even more rapidly acting drugs, drug combinations, and dosage regimens. It is now important to attempt to learn something about the rate at which the remaining viable *M. leprae* are killed, once the killing of the first 99% to 99.9% of these organisms has occurred. At this time, this can be approached only by means of the long-term trial. By this technic, patients are observed clinically, employing the microscopic examination of skin smears and the histopathologic examination of skin biopsy specimens, for at least five years. Mice are inoculated only if there is evidence of relapse or failure to improve. The measurement of the rate of killing of viable *M. leprae* is based on the presumption that viable organisms disappear no more slowly than the rate at which the nonviable organisms disappear from the skin lesions. This presumption may be incorrect, because it does not take into account persisting viable *M. leprae* that are susceptible to but unaffected by continued antimicrobial therapy. A long-term trial of acedapsone in New Guinea has recently completed its sixth year. The available results suggest that acedapsone alone represents inadequate long-term therapy for patients with multibacillary leprosy. We have recently embarked upon a long-term trial in Cebu, in which the effect of initial therapy with acedapsone, acedapsone *plus* intermittent rifampin, and daily rifampin on the outcome of long-term treatment with acedapsone or dapsone is being studied.

Patients with paucibacillary leprosy have, by definition, insufficient *M. leprae* to permit the application of the mouse foot pad technic, and some even to permit the measurement of the BI. The response of these patients to therapy can be measured only by clinical and histopathologic observations

made over the long term. In the Karamui, several hundred patients with paucibacillary leprosy have been treated with acedapsone alone for more than six years. All have responded in a satisfactory manner. Trials in paucibacillary leprosy have recently been undertaken in Cebu, in which the efficacy of therapy with acedapsone alone for one or two years will be compared with initial two or four week courses of daily rifampin *plus* dapsone to be followed by shorter courses of acedapsone alone (six to twelve months). The purpose of this trial is twofold. Information regarding the most economical means of treating patients with paucibacillary leprosy will be most useful to those countries with limited resources. Also, presuming that the burden of *M. leprae* is no smaller in these paucibacillary patients than it is in patients with inapparent infection, the results of this trial will be of great value in the design of regimens for trials of chemoprophylaxis.

During the past nine years of productive work, assisted greatly by a close relationship between laboratory and clinic, and benefiting in no small measure by the timely appearance of rifampin, the Panel's chemotherapeutic activities have progressed from their beginning in a now obsolete "protocol for chemotherapy trials in lepromatous leprosy" to the presently heavy investment in a closely coordinated battery of short-term and long-term trials in lepromatous leprosy and simultaneous trials in paucibacillary leprosy.

Time will permit presentation only of the results of the short-term trials.—[Presented by L. Levy, PHS Hospital, San Francisco, California]

Tsutsumi, S., Sakamoto, Y., Gidoh, S. and Nakagawa, H. Several findings in the metabolic fate of RFP.

The metabolic pattern of RFP (rifampicin) can be classified into two parts. The first is the formation of several established metabolites such as RFPQ, DARFP, DARFPQ, and DMeRFP¹ (dogs, rats) which can be designated as the first step metabolites; and the second is the further conjugation (the second

¹Abbreviations: Q, quinone; DA, desacetyl; DMe, desmethyl; G, glucuronyl. [If the reader will add these letter-equivalent designations to the basic RFP (rifampicin), then the letter-jargon of this technical subject will make some sense.—Editor]

step metabolism) which has already been proved by Nakagawa to be RFPG in human cases. In a strict sense, the long activity of a drug must be expressed as the action area of the drug.

Although the long activity plays an important part in the chemotherapy of leprosy and in the *in vitro* inhibitory effects of RFPQ and DARFP are nearly comparable to those of RFP, the metabolism of the first step metabolites themselves and their possible contribution to the *in vivo* inhibitory effect of RFP have not yet been elucidated.

In consideration of these problems, the following experiments were carried out.

1. The substitution of 4-OR for the naphtholic 4-OH of RFP, in which R is 1-adamantane carboxyl (AD), was achieved by the usual acetylation method under the approval of Japone Gruppo Lepetit. We expected a lowering of the *in vivo* quinone formation and the entero-hepatic circulation of RFP and hoped to gain some notion of the probable site of G in RFPG. The reaction of several other organic acid chlorides to the 4-OH of RFP was also examined. In view of these results and the steric hinderance exerted by the macrolide ring and the chromophor of RFP, the site of G could be imagined as its 4-OH. Then, ^{14}C -RFPQ and ^{14}C -DARFP were synthesized from ^{14}C -RFP which had been kindly supplied by the Daiichi Pharm. Co. Accordingly, ^{14}C -labeled 4-AD-RFP (ADP) was also synthesized.

2. The metabolism of these compounds by mice was examined through the use of whole body autoradiography and radiochemical analysis of residual frozen blocks or excreta. The results found were: a) RFP and the analogues were mainly restricted to the liver and gut. However, this restriction was more marked with the analogues than with RFP. b) The decrease of DARFP was more rapid than that of RFP or RFPQ, for example, in the liver. c) RFP was released from ADP. However, the distribution of ADP itself was inferior to that of the other compounds. Thus, ADP would not be able to not inhibit the growth of leprosy bacilli in the mouse foot pad. d) Although the total recovery, especially that from the urine, is known to be generally low in the case of RFP, the ratios of the urinary/fecal recovery rates were markedly lower with the analogues than with RFP. e) The analysis of the me-

tabolites suggested: that the oxidation of DARFP progresses more rapidly than that of RFP; that the content ratios of RFPQ and DARFPQ in the remaining radioactivity increase with the lapse of time, for example, gradually in the liver and after 7.5 hours in the gut; after 7.5 hours, the RFP released from ADP is almost undetectable in the kidney.

Mainly on the basis of the aforesaid results, the metabolic pattern of RFP will be discussed.—[National Institute for Leprosy Research and the Tokyo National Chest Hospital]

Gordon, G. R., Ghoul, D. C., Murray, J. F., Jr., Peters, J. H. and Levy, L. Plasma and tissue levels of sulfones in mice and rats receiving dietary dapsone.

The use of the mouse as an experimental animal for pharmacologic studies of antileprosy drugs as well as for the detection of dapsone-resistant *M. leprae* is well established. It also has been demonstrated that the Lewis rat can serve as a host for the multiplication of *M. leprae*. To determine the relationship between plasma and tissue levels of dapsone (DDS) and monoacetyl DDS (MADDS), we measured the concentration of these sulfones in the plasma and tissues of male Lewis rats and male BALB/c mice receiving DDS in the diet. All measurements were performed using our chromatographic-fluorometric method, which is specific for DDS and MADDS at nanogram levels.

When rats were fed a diet containing 0.005 g% DDS, mean plasma levels at seven days were 157 ng/ml and 44 ng/ml for DDS and MADDS, respectively. Nearly identical levels of both compounds were found at 21 days. Tissue-to-plasma ratios of DDS at both time periods were 0.7 for muscle and 1.0 for testes. Ratios of 1.2 to 1.8 were found for ear, nose, and body and scrotal skin. The highest ratios found were 2.2 for foot pads and tail skin. Tissue-to-plasma ratios for MADDS were similar to those found for DDS, and the extent of acetylation of DDS to MADDS in tissues was similar to that found in plasma.

Mice fed a diet containing 0.0025 g% DDS exhibited plasma levels at 7, 14, and 21 days averaging 180 ng/ml and 9 ng/ml of DDS and MADDS, respectively, with minimal differences among the various

times. Tissue-to-plasma ratios of DDS at all time periods ranged from 0.6 to 0.9 for brain, muscle, and testes and from 1.3 to 1.6 for ear, foot pads, skin, and fat. The highest ratio found was 3.5 for liver. Tissues exhibited low levels of MADDs, as did plasma.

Both species exhibited the same levels of DDS and MADDs in plasma and tissues at seven days as those found at later times, indicating rapid equilibration and no accumulation of the sulfones. The plasma levels found on these diets were consistent with our earlier observations. Because we previously demonstrated that diet and plasma levels of DDS are directly related, we would expect tissue levels to show the same direct relationship. Previous estimations of the minimal inhibitory concentration (MIC) of DDS for *M. leprae* in tissues of rats and mice were based on the assumption that tissue-to-plasma ratios are one. Our findings that most tissue-to-plasma ratios are > 1 suggest that the MICs of DDS for *M. leprae* extrapolated from plasma data are too low by a factor no greater than two.—[This research was supported in part by the U.S.-Japan Cooperative Medical Science Program, administered by NIAID (Grant AI-08214). Stanford Research Institute, Menlo Park, California and USPHS Hospital, San Francisco, California]

Peters, J. H., Gordon, G. R., Murray, J. F., Jr., Levy, L., Russell, D. A. and Shepard, C. C. Acedapson treatment of leprosy: metabolic disposition versus therapeutic response.

Studies on the disposition of DADDS (4,4'-diacetamidodiphenyl sulfone) in a small group of Filipino patients and in a large group of New Guinea patients have been performed to test possible relationships between pharmacologic parameters and therapeutic response to this drug.

In 22 multibacillary Filipino patients receiving the first intramuscular dose of 225 mg DADDS, we found that DDS and MADDs (monoacetyl DDS) were present in plasma in approximately equal quantities throughout the 77 days prior to the next treatment. Peak levels occurred between 21 and 35 days. The sum of DDS and MADDs was always approximately 90% of the total plasma sulfones, indicating extensive hydrolysis of DADDS at all times. The half-times of disappearance ($T_{1/2}$) from the plas-

ma of DDS and MADDs were 42 days, with DADDS exhibiting a half-life of 47 days. These same patients were subsequently tested for acetylator phenotype with SMZ (sulfamethazine) (10 mg/kg) and DDS (50 mg) and the disposition of DDS was also determined. The percentage acetylation of SMZ in plasma showed that 17 (77%) were rapid and 5 (23%) were slow acetylators. Correlations between acetylation of SMZ and of DDS after DDS, and between acetylation of DDS after DDS and after DADDS were highly significant, although acetylator phenotypes were not discernible after DADDS administration. $T_{1/2}$ values of DDS after DDS were variable and did not relate to acetylator phenotype. However, a positive correlation between $T_{1/2}$ of DDS after DDS and minimal plasma levels of DDS after DADDS was observed. Response to DADDS therapy was estimated from the extent of multiplication in the mouse foot pad test system of *M. leprae* obtained from biopsy specimens taken at intervals during treatment. No relationship between response and any of the pharmacologic parameters measured could be demonstrated.

In over 400 leprosy patients of all disease types in New Guinea who had received 225 mg DADDS regularly, every 77 days for five years, we determined plasma levels of DADDS, MADDs and DDS 72-75 days after a regular dose of DADDS. No significant differences were found in the plasma levels of the sulfones among the various types of disease. Of the 447 patients in the study, 438 (98%) were diagnosed as healed. In the paucibacillary group, no relationship between year of healing and current levels of sulfones was found. Similarly, in the 20 multibacillary patients, no relationship between response to DADDS and plasma levels of sulfones could be discerned.

All patients of both groups exhibited minimal levels of DDS after DADDS that exceeded the established minimal inhibitory concentration of DDS for *M. leprae*. Average minimal levels of DDS were 25 and 31 ng/ml of plasma in the Filipino and New Guinea patients, respectively. Also, average extent of acetylation of DDS in both groups was nearly identical at 42%. No substantial accumulation of sulfones in the New Guinea patients receiving DADDS for five years was observed.—[This work was supported in

part by the U.S.-Japan Cooperative Medical Science Program, administered by NIAID (Contract Number NO1 AI 02283). Stanford Research Institute, Menlo Park, California; PHS Hospital, San Francisco, California; Department of Public Health, Territory of Papua New Guinea; and Center for Disease Control, Atlanta, Georgia]

Hirata, T. and Nakayama, T. Cytomorphological studies on leprosy bacilli.

The electron microscopic observations reported here are concerned with comparative cytomorphological studies on the cellular structure of *M. leprae* and *M. lepraemurium* in the host cell.

The following problems will be discussed:

1. Peripheral parts of each organism
 - a) capsular structure
 - b) cell wall
 - c) cytoplasmic membrane
2. Intracytoplasmic organelle of each organism
 - a) intracellular membranous organelle
 - b) inclusion-granules.—[National Institute for Leprosy Research, Tokyo, Japan]

Endo, H. and Nakayama, T. Mycobacterium KNBE—its nutrition and elongation.

For the application to the studies of *M. leprae* cultivation, mycobacterial organisms isolated from leprosy lesions were investigated.

Mycobacterium KNBE, one of these organisms, was accelerated in growth and elongated when the specific substance contained in some kinds of peptones was added. This specific substance (l-methioninyl-l-leucine) was separated and purified. The effects of this substance on KNBE (e.g., elongation) and the other mycobacterial organisms were studied.—[National Institute for Leprosy Research]

Mori, Tatsuo. Red pigment produced in medium by *M. lepraemurium*.

1. *M. lepraemurium*, *M. avium* and *M. intracellulare* produce a red pigment on 1% Ogawa yolk medium.
2. The red pigment emits a red fluorescence characteristic of porphyrin under ultraviolet light.
3. Three red fluorescent spots were detected on a paper chromatogram developed from the red pigment.

4. The visible light spectrum of porphyrin extracted from each spot resembled that of coproporphyrin III.

5. The R_f value of the upper spot resembled that of protoporphyrin and hematoporphyrin, but the upper spot was an esterized coproporphyrin which was extracted in ester form from the medium. When the upper spot was hydrolyzed in 1 N HCl, it chromatographed in the bottom spot with an R_f similar to that of coproporphyrin III.

6. Newly extracted porphyrin treated quickly with 1 N HCl had the ester character by paper chromatography of porphyrin ester.

When *M. lepraemurium* was cultivated on 1% Ogawa yolk medium, secretion of red pigment was observed in the medium under the colony. On the supposition that the production of red pigment may be a characteristic property of *M. lepraemurium*, production of red pigment was compared to that of the other acid-fast bacilli, and the nature of the red pigment was studied to see if there was a relationship between the red pigment and a growth factor for *M. lepraemurium*.

Red pigment produced on 1% Ogawa yolk medium without malachite green emits a red fluorescence characteristic of porphyrin under an ultraviolet light in a dark room. As the discrimination of the red fluorescence was very clear, the presence of the red fluorescence on egg medium was tested with other cultivable acid-fast bacilli. *M. avium* 4110, *M. avium* Kirchberg 3717, *M. avium* ATCC 15769, *M. intracellulare* ATCC 15985, *M. gastri* ATCC 15754, *M. tuberculosis* H37Ra, *M. ulcerans* NCTC 10417, *M. marinum* ATCC 927, *M. bovis* Ravenal, *M. bovis* BCG Takeo, *M. kansasii* ATCC 12478, *M. xenopi* ATCC 19276, *M. nonchromogenicum* ATCC 19531, *M. terrae* ATCC 15755 and *M. lepraemurium* Hawaii were used as typical strains. *M. avium* and *M. intracellulare* produced the red pigment markedly, similarly to *M. lepraemurium*. *M. terrae* and *M. nonchromogenicum* did not produce it, but the other strains produced a little.

Porphyrin was extracted with 1 N HCl from media-grown *M. lepraemurium*, *M. avium* and *M. intracellulare* and was absorbed on talc powder according to Nicholas *et al* (R. E. H. Nicholas and A. Comfort: Biochem. M. 45 [1949] 208). Concentrated porphyrin solution was prepared by extracting with acetone-water the absorbed talc

cut off the talc column. The extracted porphyrins were developed with 2, 6-lutidine: water = 6:4 in cold room by the method of Nicholas and Rimington (R. E. H. Nicholas and C. Rimington: *Scand. J. Clin. Lab. Invest.* **1** [1949] 12). The porphyrins of each strain were separated into three spots which showed the red fluorescence under ultraviolet light. In the region of visible light the adsorption spectrum of each porphyrin solution extracted from the three spots with 1 N HCl was similar to that of coproporphyrin III. While the upper spot had an Rf value near to that of protoporphyrin which has two carboxyl groups, coproporphyrin which has three carboxyl groups did not have the same Rf. Then the porphyrin was quickly extracted with 1 N HCl and developed with chloroform-kerosin by the method of Chu *et al* (T. C. Chu, A. A. Green and E. J. Chu: *J. Biol. Chem.* **190** [1951] 643). In this method free porphyrin does not move from the original line, but porphyrin esters move according to their characteristics. The quickly extracted porphyrin of *M. lepraemurium* showed the characteristics of porphyrin ester. Free coproporphyrin may be produced gradually by splitting the ester by long extraction with 1 N HCl. The nature of coproporphyrin ester could not be clarified in detail because of insufficient material.—[Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Suita Osaka, Japan]

Nakamura, Masahiro. Essential component for the growth of *M. lepraemurium* in cell-free liquid medium and related problems.

It has been confirmed by several investigators that the NC-5 cell-free liquid medium permits the propagation of *M. lepraemurium* (*Mlm*). The purpose of this paper is to determine which component in the medium is essential for the growth of the bacilli. The growth of *Mlm* was observed in various media modified by the elimination of components from NC-5 medium. The results obtained indicate that growth equal to that obtained in NC-5 medium cannot be observed if any component in the medium was eliminated, particularly α -ketoglutaric acid.

From the results of several experiments performed so far, it could be concluded that a component essential for the propagation of *Mlm* might be α -ketoglutaric acid, and the

other components in NC-5 medium would be growth stimulators. It might be thought that α -ketoglutaric acid, which can be replaced by oxaloacetate, would act at the start of the growth of bacilli.

In addition, it was found that the growth of bacilli was stimulated by serial transfers of the slide bearing the smeared inoculum to freshly prepared medium at definite intervals. Moreover, the effect of freshness of NC-5 medium was studied. The results show that *Mlm* multiplied quite well even in medium kept at 30°C for two months. Therefore, it could be considered that the stimulating effect on growth in the slide transfer trial might not be caused by the freshness of NC-5 medium. A possibly important factor is a slightly aerobic atmospheric condition, i.e., an introduction of fresh air to culture medium during long-term cultivation.—[Department of Microbiology, Kurume University School of Medicine, Kurume, Japan]

Matsuo, Yoshiyasu. Pathogenicity of *Mycobacterium lepraemurium* maintained in mouse foot pad cell culture and association of mitosis in the cells with intracellular growth of bacilli.

The Kurume-42 strain of *Mycobacterium lepraemurium* has been maintained in mouse foot pad cell culture for more than four years covering 50 subcultures. There have been cumulative bacterial increases of $10^{20.8}$ and $10^{18.5}$ -fold during a period of 1493 days. The overall generation times were estimated at 21.6 and 24.3 days respectively.

At the 1,255th day of cultivation, the intracellular bacilli were released from a small portion of the infected cell suspension by sonication. The bacterial suspension was then serially diluted tenfold and aliquots of each dilution were inoculated subcutaneously in the abdomen of five mice. Six months later, all the mice were sacrificed and the development of murine leprosy was checked macroscopically as well as microscopically. Two of five mice inoculated with the smallest number of bacilli (8.5 cells of *M. lepraemurium*) produced a typical lesion at the site of injection. The ID₅₀ was estimated to be more than 10.7 organisms, indicating that pathogenicity of the organisms has been unchanged in long-term cell culture.

At each subculture, one ml of the preceding culture was added to each of several Leighton

tubes containing glass coverslips and incubated in parallel with the main culture. Coverslips were removed at appropriate intervals and stained by the Ziehl-Toda method. The intracellular pattern of growth of *M. lepraemurium* was similar to that previously reported. Most of the cells contained few to numerous acid-fast bacilli. Moreover, association of the process of cell division with intracellular growth of the bacilli was clearly demonstrated. Cells infected with acid-fast bacilli showed all the stages of mitosis; prophase, prometaphase, metaphase, anaphase, and telophase. These findings suggest that bacterial increase in cell cultures of *M. lepraemurium* is caused not by phagocytosis of the released bacilli from the overinfected or deteriorated cells but by a constant intracellular growth cycle of the bacilli accompanying the mitosis of the infected cells.—[Department of Bacteriology, Hiroshima University School of Medicine, Hiroshima, Japan]

Ito, T. and Kohsaka, K. *M. leprae* in mouse macrophages *in vitro*.

The morphologic change of *M. leprae* in mouse peritoneal macrophages cultured *in vitro* was studied.

Mice of the dd0 strain were immunized by the injection of 0.2 mg of lyophilized BCG vaccine twice. Peritoneal macrophages were taken from the mice of the BCG vaccinated group and nonvaccinated control group and cultured in Leighton tubes with a cover slip. On the second day, cover slips containing macrophages were infected with *M. leprae*, and cover slips were taken out weekly and examined after Ziehl-Neelsen staining. Experiments were carried out three times.

The Morphological Index of *M. leprae* decreased so rapidly that after one week of infection the Morphological Index was less than 0.5 in all experiments. No significant proliferation of *M. leprae* was observed in mouse macrophages after six weeks of infection, and no significant morphologic difference was noted between *M. leprae* in BCG vaccinated cells and in nonvaccinated control cells.—[Research Institute for Microbial Diseases, Osaka University, Osaka, Japan]

Takizawa, H., Okada, S. and Nishiura, M.

The etiology of *erythema nodosum leprosum*: a clinical approach.

Since 1912, when Murata first described the syndrome called *erythema nodosum leprosum* (ENL) as a clinical entity, we have had many papers on ENL. The characteristics of ENL that are generally accepted can be arranged as follows:

1. ENL is essentially the manifestation of antigen-antibody reactions or immune complex disease and occurs in the pure and near lepromatous patients. But some lepromatous cases escape ENL in their clinical course.
2. This condition usually starts 6 to 12 months after treatment begins.
3. ENL can be graded as slight, moderate or severe in degree. This condition is definitely more frequent and more severe in clinically more advanced patients than in those with slight lesions.
4. Although ENL has become much more common since the introduction of the sulfones, it can be caused by other antileprosy drugs including chaulmoogra oil.
5. The prognosis of patients with severe ENL is worse than that of the patients without ENL.

Our retrospective study of ENL in the lepromatous patients nearly coincided with the above described characteristics. We have tried to notice the occurrence of ENL in the lepromatous patients. The time-lag from the onset of initial skin lesions noted by the patient to the start of chemotherapy had a relationship to the rate of occurrence of ENL in the lepromatous cases ($p = 0.05$). Twenty-three of 34 new cases (68%) who had the serologic test for syphilis (STS) had positive STS at the start of chemotherapy. These biologically false positive cases of STS were confirmed by the other tests, such as RPCF (Reiter protein complement fixation test) and TPHA (*Treponema pallidum* hemagglutination test). There was only one patient who had latent syphilis. The correlation between the occurrence of ENL and positive STS in the lepromatous cases was statistically significant ($p = 0.05$). The causative agent of ENL is suggested to be lipids rather than dead or disintegrated leprosy bacilli in the lepra cells. It could be said that ENL may be related to the maturity of lepromatous leprosy.—[Leprosy Research Laboratory, Kyoto University School of Medicine, Kyoto, Japan]

Namba, M., Sasaki, N. and Ohira, K. Report on a suspected case of bulbar palsy.

This is a suspected case of bulbar palsy with an obscure diagnosis during life.¹

The patient was a 72-year-old female patient with an almost arrested infection, first diagnosed as lepromatous type in 1936. After that time progress was satisfactory. Skin smears were continuously negative for bacilli, and treatment had been discontinued for many years. In November of 1972 she complained of dyspnea and visited our clinic but nothing abnormal was detected.

On 1 June she visited the clinic again and asked for medication because she was afraid of relapse, and daily doses of 25 mg of DDS were then administered regularly. Six months elapsed quietly. On 30 November 1973, in the middle of the night, she complained of sudden intense choking, but no stricture was detectable laryngoscopically beyond moderate pooling of saliva in the hypopharynx. This choking sensation was relieved after four hours, but other symptoms grew worse and dyspnea, fever and stridor appeared. On 6 December, tracheotomy was performed to prevent asphyxia, but all was not improved.

Pathologic findings. Up to the present no remarkable changes were seen histopathologically in the medulla oblongata except perivascular lymphocytic infiltration and slight localized proliferation of microglia cells centering on and around the nucleus ambiguus. There were a few solid mycobacteria in the hypoglossal nuclei.

Further research is being conducted.—[National Institute for Leprosy Research; and National Leprosarium Tama Zensho-en]

Jacobson, R. R., Hastings, R. C., Morales, M. J. and Slack, C. E. The treatment of leprosy with transfer factor.

Considerable interest has been generated by reports of several investigators who have administered immunotherapy to patients with leprosy (Paradisi *et al*, *N. Engl. J. Med.* **280** [1969] 859; Bullock, *Immunopathol.* **1** [1972] 122). Concern has been justifiably expressed by some workers as to the potential hazards of this new approach to therapy (Godal *et al*, *N. Engl. J. Med.* **288** [1973]

741). The purpose of the present report is to present preliminary toxicity data gathered during the course of treating three polar lepromatous leprosy patients and one borderline lepromatous patient with dialyzable transfer factor (Lawrence and Valentine, *Am. J. Pathol.* **60** [1970] 437). The material was prepared from healthy lepromin (Fernandez as well as Mitsuda) skin test positive hospital personnel by modifications of the method of Schulkind *et al* (*Cell. Immunol.* **3** [1972] 606). The donors were screened for the presence of hepatitis-associated antigen by counter-electrophoresis (Hyland Laboratories). Thyoglycollate cultures were made at five steps in the procedure and sterility in preparation was ensured prior to administration of the material. Each injection was millipore filtered (0.45 micron pore size) prior to lyophilization; a two step (48 hours followed by 24 hours) dialysis was employed; and a final millipore filtration (0.2 micron pore size) was performed immediately after reconstitution with 3.0 ml sterile, nonpyrogenic water and before subcutaneous injection into the subjects. Each patient received transfer factor (TF) from approximately 7×10^9 lymphocytes given in 36 divided doses over a 12 week period.

The three polar lepromatous patients improved both clinically and bacteriologically, but the borderline lepromatous case did not improve to a greater extent than would have been expected from her dapsone therapy alone. The patients who improved experienced histologically documented reversal reactions with onset at 19, 23, and 35 days after TF injections were begun. In each case the reversal reaction continued for the remainder of the trial (and beyond).

We have found no evidence of inherent toxicity in the material either locally or systemically. The potentially hazardous reversal reactions which occurred in all three polar lepromatous patients were mild, have required no specific therapy, and have resulted in no lasting adverse effects. Given the experience that corticosteroids are effective in suppressing spontaneous or chemotherapy-associated reversal reactions, and in reversing damage incurred by these reactions when given promptly and in adequate dosages, we feel that dialyzable TF can be administered with reasonable safety to leprosy patients under intensive medical supervision. In our

¹This instance is additional to the previous reports at these conferences.—Editor.

opinion the recognized hazards of TF and reversal reactions do not appear to be excessive under these conditions in view of the potential benefits to the patient-subjects involved.—[USPHS Hospital, Carville, Louisiana]

✓ **Abe, M., Minagawa, F., Yoshino, Y. and Itoh, M.** Immunoglobulin levels and antibody titers in nasal secretion versus serum of leprosy patient.

Using the indirect fluorescent antibody test (FLA-ABS test) we reported that the IgG and IgM antibodies reacting specifically with *M. leprae* were found in the serum of leprosy patients. However, IgA antibodies were rarely detectable. Since IgA is known as a secretory immunoglobulin and the nose may be a route of primary infection with *M. leprae*, it would be of interest to learn if IgA antibodies to *M. leprae* are found in the nasal secretion of leprosy patients. Serum and nasal secretions were obtained from five patients with tuberculoid leprosy, seven with lepromatous and five with *erythema nodosum leprosum* (ENL). Nasal washings were centrifuged at 10,000 rpm for 30 minutes and concentrated approximately 100-fold by ultrafiltration. The concentrations of IgG, IgM and IgA were determined with Hyland's immunoplates. The IgE level was measured by Pharmacia's "Phadebas" IgE test. FLA-ABS titers were expressed as the maximum dilution of serum or nasal fluid showing 2+ fluorescence of bacilli in the smears stained with fluorescent antibodies to human gammaglobulins and to each class of immunoglobulins.

Each average level of IgG, IgM, IgA and IgE in the serum of leprosy patients was far beyond the range observed in normal serum. No IgM was found in the nasal secretions of tuberculoid patients. The ratio of IgA/IgG in nasal secretions to IgA/IgG in serum was higher in lepromatous patients than in tuberculoid. This fact seems to suggest excessive secretion of IgA from the nasal mucous membrane of lepromatous patients. FLA-ABS titer of IgG in the serum was higher in lepromatous patients than in tuberculoid. Compared with the high IgG and IgM antibody titers in serum, the titers in the nasal secretion were very low in some lepromatous patients and negative in the others; the titers were negative in almost all tubercu-

loid patients. IgA antibodies in the serum were not found except in two lepromatous patients which had low IgA titers in the serum. On the contrary, IgA antibodies in nasal secretions were found in every case with tuberculoid leprosy and in two separate cases with lepromatous. The minimum concentration of IgA in nasal fluid causing positive FLA-ABS test was as low as that of IgM in the serum of lepromatous patients. IgE antibodies were found in the seven lepromatous patients only one of whom suffered from ENL. IgE antibodies were not detected in the nasal secretions of any leprosy patients examined. Middlebrook-Dubos' passive hemagglutination test with nasal secretion was positive in all of the lepromatous patients and antistreptolysin O titers with 50 or higher Todd units were found in the nasal secretions of five lepromatous cases.

These observations seem to indicate that IgA antibodies reacting specifically to *M. leprae* are absent or nearly so in both nasal secretions and sera of lepromatous patients. This deficiency may be related to the pathogenesis of nasal lesions in lepromatous leprosy.—[National Institute for Leprosy Research; and National Leprosarium Tama Zensho-en]

✓ **Morrison, N. E. and Collins, F. M.** Thymosin reconstitution of antimycobacterial immunity in the T cell depleted mouse.

Vaccinating doses of BCG will produce a fatal mycobacteriosis in mice depleted of T cells by adolescent thymectomy, lethal irradiation and bone marrow transfusion. An increased number of BCG organisms was found in the lungs, spleen, liver, bone marrow and lymph nodes of depleted animals. A persistent bacteremia was also present that was not detectable in control animals. The tempo of infection was slower in aerogenically-challenged as compared to intravenously-challenged animals. Aerogenically infected animals did not develop antituberculosis immunity against intravenous rechallenge by *M. tuberculosis*. Furthermore, the T cell depleted animal did not develop tuberculin hypersensitivity when measured in the foot pad.

In view of the fact that thymosin, an active polypeptide from the thymus gland, can influence the maturation of T cells, attempts were made to see if thymosin treatment

would influence the fatal course of BCG infections in T cell depleted animals.

Thymosin was injected into normal and T cell depleted animals and the multiplication of BCG was followed over a 90 day period. The effect of varying thymosin doses was also investigated by determining its ability to restore delayed hypersensitivity to sheep red blood cell antigens in the T cell depleted mouse. Thymosin treatment prevented the occurrence of a fatal BCG mycobacteriosis in that no deaths occurred during the experimental period in the T cell depleted animal. The viable organ counts of BCG indicated the eventual emergence of an immune response that controlled the infection. Furthermore it was found that thymosin was capable of restoring in some measure a delayed hypersensitivity response to sheep red blood cells in the T cell depleted mouse. These results are consistent with the claim that a thymosin-sensitive subpopulation of T cell precursors can be matured into a functional state in the absence of the thymus gland to express some aspects of cell-mediated immunity.—[Johns Hopkins University-Leonard Wood Memorial Leprosy Research Laboratory, Baltimore, Maryland; and Trudeau Institute, Saranac Lake, New York]

Bullock, W. E., Evans, P. D. and Wyatt, C. R. Depletion of lymphocyte subpopulations in the spleen by murine leprosy.

Murine leprosy is a chronic granulomatous infection of lymphoid tissues and involves periarteriolar lymphocyte sheaths of the spleen. C³H mice were inoculated iv with 5×10^7 *Mycobacterium lepraemurium*. At 8, 16 and 23 weeks post-inoculation, infected and age-matched control mice were sacrificed. Whole spleens were prepared for total nucleated and differential cell counts. Lymphocytes bearing the θ -isoantigen were quantitated by a ⁵¹Cr release cytotoxicity assay and the response of spleen cells to phytohemagglutinin P (PHA) was measured. Mean numbers of nucleated cells increased to a maximum of 3.1×10^8 at 16 weeks in infected spleens but had declined by 23 weeks (1.6×10^8 cells) to near control levels (1.4×10^8 cells). The proportion of lymphocytes by differential count in infected spleens at 23 weeks was 25% vs 77% in normal spleens. Frequencies of θ -positive cells (% lysis) declined after the 16th week of infec-

tion to a mean of 5.5% vs 22.9% in control spleens at 23 weeks. The results indicate that murine leprosy causes progressive loss of lymphocytes including T cell subpopulations from the spleen.—[University of Kentucky College of Medicine, Lexington, Kentucky]

Shepard, C. C. Immunity to *M. leprae* infections in mice caused by GVH reactions and by injections of *M. leprae* and BCG.

Protective effect was provided against *M. leprae* infections in mice by graft-versus-host (GVH) reactions and by injections of BCG and *M. leprae*. The mechanisms by which the protection was induced were probably different with the different antigens. Chronic GVH reactions were accomplished by injections of parental spleen cells into C3H/Hej \times DBA/2J F1 hybrids. No protection against challenge with *M. tuberculosis* or *M. marinum* was observed. The protection against challenge with *M. leprae* was thought to be caused by the generalized activation of macrophages that accompanies GVH reactions, and it was manifested by a delay in the appearance of the growth of *M. leprae*. After the delay, the new growth proceeded at a normal rate and continued to normal plateau value.

The protective effect of *M. leprae* and BCG was studied in another experiment. Some of the mice were inoculated in one rear foot pad with *M. leprae*, and when the infections had developed, the *M. leprae* was harvested from some of these, purified and concentrated so it could be given as a foot pad injection to previously uninfected mice. BCG was given by foot pad or intradermal injection. The protective effect provided by BCG resembled that associated with GVH reactions (delayed onset of growth of *M. leprae*), thus suggesting that at least part of its effect was accomplished by the generalized activation of macrophages that is known to accompany BCG infection. The protective effect provided by *M. leprae* injection or by prior foot pad infection was characterized by distinctly lower plateau levels but only moderate delay in the appearance of bacillary growth. Presumably this type of protection was caused by T cell sensitization to *M. leprae* antigens with later activation of macrophages in the local area of the challenge infection. The protective effect

of certain combinations of *M. leprae* and BCG was superior to that provided by either antigen alone.—[Supported in part by the U.S.-Japan Cooperative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases (NIAID) by means of an interagency agreement between NIAID and the Center for Disease Control, U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Atlanta, Georgia 30333]

✓ **Matsubishi, T. and Usui, M.** Specific suppression of antibody production to a given antigen by the injection of oil droplets enclosing a mixture of the antigen and an immunosuppressant.

One of the most important problems in immunology is the regulation of the immune response, especially the specific immune suppression to a given antigen while maintaining normal responsiveness to other antigens. In attempts searching for the way to suppress immune reaction specifically to a given antigen, we found that the induction of antibody production to a given antigen can be specifically suppressed by giving an injection of water-in-oil-in-water emulsion (w/o/w) in which the oil phase contains a mixture of an antigen and dexamethasone phosphate (DMP) within a droplet.

When mice were injected with HGG-DMP w/o/w and BSA w/o/w, the antibody response was greatly reduced to HGG but not to BSA. By injecting mice with BSA-DMP w/o/w and HGG w/o/w, the reverse result was observed. We could not get such a specific immunosuppressive effect in the experiments employing lipopolysaccharide (LPS) from *E. coli* as a thymus independent antigen. However, when w/o/w emulsion of which the oil phase containing LPS together with daunorubicine or cyclophosphamide was given to mice, the antibody response to LPS was selectively suppressed. Further results of such experiments will be reported.—[Department of Allergology, Institute of Medical Science, University of Tokyo]

✓ **Nakamura, K., Yogi, Y. and Matsuoka, M.** Studies on *Mycobacterium leprae* transmission into various animals—transmission into the foot pad of the bank vole (*Apodemus speciosus*) and field mouse (*Microtus montebelli*).

mus speciosus) and field mouse (*Microtus montebelli*).

Studies representing an attempt to search for a species of laboratory animal with a high susceptibility to leprosy bacilli are in progress in our laboratories. They are carried out by inoculating *M. leprae* into the foot pads of various animals and comparing the results of inoculation with those in laboratory mice.

This report summarizes the findings noted in a study of bank voles (*Apodemus speciosus*) and field mice (*Microtus montebelli*), in which the time-course of bacillary counts in the food pad of inoculated bank voles and field mice was followed. Bank voles and field mice were inoculated with leprosy bacilli into the foot pad (10^4 bacilli per foot pad) and at the same time control groups of laboratory mice were inoculated with the same strain of organism and in the same inoculum size, for comparison. All procedures of examination for mice, golden hamsters, rats, chipmunks, Mongolian gerbils, etc. were the same as described in a previous report. When acid-fast bacilli were found in the foot pad tissue of an inoculated bank vole and field mouse, they were cultured on a modified egg yolk medium (Nemoto *et al*) as well in order to distinguish them from *M. lepraemurium*.

In the foot pad tissue of inoculated bank voles and field mice, approximately 10^5 bacilli in the case of bank voles, and 10^3 – 10^4 bacilli in the case of field mice were found per animal five to seven months after inoculation. The microscopic examination demonstrated microcolonies seemingly characteristic of *M. leprae*. Virtually the same number of bacilli still were demonstrable in the foot pad of animals 10–13 months after inoculation. The organisms at these various stages were transferred by inoculation of ground tissues into foot pads of laboratory mice and also were cultured by our technic. The mouse foot pad test was positive, whereas the cultures failed to demonstrate any growth of acid-fast organisms. Bacterial counts of foot pad tissue of the inoculated animals were slightly lower than those in laboratory mice. However, these findings have led to the conclusion that bank voles and field mice are susceptible to leprosy bacilli. Further investigation into various wild animals, such as the Japanese vole, is under way to look for a species with higher susceptibility.—[National Institute for Leprosy Research]

Sasaki, N., Kawatsu, K., Minagawa, F. and Matsuoka, M. The effect of radioisotope ^{131}I combined with immunosuppressive agents on experimental leprosy.

We reported previously that the infection by *Mycobacterium lepraemurium* or *M. leprae* in mice was increased with the use of large doses of ^{131}I alone and combined with other immunosuppressive agents.

The present study has been carried out as a series of experiments using 100 uCi ^{131}I combined with ATG (anti-thymocytic globulin) and cortisone for the purpose of obtaining *M. leprae* infection of the whole body of mice and observing the host reaction of infected animals. The frequency and the period of administration of ^{131}I , ATG and cortisone were examined in four groups of mice as follows:

- A. ^{131}I + ATG + cortisone group
- B. ^{131}I + cortisone group
- C. ^{131}I ATG, or cortisone alone group
- D. Untreated control group

The results showed that the combined group A (^{131}I + ATG + cortisone) had more bacilli than each single group C (each treatment alone) and group D (untreated controls). In the foot pads of the A group the number of *M. leprae* reached 10^6 to 10^7 , in contrast to 10^5 in group D, one year after the inoculation with 10^4 bacilli. Histopathologically, apparent lepromatous granulomas with many bacilli in and around macrophages were observed in the inoculated site. The body temperature of ^{131}I -treated mice was generally 1° to 4° lower than that of control mice.

Lymphocytes in peripheral blood of group A were less than 35% as compared to approximately 60% in the control group. These observations seem to indicate the depletion of T lymphocytes. Preliminary results of lymphocyte transformation tests using phytohemagglutinin or Dharmendra antigen and immune responses against sheep red blood cells, confirm this impression.—[National Institute for Leprosy Research]

Sushida, Kiyo. Host-parasite relationship in the experimental transmission of leprosy bacilli in mice.

I recently read a paper on diminished natural resistance in mice which had been injected with sodium iodide. In that report I stated that iodide-treated mice had a dimin-

ished natural resistance to leprosy infection.

This paper contains data concerning the experimental transmission of leprosy bacilli into first generation mice (F1) that were the offspring of iodide-treated female parents. The first group of F1 mice was born of female parents that had been injected with 100 μC sodium iodide "during" pregnancy. A second group of F1 mice was of female parents injected with sodium iodide eight or ten weeks "before" pregnancy. The first group of 34 mice are designated "during pregnancy" mice. The second group of 38 F1 mice are designated "before pregnancy" mice. Seventy-three normal male mice were injected similarly with *M. leprae* as a control for the "during" and "before" pregnancy groups. The leprosy bacilli, approximately 5×10^3 *M. leprae*, were injected into each testis. The leprosy bacilli used were the six strains, LL28, LL32, LL33, LL34, LL35, and LL36. These strains had been isolated from six lepromas of four patients at different times. Four of the six strains were taken from patients who had not received any anti-leprosy drugs, viz., LL28, LL32, LL33, and LL34. The remaining two strains of LL35 and LL36 were taken from patients who were being treated with drugs. Acid-fast bacilli were demonstrated on the stamp-smear samples of testes with Ziehl-Neelsen staining. When acid-fast bacilli in the condition known as globi were packed within a cell, this positive result was designated as +G. In 11 (32%) of 34 mice in the first group, globi were present. The strains used were LL28, LL35 and only a part of the total leproma of strains LL32 and LL33. However, LL35 was negative. Thirty-eight mice belonging to the second group ("before pregnancy") were all negative. The four strains used in this second group were LL34 and LL36, and the remaining parts of the leproma of strains LL32 and LL33. The 73 control mice in both groups were also negative. Because of these demonstrated facts, the "during pregnancy" group was more susceptible than the second group.

The positive results (+G) were in the combination A, meaning the use of "during pregnancy" group mice as host and then injecting them with leprosy bacilli isolated from the nontreated patients. All other combinations showed negative results in this investigation. In the F1 mice the thyroid tissue

was not destroyed, and there were few changes in their body temperatures.

The above investigations would indicate that this is an excellent way to develop more susceptible mice for leprosy infection. This is done by using mice a) born of females which had been treated with some immunosuppressive material, or b) born of females which had suffered diminution of natural resistance "during" their pregnancies.—[Department of Microbiology, Tokyo Women's Medical College]

Fieldsteel, A. Howard. Detection of a few viable *M. leprae* in an inoculum of 10^7 dead *M. leprae* by inoculation into foot pads of neonatally thymectomized rats.

We have shown that the cooler sites on the body of neonatally thymectomized Lewis rats are highly susceptible to infection with *M. leprae* after either local or intravenous inoculation. After foot pad infection, 100 to 1,000 times more *M. leprae* can be recovered from neonatally thymectomized rats than from intact rats or mice. Since the absolute ceiling of multiplication in foot pads of intact mice and rats is about 2×10^6 *M. leprae*, only a limited number of organisms can be inoculated to demonstrate multiplication. This limits the sensitivity of the intact mouse for evaluation of the results of chemotherapy, since a patient with lepromatous leprosy may harbor from 10^{11} to 10^{12} *M. leprae*, and a skin biopsy may contain up to 10^8 organisms. It thus appeared that the neonatally thymectomized rat might have a better potential for detecting smaller numbers of surviving *M. leprae* during or after chemotherapy.

Although we knew that *M. leprae* would multiply to a higher level in the thymectomized rat than in the intact rat or mouse, we did not know the minimum number of viable organisms necessary to infect thymectomized rats, nor did we know whether small numbers of viable *M. leprae* would multiply in the presence of large numbers of dead *M. leprae* in these animals. We have now shown that an inoculum of *M. leprae* containing as few as two to three viable organisms reached a level of 6.86×10^6 per foot pad by eight months after inoculation, and at the end of a year the number had reached 4.81×10^7 . When two to three viable *M. leprae* were mixed with 10^7 heat-killed *M. leprae* and also inoculated into the hind foot pads, a peak of 6.40×10^7 per foot pad was obtained at the

end of a year. The viability of these organisms was confirmed by passage into mouse foot pads. It thus seems likely that the neonatally thymectomized rat may prove to be a useful and highly sensitive host in experiments designed to test the efficacy of drugs against *M. leprae* infection.—[This work is supported by the U.S.-Japan Cooperative Medical Science Program, Grant AI-08417. Life Sciences Division, Stanford Research Institute, Menlo Park, California, U.S.A.]

Evans, Michael J. Mononuclear cell turnover in mouse foot pads infected with *Mycobacterium leprae*.

The purpose of this research was to study the relationship of macrophages to *M. leprae* during the course of the infection in the mouse foot pad. Specifically, we are studying the kinetics of macrophage turnover as the cellular infiltrate develops in the foot pads.

The experiments to date cover three points on the growth curve, early multiplication (79 days post-infection), multiplication (121 days), and the stationary phase (156 days). In each experiment the animals were inoculated in both hind foot pads with 5×10^3 *M. leprae* in Dr. Levy's laboratory in San Francisco. At each point studied, a group of 15 mice were injected with $100 \mu\text{c}$ of $^3\text{H-TdR}$. At intervals of one hour, 1, 5, 11, and 14 days, groups of three mice were sacrificed and both hind foot pads prepared for light autoradiography. Some tissues were also prepared for electron microscopic autoradiography. The number of labeled and unlabeled macrophages were determined from both foot pads in each animal and expressed as labeled cells per 1,000 cells.

In animals from the 79 and 121 day experiments, there were no large cellular infiltrates in the foot pad and the labeling indexes were low at one hour (1.6 and 1.2). Most labeled cells found were associated with small groups or "islands" or mononuclear cells near blood vessels and nerve bundles. In the group at 156 days, a large cellular infiltrate was present throughout the connective tissue and the labeling index was considerably higher (13.7). No *M. leprae* were found in labeled cells at 79 and 156 days. At 121 days several labeled cells at each time interval contained organisms.

In animals from the 79 and 121 day experi-

ments the labeling index at 5 and 11 days increased about fivefold over one hour values. In animals from the 156 day experiment the labeling index increased less than twofold at 5 and 11 days. These results indicate that cell division at the site, and not migration from the vascular system, is the principle source of cells in the infiltrate at

156 days. However, at 79 and 121 days there is little measurable cell division at the site and most of the infiltrate cells are derived from cells migrating to the site.—[This work is supported by the U.S.-Japan Cooperative Medical Science Program, Grant AI-10110. Life Sciences Division, Stanford Research Institute, Menlo Park, California, U.S.A.]