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ABSTRACTS

FOURTEENTH JOINT LEPROSY RESEARCH CONFERENCE

Dauphine Orleans Hotel
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24–26 September 1979

U.S.–Japan Cooperative Medical Science Program

OPENING REMARKS

I want to welcome you all here to the Fourteenth Annual U.S.–Japan Leprosy Research Conference. We are particularly gratified and very pleased that this meeting could be held in the beautiful and historic city of New Orleans. I think that for that a tremendous amount of thanks is due to a number of people, particularly Dr. Hastings, who has been instrumental in securing this lovely hotel and in doing a great deal of the work that has made this meeting possible, and also to the staff of Carville, which was the purpose of meeting here: to make their facilities available for the people at this meeting. Not least, by any means, our thanks go to Drs. Gwinn, Beck, and Jordan, of the National Institute of Allergy and Infectious Diseases of the NIH, for their on-going support and for having permitted us to meet in this most interesting city.

—Barry R. Bloom, *Chairman*
U.S. Leprosy Panel

Photo courtesy of USPHS Hospital, Carville, Louisiana.
The participants at the U.S.–Japan Fourteenth Joint Leprosy Conference

Photo courtesy of USPHS Hospital, Carville, Louisiana.
Drs. Masashi Namba and Barry R. Bloom

PROGRAM OF THE FOURTEENTH JOINT LEPROSY RESEARCH CONFERENCE

Contents

24 September 1979, Monday

Opening Remarks: Dr. Barry R. Bloom, Chairman, U.S. Leprosy Panel

Session I: *Co-Chairmen:* Dr. Seitaro Okada
Dr. A. Howard Fieldsteel

Okada, S. Basic research on the electron microscopy of leprosy.

Kirchheimer, W. F., McCormick, G. T. and Sanchez, R. M. Identification of *Mycobacterium leprae.*

Kusaka, T., Kohsaka, K. and Akimori, H. Studies on analysis of mycolates in *Mycobacterium leprae* and *Mycobacterium lepraemurium,* using high performance liquid chromatography and mass-spectrometry.

Nomaguchi, H., Kohsaka, K., Yoneda, K. and Mori, T. Growth of *M. lepraemurium* in mouse, human and chick cells by soft agar technique.

Hanks, J. H., Dhople, A. M. and Funk, H. B. Continuous *in vitro* growth of *M. lepraemurium* during one year at 38°. Taxonomic validation.

Fukunishi, Y., Okada, S., Nishiura, M. and Kohsaka, K. The figures of multiplication of acid-fast bacilli in the macrophage of nude mice—electron microscope findings.

Session II: *Co-Chairmen:* Dr. Masahiro Nakamura
Dr. Norman E. Morrison

Nakamura, M. Subsequent results of cultivation trial of *M. leprae* in cell-free liquid medium.

Fieldsteel, A. H., Sato, N. and Moeckli, R. A. Studies on *Mycobacterium leprae* in tissue cultures of the nine-banded armadillo (*Dasypus novemcinctus*).

Namba, M., Ozawa, T., Sasaki, N., Fukushima, K., Sanada, K. and Koseki, M. Malignant neoplasm among leprosy patients in relation to carcinogenicity of DDS.

Morrison, N. E., Klayman, D. L. and Collins, F. M. Antimycobacterial activity of thiosemicarbazones in relation to their chemical structure.

Brand, P. W. and Beach, R. B. Plantar ulceration in leprosy.

MacMoran, J. W. and Brand, P. W. Bone absorption in fingers in leprosy: a ten-year radiologic study.

Hastings, R. C., Morales, M. J., Belk, S. E. and Shannon, E. J. Thalidomide analogs with potential activity in erythema nodosum leprosum.

25 September 1979, Tuesday

Session III: *Co-Chairmen:* Dr. Kenji Kohsaka
Dr. Robert C. Hastings

Kohsaka, K., Yoneda, K., Mori, T. and Ito, T. Study of chemotherapy of leprosy with nude mouse.

Gillis, T. P. and Buchanan, T. M. Fractionation of antigens of *Mycobacterium leprae*.

Shannon, E. J., Morales, M. J., Christy, S. A. and Hastings, R. C. Antigenic interrelationships among selected mycobacterial species relative to *Mycobacterium leprae* as manifested by cell-mediated immunity in guinea pigs.

Watson, S. R. and Collins, F. M. Suppressor T-cell production in heavily infected mice.

Wong, J. F. Sensitized T-cell proliferative response to lepromin.

Bullock, W. E., Havens, R. A. and Nickerson, D. A. Splenic suppressor cell populations in *M. lepraemurium* infected mice that suppress cell-mediated lymphocytotoxic activity.

Session IV: *Co-Chairmen:* Dr. Takashi Kusaka
Dr. Barry R. Bloom

Shepard, C. C. Vaccination experiments with *M. leprae* preparations and with cultivable mycobacteria.

Lefford, M. J. Interaction of *M. lepraemurium* and BCG infections in mice.

Patel, P. J. Systemic macrophage activation in *M. leprae* immunized mice.

Colston, M. J., Fieldsteel, A. H., Lancaster, R. D. and Dawson, P. J. The athymic rat: immunological status and infection with *Mycobacterium leprae*.

Mehra, V., Mason, L. H., Wells, L. M. and Bloom, B. R. Lepromin induced suppressor cells in leprosy patients: evidence for mediation by TH_2^+ T-cell subset.

Artz, R. P., Bullock, W. E. and Jacobson, R. R. Decreased suppressor cell activity in leprosy and other disseminated granulomatous infections.

Rea, T. H. and Terasaki, P. I. HLA-DRW antigens in Mexican patients with leprosy.

26 September 1979, Wednesday

Session V: *Co-Chairmen:* Dr. Masashi Namba
Dr. Wayne M. Meyers

Gordon, G. R., Murray, J. F., Jr., Peters, J. H., Gelber, R. H. and Jacobson, R. R. Studies on the urinary metabolites of dapsone in man.

Peters, J. H., Murray, J. F., Jr., Gordon, G. R., Tatsukawa, H. and Matsuo, Y. Thioamides and thioamide-S-oxides for leprosy chemotherapy.

Jacobson, R. R. Rifampin for the treatment of lepromatous leprosy.

Collins, F. M., Klayman, D. L. and Morrison, N. E. Antimycobacterial activity of thiosemicarbazone derivatives *in vitro* and in chronically infected mice.

Meyers, W. M., Heggie, C. D., Kay, T. L., Staple, E. M. and Kvernes, S. The Ridley-Jopling five-group classification of leprosy—correlations of parameters of the classification in 1429 leprosy patients.

Gelber, R. and Gibson, J. The killing potential of various aminoglycoside antibiotics for *M. leprae*.

Closing Remarks: Dr. Masashi Namba, Chairman, Japanese Leprosy Panel

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ABSTRACTS OF LEPROSY CONFERENCE

Okada, S. Basic research on the electron microscopy of leprosy.

Epon 812, a kind of epoxy resin, is used most widely for electron microscopic study by the ultrathin-section method in biology because it has many good points. It, however, has a weak point in the study of leprosy. It can penetrate well into the cytoplasm and leprosy bacilli in tuberculoid lesions or lesions having some bacilli which are not in foamy structures, i.e., existing freely in the cytoplasm. But in lepromatous lesions, Epon does not penetrate well into foamy structures and leprosy bacilli in them. Therefore, Epon is not polymerized uniformly in the cell, and some parts of foamy structures and some bacilli in them are left out at the time of ultrathin-sectioning to create a large hole in the section. Additionally, some of the bacilli which are retained in the foamy structure are homogeneously dense, their inner structures not being differentiated. Vestopal, polyester resin, and Araldite, Maraglas, and Epok 533, epoxy resin, are stable in an electron beam but are not as widely used as Epon 812. They also have the same weak point as Epon 812 in the study of leprosy.

Methacrylate is not commonly used because it has several weak points. On the other hand, it has very low viscosity in contrast to Epon, which has high viscosity, and therefore methacrylate can penetrate well into foamy structures and leprosy bacilli. The resin is polymerized as uniformly inside foamy structures and leprosy bacilli as at other parts. Accordingly, foamy structures and leprosy bacilli are ultrathin-sectioned well, keeping their fine structures, and electron microscopic observation reveals clear figures of those fine structures. In fields other than leprology, students of the morphology of yeast still use methacrylate because Epon cannot penetrate into yeast cells through the thick cell wall.

A low viscosity epoxy resin has been used in the field of botany and yielded good results. The plant cell has a hard cell wall, and the usual resins, especially viscous Epon, Araldite, etc., cannot penetrate well into it. In contrast, Spurr's resin mixture including ERL 4206, which has very low viscosity, penetrates well into plant cells and yields good electron microscopic figures. Additionally, the resin is said to be stable in an electron beam. For these reasons, this resin mixture was tried for the study of lepromatous lesions. A part of this trial was done in cooperation with Drs. Mukherjee and Ramu at the Central JALMA Institute for Leprosy in India. Lepromata were removed from lepromatous patients, fixed in glutaraldehyde, washed with buffer, postfixed in OsO_4, and dehydrated with a graded ethanol series. Then, a part of each leproma was embedded in methacrylate, another part in Epon, and the remaining part in Spurr's resin mixture. Additionally, a part of a leproma was embedded in Quetol 651, which is a new epoxy resin having a lower viscosity than Epon. As for substituter, DMSO (dimethylsulfoxide) or DMF (dimethylformamide) were used after dehydration with the graded ethanol series, being compared with PO (propylene oxide). In regard to the mixing

ratio of resin, the standard mixture, namely ERL 4206, 10.0; DER 736, 6.0; NSA, 26.0; and S-1, 0.4; was found to be good. The sections were stable in the electron beam, and therefore sections mounted on a copper grid without supporting film could be observed under the electron microscope. The sections were stained with uranyl acetate and lead citrate. The contrast of figures is lower than that of the section embedded in methacrylate, but because membranous structures can be observed clearly, photographing is easy. In the lepromata used in this study, the penetration of Epon into foamy structures and leprosy bacilli was not good, and a large part of the inside of the foamy structure was left out at the time of ultrathin-sectioning to form large holes in the section. In one of the three lepromata examined, Spurr's resin mixture penetrated completely into the foamy structures and leprosy bacilli in them, and no hole could be found in the foamy structure after ultrathin sectioning similar to those embedded in methacrylate. In the other two lepromata, a small hole could be found in some foamy structures, but generally speaking, the penetration of this resin was much better than Epon. It could penetrate well into leprosy bacilli in foamy structures, and the fine structures of these bacilli were preserved very well. As for substituter, the fine structures of bacilli were better preserved in the specimen for which DMF was used than in that for which PO was used although the difference was not marked.

Spurr's resin mixture has the same strong point as methacrylate in penetrating well into foamy structures and leprosy bacilli in them in contrast to Epon which cannot penetrate well. On the other hand, it has another strong point; like Epon it is stable against the electron beam. Besides, the specimen embedded in it can be ultrathin-sectioned easily. In some specimens, a small hole may be formed in the foamy structure. Further study on the process of dehydration and embedding in it is necessary to eliminate the appearance of these small holes. It can be said that Spurr's resin mixture is a resin suitable for the electron microscopic study of leprosy, especially lepromatous leprosy and leprosy bacilli.—[Leprosy Research Laboratory, Kyoto University School of Medicine]

Kirchheimer, W. F., McCormick, G. T. and Sanchez, R. M. Identification of *Mycobacterium leprae.*

At the time of the IX International Leprosy Congress in 1968 in London, the need for identifying *Mycobacterium leprae* concerned mainly mycobacteria growing in cultured cells or bacteriologic culture media. Some areas of epidemiology such as human carriers and the role of arthropods in the transmission of leprosy were also part of the picture. Since then, experimental leprosy in armadillos, a controversial enzootic of unresolved origin and significance in this mammal, worldwide transmission-experiments in indigenous mammals, and the possible existence of plant reservoirs of *M. leprae* made its correct identification of added importance. In this paper Carville's experience with the pyridine extraction test and the D-dopa oxidation test are presented. Mycobacteria tested were *M. leprae* A, *M. leprae* H, 18 culturable mycobacteria obtained from ATCC, a cultivable mycobacterium from an armadillo, and Skinsnes' mycobacterium strain HI-75. Eight-five (96%) of the 88 *M. leprae* A isolates from experimentally infected armadillos gave unequivocally positive pyridine extraction and dopa oxidase tests. One strain (1.1%) gave neither a positive pyridine extraction test nor a positive dopa oxidation test. In one isolate (1.1%) the pyridine extraction test was positive and the dopa oxidase test negative. In an additional isolate with a positive pyridine extraction test, the dopa oxidase reaction was repeatedly questionable (1.1%). Negative pyridine extraction and positive dopa oxidase tests were not observed in any one of the 88 isolates.

On the other hand none of the culturable mycobacteria gave either positive pyridine or dopa tests.

In the eighth edition of Bergey's *Manual of Determinative Bacteriology*, 1974, numerical characters used for typing mycobacteria are listed as positive when more than 84% of the strains tested are positive. Following this reasoning, the pyridine extraction test in *M. leprae* A and the dopa oxidation test are well supported positive characteristics of *M. leprae.*

It is suggested that difficulties in identi-

fying "cultured *M. leprae*" can be resolved because a profusion of *M. leprae* from infected armadillos is available for preparation of DNA. Relatedness should be based on the extent of reassociation between ^{14}C-labeled DNA of the supposedly cultured leprosy bacilli and DNA of *M. leprae*.—[U.S. Public Health Service Hospital, Carville, Louisiana]

Kusaka, T., Kohsaka, K. and Akimori, H. Studies on analysis of mycolates in *Mycobacterium leprae* and *Mycobacterium lepraemurium*, using high performance liquid chromatography and mass-spectrometry.

Mycolic acids, a series of high molecular weight 3-hydroxy fatty acids with a long chain alkyl branch at the 2-position, are well known as one of the main components of cell walls of mycobacteria as well as related taxa.

Though physiological functions of mycolates are not yet elucidated completely, their taxonomical importance is becoming more and more evident recently, due to a remarkable progress in analytical procedures, especially in mass-spectrometry combined with gas-chromatography.

From the viewpoint of chemical identification of *Mycobacterium leprae*, it seemed very important to elucidate if one can detect any mycolate in this organism and elucidate its structural character. Under these circumstances, we have been able to find a new method to isolate mycolates efficiently from a small amount of bacterial cells using high performance liquid chromatography. The method was subsequently applied to analysis of mycolates in *M. leprae*. In consequence, at least two series of mycolic acids, one of which belonged to α-mycolate having C_{20}-branch at the 2-position, could be detected for the first time from an animal tissue containing only *M. leprae* as a parasite.

With reference to a parallel study on *Mycobacterium lepraemurium*, the applicability of the method here described to a chemical identification of *M. leprae* was discussed.—[Department of Biochemistry, Kawasaki Medical School; Institute of Microbial Diseases, Osaka University; Naka Works, Hitachi Ltd., Japan]

Nomaguchi, H., Kohsaka, K., Yoneda, K. and Mori, T. The growth of *M. lepraemurium* in mouse, human, and chick cells by soft agar technique.

M. lepraemurium grew well in a Balb/c 3T3 recloned cell line (A31). The average generation time of *M. lepraemurium* in A31 cells was 5.3 days at 37°C and 9.4 days at 35°C. A31 cells were very sensitive to infection with *M. lepraemurium*. Only 6 bacilli were inoculated into an A31 monolayer, and an increased number of bacilli was clearly evident microscopically 30 days after inoculation. The intracellular bacilli were readily transferred without loss of the number of organisms by host cell transfer.

The growth of intracellular bacilli was inhibited by streptomycin 100 μg/ml, clindamycin 25 μg/ml, INH 5 μg/ml, and rifampin 5 μg/ml. When streptomycin or clindamycin was removed from the culture medium on day 40, and the cultivation carried on with drug free medium, the intracellular bacilli began to grow immediately. In contrast, INH and rifampin treated bacilli did not grow again immediately after removal of these drugs.

Next we attempted to apply the soft agar technique for the cultivation of host cells for *M. lepraemurium* because normal cells stop cell division in semisolid agar medium (0.33%), and transformed cells stop cell division in more solid agar medium (0.5%). *M. lepraemurium* grew well in A31, A31 transformed by polyoma virus, nude mouse footpad cells, chick embryo cells, and human neuroblastoma cells by applying the soft agar technique. In monolayer culture, A31 cells do not remain in good condition for more than 2 months without transfer of the cells. However, A31 in agar remained viable for more than 5 months as shown by their ability to absorb methylene blue. These data provide useful clues for the cultivation of *M. leprae*. We are investigating the possibility of applying the soft agar technique to the cultivation of *M. leprae*.—[Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita City, Osaka, Japan]

Hanks, J. H., Dhople, A. M. and Funk, H. B. Continuous *in vitro* growth of *M. lepraemurium* during one year at 38°. Taxonomic validation.

Having confirmed that Mlm (*M. lepraemurium*) is capable of a single, non-transferable cycle of growth in the Nakamura system at 30°, we have:

a) eliminated the abnormal elongation of cells;
b) replaced the absolutely required, but labile, compounds with stable related compounds or precursors;
c) determined the complex nutriments required for continuous growth at 38°;
d) accomplished serial transfers for a period of one year at 38°;
e) validated the cells thus grown by means of specific genetic markers and
f) are now initiating investigations on the physiology and cultivation of *M. leprae*.

30°, semi-synthetic media. The factor required to facilitate normal cell division was Mg^{++} at 20× the usual concentration. The labile key compounds were replaced as follows: malate for α-ketoglutarate, the heme precursor, delta-aminolevulinic acid, for hemin; and two sulfhydryls, thioglycolate and dithiothreitol, for cysteine or thioglycolate alone. Growth rates during 4 weeks were 8× those in highly susceptible mice in which the doubling time is seven days. Nevertheless, the semi-synthetic media were unable to maintain serially transferable growth. The growth potential declined progressively following each transfer.

38°, complex media. In the semi-synthetic media above, continuous growth required the excess Mg^{++} mentioned plus unknown factors provided by a combination of yeast extract and tryptic digest of casein. The continuous growth may be characterized as follows:

a) growth rates were 12× those in susceptible mice;
b) after growing one year at the standard rates, each inoculated cell had given rise to 3×10^{21} new cells;
c) there was no decline in growth rates or growth potential;
d) there was an increase in growth rates and no relaxation of the original specifically required conditions and growth factors.

Items c and d seem to refute the claims of Skinsnes, Kato, and others, namely the Mlm *in vitro* readily gives rise to growth competent mycobacteria (which to date have had genetic markers that differ from those of a legitimate Hawaiian strain of Mlm).

The experience with ATP and Mlm seems at last to have laid a scientific basis for our current investigations with *M. leprae*.—[Department of Pathobiology (Division of Tropical Diseases) Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland]

Fukunishi, Y., Okada, S., Nishiura, M. and Kohsaka, K. The figures of multiplication of acid-fast bacilli in the macrophage of nude mice—electron microscope findings.

In order to study differences in ultra-structural features of the multiplication of *M. leprae* and *M. lepraemurium* in the cytoplasm of macrophages of nude mice, we have inoculated 10^7 *M. leprae* and 10^7 *M. lepraemurium* into the footpads of nude mice.

M. leprae grew in the phagolysosomes of macrophages of the nude mice, and at the same time small spherical droplets of lipidic material accumulated in the same phagolysosomes. This ultra-structural feature is quite the same as that seen in human lepra cells in human lepromatous lesions.

On the other hand, in the case of the multiplication of *M. lepraemurium*, bacilli growing in the phagolysosomes of macrophages of nude mice produce a crystalline substance around the bacillary bodies just like that of murine leprosy bacilli growing in C3H strain mice.

This experiment showed that the peribacillary substances, which *M. leprae* and *M. lepraemurium* produce in macrophages of their natural host cells (human and murine cells), did not change at all when they were inoculated into the nude mouse.

Also, these results suggest the possibility of using these ultra-structural features of peribacillary substances around the respective mycobacteria as criteria for the identification of these bacilli.—[Leprosy Research Laboratory, Kyoto University; Research Institute for Microbial Diseases, Osaka University, Japan]

Nakamura, M. Subsequent results of cultivation trial of *M. leprae* in cell-free liquid medium.

Enigmatic and curious growth features of *M. leprae* cultivated in a liquid cell-free medium were reported at the previous U.S.–Japan meeting in Osaka. When *M. leprae* were inoculated into ND-5 medium containing tyrosine and vitamin C and cultivated at 30°C, it seemed that the number of bacilli increased, and microscopical features resembling genuine growth were frequently observed three or six months after inoculation.

These morphological patterns, however, were not reproducible and depended upon the inoculum size. If a small number of *M. leprae* was inoculated, such a growth pattern was never recognized.

From these results, discussion should occur concerning the origin of such findings. In order to clarify and analyze the results, the following experiments might be necessary:

1) Evaluating experiments by using bacillary counting.
2) Directly observing the growth pattern by using a slide culture method.
3) Morphological observation by electron microscopy.
4) Estimation of viability of cultured *M. leprae* by ATP measurement and by mouse footpad inoculation.

The results obtained by the slide culture method, the bacillary counting method, and morphological observation, provide no evidence so far that multiplication of *M. leprae* takes place in the culture system in which *M. lepraemurium* easily and consistently multiplies.—[Department of Microbiology, Kurume University School of Medicine, Kurume, 830 Japan]

Fieldsteel, A. H., Sato, N. and Moeckli, R. A. Studies on *Mycobacterium leprae* in tissue cultures of the nine-banded armadillo (*Dasypus novemcinctus*).

For many years we have attempted to cultivate *Mycobacterium leprae* in a variety of mammalian tissue cultures without success. It is now known that *M. leprae* replicates, in many instances, better in the armadillo than in patients with lepromatous leprosy. Therefore, we have attempted to cultivate *M. leprae* in various tissues of the armadillo.

Initially, we obtained an armadillo, and from it we established cultures from 14 separate tissues. Three of the armadillo cultures (ear, nose, and footpad) were infected with *M. leprae* obtained from neonatally thymectomized Lewis rats (NTLR). *M. leprae* survived at least 14 days in all of the cultures, as determined by infectivity of the organisms for mouse footpads. Only in the armadillo nose culture did *M. leprae* survive longer (28 days) with no evidence that multiplication had occurred.

Since armadillos are not uniformly susceptible to *M. leprae* infection, it is possible that the armadillo from which these tissues were obtained was resistant to infection with *M. leprae*. We therefore initiated cultures from skin of an armadillo that had been inoculated intravenously with *M. leprae*. This animal had disseminated leprosy and the skin contained approximately 6×10^7 *M. leprae* per gram. Fluids were removed from these cultures at approximately semimonthly intervals, counts made, and organisms passaged into the foot-pads of BALB/c mice to test for viability. We have now demonstrated survival of *M. leprae* in these cultures for 94 days. There appears to be no evidence of multiplication, however. Details of these and other tissue culture experiments with both armadillo skin and human epidermal keratinocytes infected with *M. leprae* will be presented.—[Life Sciences Division, SRI International, Menlo Park, California]

Namba, M., Ozawa, T., Sasaki, N., Fukushima, K., Sanada, K. and Koseki, M. Malignant neoplasm among leprosy patients in relation to carcinogenicity of DDS.

Leprosy, being one of the chronic infectious diseases, normally requires a prolonged period of treatment. Recently, an increased incidence of malignant neoplasms has been noted among leprosy patients, and a possible connection with their immunodeficiencies, particularly in regard to cellular immunity, is being questioned. At the same time, there are a number of reports on the results of animal experi-

ments on the carcinogenicity of DDS, one of the main anti-leprotic drugs.

The study reported here was undertaken to find a possible pointer for further epidemiological investigation on the above questions by correlating the deaths due to malignancy among leprosy patients in the National Leprosarium, Tama Zensho-en, in recent years with the history of their sulfone intake in the past.

For our study, all deaths among the patients in Tama Zensho-en which took place during a seven year period since 1971 were investigated.

The 125 deaths which occured among the in-patients of the National Leprosarium Tama Zensho-en in the recent seven year period were investigated in regard to the incidence of malignant neoplasms recorded in autopsy reports, and their mortality rate and incidence of malignant neoplasms were compared with the expected values in the National Demography Statistics.

Of the deaths due to malignant neoplasm among the in-patients, possible correlation between cancer incidence and the amount of sulfone intake in the past was investigated.

From the present study, the following conclusions were drawn:

a) mortality rate of in-patients approximates the national expected value very closely;
b) deaths due to malignant neoplasms among the in-patients are slightly higher than the expected numbers, but these differences are not necessarily significant statistically;
c) of the cancer groups, it was suspected that in males, there is a statistically significantly larger number of patients who had greater intake of DDS;
d) Promin and other DDS derivatives seem to have no influence on cancer incidence;
e) there seems to be no fixed relationship between cancer incidence and disease types as well as sex differences.

—[National Institute for Leprosy Research; and National Leprosarium, Tama Zensho-en, Japan]

Morrison, N. E., Klayman, D. L. and Collins, F. M. Antimycobacterial activity of thiosemicarbazones in relation to their chemical structure.

Thiosemicarbazone derivatives, such as thiacetazone, are known to have antimycobacterial activity against *M. leprae* and *M. tuberculosis*. Recent investigations have resulted in the recognition of a new class of potential antimalarial agents, namely the 2-acetylpyridine thiosemicarbazones. A large number of compounds in this series have now been screened *in vitro* to establish their MIC values against *M. smegmatis* #607 plus a cross section of atypical mycobacterial strains including, *M. kansasii, M. simiae, M. avium*, and *M. intracellulare*. The results of this screening have resulted in structure-activity correlations for antimycobacterial activity and in the selection of compounds with potential candidate drug activity against highly drug-resistant atypical infections in the mouse.

In comparing the significance of structural parameters for MIC's, it was found that the lipophilicity of the various 2-acetyl-pyridine thiosemicarbazones was an important factor in assessing antimycobacterial activity. Lipophilicity, as calculated from log P values for octanol-water partitioning, indicated an activity distribution range for log P values of +3.5 to +4.5 with the optimum log P_o value around +4.0. This log P_o is identical to that found by other investigators for gram negative organisms. Thus it is concluded that the high concentration of lipid in the mycobacterial cell wall-membrane complex is a significant factor in the penetration of 2-acetylpyridine thiosemicarbazones to attain active MIC's. A consideration of lipophilicity parameters has resulted in the synthesis of a second class of substituted thiosemicarbazones, namely the 2-acetylquinazoline thiosemicarbazones which have been found to exhibit high antimycobacterial activity in the preliminary *M. smegmatis* #607 screen. Compounds with appropriate log P values are at present being tested against *M. leprae* in the Shepard mouse footpad assay.—[Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland; Walter Reed Army Institute of Research, Washington, D.C.; Trudeau Institute, Saranac Lake, New York]

Brand, P. W. and Beach, R. B. Plantar ulceration in leprosy.

The success or failure of leprosy control in endemic areas is often related to the perceived ability of the staff to overcome secondary problems such as plantar ulcerations, which are regarded by patients and families as manifestations of the disease.

Plantar ulceration is not hard to understand or to prevent when it is the result of direct injury from objects that penetrate the skin or when it results from ischemic necrosis from tight footwear. However, the majority of plantar ulcers have no such history and appear to result as a simple consequence of normal walking. This gives the feet of leprosy patients an image of fragility and incompetence which is discouraging to patients and staff.

In order to explore the etiology and pathology of this ulceraction, we have created an animal model and a machine to imitate the pressures of normal walking. The model is the hind footpad of a Sprague-Dawley rat, and the machine is an extension of a viscoelastometer apparatus which can apply a measured amount of pressure repetitively to the same spot on the footpad of an anesthetized rat for thousands of repetitions, simulating the repetitive stress of walking.

Some rats were normal and others were surgically denervated. The footpads were subjected to various levels and numbers of repetitive stresses over numbers of days. The rats were sacrificed at various days so that the histology of the footpad could be studied.

Levels of pressure and numbers of repetitions were found which would result in ulceration of the skin after a number of days. The histology showed a progressive inflammation that developed as the result of repetitive moderate stress over a period of days. The inflammation was aseptic and resulted in increased turgidity of the tissues and loss of compliancy. Subsequent stress at the same level then resulted in deep necrosis and then ulceration.

The normal and denervated rats developed the same type of change though it occurred faster in the denervated pads.

The study is presented as evidence that both normal and denervated feet are vulnerable to this kind of damage. The normal feet avoid it by awareness of the early stage ("soreness") and then by a reduction of exposure to stress. Both normal and denervated feet develop localized high temperature of the inflamed area for some time before breakdown. This early sign of inflammation may be used to warn patients who have insensitive feet that they are in imminent danger of breakdown.—[Rehabilitation Branch, USPHS Hospital, Carville, Louisiana]

MacMoran, J. W. and Brand, P. W. Bone absorption in fingers in leprosy: a ten-year radiologic study.

One of the best known and most stigmatizing features of leprosy is the progressive loss of digits in the hand.

There are at least three possible causes for this. The first is that the bones may be infiltrated and softened or actually destroyed by *M. leprae* or by the response of the tissues to the presence of *M. leprae*. The second is that the loss of peripheral nerves results in some "trophic" change which either renders the bones more fragile or allows actual absorption in association with excessive force used by the insensitive hand. The third is that the bones are destroyed by secondary infection which enters through wounds in the skin and causes osteomyelitis and sequestration.

Even though more than one cause may operate to produce absorption, it was recognized that if one cause could be clearly identified as the major cause of absorption, it would be helpful in the planning and implementation of a program of prevention.

Method. For a five-year period the hands of every patient at the U.S. Public Health Service Hospital at Carville were X-rayed at yearly intervals to determine if any progressive absorption of bones was taking place. For a further five years, the hands of those who had been studied in the first five years were further X-rayed annually so long as they remained under observation. No new cases were added in the second five-year period.

Since patients were being admitted and discharged during those years, and since a few patients declined to participate, only 206 had two or more radiographs for com-

parison. Fifteen hundred fifty-four patient years were completed, and 1100 mm of absorption were recorded. Those patients were completely unselected except that they represented the older and more severely sick and deformed of the patient body since the less involved were discharged sooner.

The radiographs were made with special projections to display the full length of each phalanx even when the fingers were clawed or otherwise distorted. A research technician measured each phalanx, under magnification when necessary, and recorded every change from year to year. A senior specialist radiologist visited, reviewed the films, and made an independent evaluation of the cause of any bone changes. The medical record of each patient was also screened to determine the timing of wounds and infections and also of changes in bacterial indexes, reactions, and major medication that might be related to bone change.

Results. The major conclusion of this ten-year study was that more than 90 percent of all bone loss was directly traceable to secondary infection. There was almost no change in the length of bones in the absence of soft tissue wounds of the fingers. The shortening associated with *M. leprae* infiltration of phalanges was rare, but when it occurred was readily diagnosed by radiograph and was usually proximal whereas the secondary infection usually resulted in distal phalangeal absorption or sequestration.—[Department of Radiology, Germantown Hospital, Philadelphia, Pennsylvania; USPHS Hospital, Carville, Louisiana]

Hastings, R. C., Morales, M. J., Belk, S. E. and Shannon, E. J. Thalidomide analogs with potential activity in erythema nodosum leprosum.

The major drawback to the widespread use of thalidomide in erythema nodosum leprosum (ENL) is its well-known teratogenicity. The drug is unique in having immunosuppressant-antiinflammatory activity in ENL and not sharing characteristics of other known drug classes expected to show such activity, namely the cytotoxic immunosuppressants, the acidic non-steroidal antiinflammatory agents, and the corticosteroids. Studies with thalidomide have suggested that it has 2 clinically relevant sites of action in ENL, inhibition of IgM antibody synthesis and inhibition of neutrophil chemotaxis. Reasoning that a thalidomide analog which would have efficacy in ENL clinically must have similar activities, we have screened a number of thalidomide derivatives in two *in vivo* models which appear to reflect these two relevant sites of action of the parent compound. As a measure of the ability of the compounds to inhibit IgM antibody synthesis we have utilized the assay of direct plaque-forming-cells (IgM antibody synthesizing cells) (PFC) in the spleens of Swiss-Webster mice 4 days after i.v. immunization with sheep erythrocytes. As an *in vivo* measure of the ability of the test compounds to inhibit neutrophil chemotaxis, we have utilized the late (6 hour) carrageenan rat paw edema assay.

To date, 10 phthalimide derivatives have been screened in both assays. Phthalimide itself and two N-alkyl substituted phthalimide derivatives, N-ethyl-phthalimide and N-n-butyl-phthalimide are probably non-teratogenic and have no thalidomide-like activity in the PFC or carrageenan assays. Similarly N-hydroxyphthalimide and N-(3-hydroxypropyl) phthalimide lack activity in the screens and are probably non-teratogenic as are 4-nitrophthalimide and 3,6-dihydroxy-phthalimide. The thalidomide metabolites, phthalyl-DL-glutamic acid, N-α-phthalylglutamine and N-α-phthalylisoglutamine are inactive in carrageenan but inhibit PFC. They are generally considered non-teratogenic although there is some controversy that the L isomers may be. The marginally teratogenic, N-phenylphthalimide is inactive in both screens. Of considerable interest is the lack of activity in the screens of 2-N-phthalimidophthalimide, a compound with teratogenic properties said to be equivalent to those of thalidomide itself. This suggests that the antiinflammatory/immunosuppressive activity of the thalidomide structure (apparently lacking with 2-N-phthalimidophthalimide) is separable from the teratogenic activity of the thalidomide structure (apparently present with 2-N-phthalimidophthalimide).

To date, glutarimide itself is inactive in carrageenan, and glutamic acid is inactive

in PFC. Two B-phthalimido substituted glutarimides, E 350 and E 565, have anti-tumor activity and lack anti-carrageenan activity. E 350 inhibits PFC but not E 565 to date (although the data are suggestive). Of the α-substituted glutarimides, glutethimide shows marginally significant activity in the carrageenan assay at the lowest dose but not in higher doses tested; activity to enhance PFC at the highest dose screened but not at lower doses; is not known to be teratogenic; and lacks anti-ENL activity clinically.

Two α-phthalimidoglutarimide derivatives with substitutions in the 5 membered phthalimide ring system have been studied. EM 8, which is non-teratogenic, lacks activity to date. EM 12, which is more teratogenic than thalidomide, has marginal activity in the carrageenan assay with activity at the highest dose tested but no activity in lower doses, and lacks activity in the PFC assay. The results with EM 12, like those with 2-N-phthalimidophthalimide, suggest that teratogenicity is separable from anti-inflammatory/immunosuppressant activities in the thalidomide structure.

Three thalidomide derivatives with substitutions in the 6-membered ring of the phthalimide moicty have been screened. A non-polar sulfur substituted derivative, E 35, is inactive in the carrageenan assay but inhibits PFC in the 2 higher concentrations tested. It has the structure probably required for teratogenicity. An even less polar, chlorine substituted compound, E 48, is probably teratogenic and lacks activity in both screens to date. The more polar, carboxy-substituted compound, E 122, lacks activity in both assays. This suggests that a prerequisite for a compound with anti-ENL activity clinically will probably be non-polarity similar to thalidomide itself.—[Pharmacology Research Department, USPHS Hospital, Carville, Louisiana; this investigation received support from the Therapy of Leprosy (THELEP) component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases; E 35, E 48, E 122, E 350, E 565, EM 8, EM 12, N-α-phthalylglutamine, N-α-phthalylisoglutamine, and 2-N-phthalimidophthalimide were gifts of Chemie Grünenthal, G.M.B.H., Stolberg, West Germany]

Kohsaka, K., Yoneda, K., Mori, T. and Ito, T. Study of chemotherapy of leprosy with nude mice.

We are attempting the application of experimental leprosy in nude mice for the study of chemotherapy of leprosy. At the last meeting in Osaka, a part of the results was previously reported that rifampin showed a tremendous initial killing effect on *Mycobacterium leprae*. Further results of the experiment will be shown in this paper.

A BL patient was treated with rifampin 450 mg daily, and biopsies were taken before treatment, on day 4 with 2 days' intermission after 2 days' treatment, on day 9 with 2 days' intermission after one week's treatment, and after one month of treatment. Another BL patient was treated with rifampin 450 mg daily and biopsied before treatment and on day 4 with 2 days' intermission after 2 days' treatment. The third case, a LL patient, has been treated with DDS or Promin for over 20 years, was biopsied, and *M. leprae* from this patient were inoculated into right hind footpads of nude mice. The infected mice were given 0.01% DDS in the diet for 6 months and then were given 0.2 mg daily of rifampin for 6 days orally. In an experiment involving chemoprophylaxis, infected nude mice were given 0.03 mg of minocycline orally, 6 days a week for 3 months; the dose of 0.03 mg per mouse is equivalent to nearly 100 mg per man. Nude mice (BALB/c-*nu/nu*), bred in our laboratory under SPF conditions, were used in the experiment, and they were maintained in plastic-isolators or in a well controlled environment. Bacilli in the infected footpad were counted, and the footpads were also examined histopathologically.

The results of the experiments supported our preliminary report that rifampin showed a tremendous initial killing effect on *M. leprae*. The bacilli lost infectivity for nude mice after only 2 days' administration of 450 mg of the drug daily to man. *M. leprae* from the patient treated with DDS or Promin for over 20 years were determined to be highly resistant to DDS in the infected nude mice. It is expected that further multiplication of *M. leprae* will be prevented by the treatment with rifampin. Chemopro-

phylactic administration of minocycline in a dose of 0.03 mg for 3 months does not depress the growth of *M. leprae* in nude mice. Further work is in progress.—[Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita-shi, Osaka, Japan]

Gillis, T. P. and Buchanan, T. M. Fractionation of antigens of *Mycobacterium leprae*.

Acetone-killed *M. leprae* separated from infected armadillo liver tissue without the use of proteases were treated with a 0.2 M lithium acetate, 20 mM EDTA, pH 8.8 solution. The extract was centrifuged for 15 minutes at 10,000 × g to remove whole cells. The supernatant fluid was centrifuged for 20 minutes at 30,000 × g to remove any remaining cells and large membrane fragments. The extract was electrophoresed over polyacrylamide gels (PAGE) in the absence of sodium dodecyl sulfate (SDS) and contained 6–7 protein bands plus nucleic acid and carbohydrate components. Differential centrifugation of this mixture allowed separation of a complex that gave a single high molecular weight band on non-SDS-PAGE. On SDS-PAGE this complex dissociated into 4–6 protein bands with subunit molecular weights ranging between 78K and 18K daltons, and a low molecular weight polydisperse material which exhibited staining characteristics not unlike a lipopolysaccharide macromolecule. This partially purified complex contained protein antigen that reacted with human lepromatous leprosy (LL) sera to make it "specific" for *M. leprae*. It also contained antigens not specific to *M. leprae* that reacted with rabbit anti-BCG serum and serum from patients with tuberculosis as tested by Ouchterlony immunodiffusion precipitation and quantitative ELISA immunoassay. Material obtained from identical extracts from *M. lepraemurium* (Mlm) showed immunoreactivity with unabsorbed leprosy sera and produced a line of identity in immunodiffusion tests with *M. leprae* extracts and the leprosy sera absorbed to make it "specific" for *M. leprae*. Studies are in progress to determine which of the proteins in the complex contains the *M. leprae*-Mlm antigen and whether this molecule when fully purified contains any antigens shared with other Mycobacteria. This should allow determination of whether the purified molecule is useful as a serodiagnostic test to indicate exposure to the leprosy bacillus.—[Departments of Pathobiology and Medicine, University of Washington, and Immunology Research Lab, USPHS Hospital, Seattle, Washington]

Shannon, E. J., Morales, M. J., Christy, S. A. and Hastings, R. C. Antigenic interrelationships among selected mycobacterial species relative to *Mycobacterium leprae* as manifested by cell-mediated immunity in guinea pigs.

Polar lepromatous leprosy (LL) is characterized by a total lack of cell-mediated immunity (CMI) to *M. leprae*, and we believe that this anergy may likely be on a genetic basis. If genetic, this specific immune deficit cannot be circumvented by a bacterin consisting of *M. leprae* itself. The most promising candidate for a vaccine for pre-LL individuals would be a mycobacterial species sharing antigenic properties with *M. leprae* yet possessing immunogenic CMI epitopes for these pre-LL individuals. As an initial step in identifying such a vaccine candidate, a ranking as to antigenic relatedness of 28 mycobacterial species to *M. leprae*, in *M. leprae* immunized guinea pigs was undertaken.

As reported in 1978 at the Thirteenth Joint Conference on Leprosy Research in Osaka, we systematically attempted to sensitize guinea pigs to *M. leprae*. We concluded: 1) that inocula of heat killed *M. leprae* consisting of from 10^4 to 10^9 organisms suspended in Freund's Incomplete Adjuvant (FIA) or Hank's Balanced Salt Solution (HBSS), showed only mild evidence of eliciting delayed hypersensitivity (DTH); 2) that inocula of 10^7 organisms and above suspended in HBSS elicited better DTH than similar inocula suspended in FIA; 3) that boosting with autoclaved *M. leprae* enhanced anamnestic recall phenomena in skin testing as compared to single doses of leprosy bacilli; 4) that skin test sites in animals immunized and boosted with autoclaved *M. leprae* were histologically characterized by prominent neutrophilic infiltrates indicating considerable humoral hypersensitivity as well as DTH; 5)

that sensitization of guinea pigs with a single inoculation of *M. leprae* prepared from the spleen of a freshly-sacrificed, infected armadillo imparted reasonable evidence for specific sensitization by skin tests with essentially no neutrophilic infiltrates histologically; and 6) that guinea pigs injected with cryopreserved (non-autoclaved) *M. leprae* and boosted with cryopreserved *M. leprae* showed enhanced levels of DTH as compared to all other variations in which *M. leprae* antigens were presented.

In summary, past work has shown that highest levels of DTH sensitization have been achieved in guinea pigs inoculated in the footpads with 10^8 cryopreserved *M. leprae* and boosted with 10^7 cryopreserved *M. leprae* intradermally three weeks before sacrifice. Sensitization to antigens of *M. leprae* and to cross-reactive antigens shared by other mycobacterial test organisms occurred concomitantly. Due to persisting humoral inflammatory components, macroscopic skin tests appeared to detect predominantly serologically cross-reactive antigenic determinants.

More recent work has dealt with a number of approaches which come to mind for circumventing the undesired humoral sensitization occurring concomitantly with the desired DTH in these animals. Two of the more simple of these approaches have been undertaken. The first of these was to lower the immunizing dose of cryopreserved *M. leprae* in the hope of selectively inducing adequate DTH with amounts of antigen which are subthreshold for inducing significant humoral antibody formation. Guinea pigs were immunized with 10^7 cryopreserved *M. leprae* and boosted with 10^6 organisms. In addition to using the usual 10^7 skin test does of the mycobacteria, we reduced the challenge inocula to 3.16×10^6 and 10^6 acid-fast organisms as well. The lower immunizing dose induced unsatisfactory DTH to *M. leprae* as measured by lymphocyte blast transformation (LBT). On the other hand, responses of inoculated animals were clearly different from responses of uninoculated controls to many of the challenge mycobacterial species indicating that the animals were indeed immunized, and suggesting that *M. leprae* itself was merely a relatively poor elicitor of this DTH. This is not surprising in view of the *in vivo* origin of *M. leprae* and the *in vitro* origin of the bulk of the other mycobacteria tested. Although encouraging, the low level of sensitization achieved in these animals appeared to make this approach impractical.

A second, simple approach to circumventing the concomitant humoral hypersensitivity occurring in these *M. leprae*-immunized guinea pigs has been undertaken, and is based on the expectation that humoral sensitivity of the Arthus type might be expected to be shorter lived than DTH. Guinea pigs have been immunized with 10^8 cryopreserved *M. leprae* and boosted with 10^7 cryopreserved *M. leprae* as before, but sacrifice and testing of the animals is being delayed until the levels of anti-mycobacterial antibodies have decayed to levels low enough to be incapable of causing an Arthus type hypersensitivity. Work is in progress measuring anti-mycobacterial antibody titers in *M. leprae*-immunized guinea pigs using a quantitative indirect fluorescent technique adapted from Gillis and Thompson (J. Clin. Microbiol. **7** [1978] 202–208).

An incidental additional problem has been investigated recently. Erratically positive 4 hour skin tests have been seen with various mycobacterial species in unimmunized animals. To investigate this phenomenon we have found that there is a positive correlation between the ability of sonified mycobacterial species to activate complement in the serum of unimmunized guinea pigs *in vitro* and their ability to elicit an early (4 hour) inflammatory skin test response in unimmunized guinea pigs *in vivo*. Thus, activation of complement by naturally occurring antibodies to mycobacteria or by the activation of the alternate pathway induced by bacterial polysaccharides in the absence of antibody occurs to a slight extent under these conditions. Guinea pigs immunized with *M. leprae* are, however, clearly subject to immunologically specific inflammatory events much over and above these nonspecific inflammatory reactions seen in naive animals.—[Pharmacology Research Department, USPHS Hospital, Carville, Louisiana; this investigation received support from the Immunology of Leprosy (IMMLEP) component of the UNDP/World Bank/WHO Special Pro-

gramme for Research and Training in Tropical Diseases, and the Victor Heiser Award]

Watson, S. R. and Collins, F. M. Suppressor T-cell production in heavily infected mice.

Specific pathogen free B6D2 mice were infected intravenously with 10^6 or 10^8 viable BCG Pasteur or *M. habana* (*simiae* serotype II), and the level of tuberculin hypersensitivity to 2.5 mg of PPD or *M. habana* cytoplasmic protein antigen (CPA) was determined using the footpad swelling test 14, 28, and 90 days later. The growth or persistence of the mycobacterial populations within the liver and spleen was correlated with the amount of footpad reactivity to the homologous and heterologous mycobacterial antigens.

Spleen cells were harvested from the 4 groups of mice at increasing time intervals. The T-cell population was enriched by nylon-wool filtration and the level of blast transformation following exposure of the cells to PHA, PPD, or *M. habana* CPA was measured *in vitro*. Early in the mycobacterial infections (day 14), thymidine incorporation was significantly enhanced, followed by a profound depression in ^{3}H-TdR uptake as the infection progressed. Throughout this, the mice were almost completely unresponsive to the footpad test antigens.

Cell mixing studies were carried out using day 28 or day 90 spleen T-cells (anergic donors) added to day 14 indicator cells which were exposed to PPD or *M. habana* CPA. The thymidine uptake of the indicator cells in response to PHA or CPA stimulation was markedly depressed by the presence of an equal number of day 90 spleen cells. This suppression was greater than would have been expected by halving the number of cells in culture. The suppressive activity of the day 90 T-cell enriched suspension was almost totally ablated by prior treatment of the cells with anti-Thy-1 antiserum and complement. There was no evidence for suppressor T-cells in the day 14 *M. habana*-infected mouse spleens whereas large numbers of suppressor cells were present by day 28.

Suppressor T-cells could be induced in day 14 *M. habana* spleen cell suspensions by exposure of the filtered spleen cells *in vitro* to 4 μg of concanavalin A or to 4 μg of *M. habana* CPA for 48 hours. The cells were washed to remove the inducer and the spleen cells cultured alone or with equal numbers of normal or day 14 *M. habana* indicator T-cells. The Con A treated splenic T-cells brought about a significant reduction in tritiated thymidine uptake by the indicator cells when exposed to PHA or to *M. habana* CPA. However, the suppressor cells induced by the *M. habana* CPA were only able to affect thymidine uptake by the indicator cells exposed to *M. habana*-CPA but not to PHA. The timing of the emergence of the specific suppressor T-cells in the heavily infected spleen bears a striking relationship to the loss of footpad hypersensitivity to the CPA by the *M. habana*-infected host. The possible significance of the suppressor T-cell population with respect to the persistence of *M. habana* in the livers, spleens and lungs of heavily infected animals will be discussed.—[Trudeau Institute, Inc., Saranac Lake, New York]

Wong, J. F. Sensitized T-cell proliferative response to lepromin.

A very sensitive, antigen-induced, T-cell proliferative response assay was developed and subsequently used to determine whether a relatively rapid growing, chromogenic, leprosy-derived isolate from a leproma was similar to the acid-fast bacilli in lepromin. Splenic, murine T-cells sensitized to this leprosy-derived isolate were cultured for 72 hr *in vitro* with either lepromin, a closely related mycobacterium, an unrelated propionibacterium, or the sensitizing organism and assayed by the uptake of ^{3}H-thymidine which was introduced into the cultures 18–20 hr prior to termination of the cultures. The initial results showed that lepromin induced a very low proliferative response in the immunized cultures. However, it was later demonstrated that this low response was attributed to either the partial masking of the organisms' antigenic determinants by the tissue debris of the lepromata or possibly by the alteration of the critical surface determinants during the processing of the infected tissues for lepromin. If the sensitizing organisms were phagocytized by thioglycollate-induced, murine, peritoneal

exudate cells *in vivo*, and subsequently harvested, lysed, and processed by the same methods used for lepromin, it was found that the resultant suspension of acid-fast bacilli could no longer induce a high proliferative response. Instead, the response was similar to that found with lepromin. Moreover, merely autoclaving a pure suspension of the sensitizing organisms—which is routinely carried out in the processing of lepromin—greatly reduced the cultures' ability to undergo blatogenesis.—[Memorial Sloan-Kettering Institute for Cancer Research, New York, New York]

Bullock, W. E., Havens, R. A. and Nickerson, D. A. Splenic suppressor cell populations in *M. lepraemurium* infected mice that suppress cell mediated lymphocytotoxic activity.

Previously, we have demonstrated the sequential development of two cell populations in the spleens of *M. lepraemurium*-infected mice that suppress the T-lymphocyte dependent primary antibody response to sheep red blood cells by normal syngeneic splenocytes *in vitro*. Suppression was mediated by macrophage-like cells prior to the eleventh week of infection and thereafter was augmented by a second population of suppressor T-cells (J. Immunol. **120** [1978] 1709–1716).

In this study, spleen cells from normal C3H/Anf mice (H-2^{k}) were placed in a one-way mixed lymphocyte culture (MLC) system with semiallogeneic cells (mitomycin C treated) from C3D2F mice (H-$2^{k,d}$). To these cultures were added splenocytes from either normal or infected C3H/Anf mice. The cytotoxic activity of effector cells generated in MLC was assayed by measuring release of radioactivity from ^{51}chromium-labeled P815 mastocytoma cells (H-2^{d}) and expressed as percent corrected lysis.

Suppression of normal cell mediated lymphocytotoxicity (CML) by splenocytes from infected mice was observed by week 11 and became maximal by week 16. At this time, addition of 3×10^6 splenocytes from infected mice to 3×10^6 normal spleen cells reduced CML to 12.6 ± 3.7% lysis as compared with 48.0 ± 5.1% by equal numbers of normal spleen cells. Splenocytes from 16 week-infected animals were placed on nylon wool columns and either the adherent or passed T cell fraction was co-cultured with normal spleen cells. The nylon-passed T cells suppressed the CML of normal splenocytes (20.0 ± 3.6%) as compared with cultures to which equal numbers of normal splenic T cells had been added (52.5 ± 4.0%). Nylon adherent cells also suppressed the CML of normal spleen cells (21.0 ± 5.1%) as compared to control cultures (57.0 ± 5.8%). Double treatment of adherent cells with anti-Thy 1.2 serum failed to reverse the suppressor activity.

In control experiments, suppression of normal CML activity could not be overcome by varying either the degree of alloantigen stimulation or the time of the MLR. Cytotoxic activity by normal effector cells was not suppressed by direct addition of splenocytes from infected mice to the CML assay system. Evidence will be presented that *M. lepraemurium*-induced suppressor cells exert their effects during the proliferative phase of a cytotoxic effector cell generation.

In summary, these studies demonstrate that two cell populations are generated within the spleens of *M. lepraemurium*-infected mice which depress CML effector function by normal splenic T cells. One suppressor population is composed of T lymphocytes and the other of macrophages or, possibly, B cells. The activity of the two populations acting together in whole spleen cell preparations usually is greater than the activity of either cell population alone.—[Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky; this work was supported by USPHS Grant AI-10094]

Shepard, C. C. Vaccination experiments with *M. leprae* preparations and with cultivable mycobacteria.

The search for a vaccine against leprosy continues along two lines: a) The use of *M. leprae* itself. The relative abundance of armadillo-grown *M. leprae* and the heat-stability of *M. leprae's* immunogenicity offer promise that a heat-killed product may be effective. For use in humans, the product should be free of armadillo antigens but fully immunogenic. b) The use of a cultivable mycobacterium. If a culture can be found that provides good protection against *M.*

leprae, the supply and production problems would be greatly simplified.

Our methods were those described previously. Mice were immunized intradermally, and local reactions and regional lymph node enlargement were measured. At +28 days the mice were challenged in the footpad with a suspension of heat-killed *M. leprae* for elicitation of footpad enlargement (FPE) or with an infectious suspension to measure protection against infection.

The preliminary results of an experiment in collaboration with Draper and Rees were reported at last year's conference. The final results now confirm that a 24-hour treatment with trypsin and chymotrypsin or a 2-hour treatment with 0.1 N NaOH was harmful to immunogenicity. Gamma irradiation with 2.5 megarads or separation in a two-phase aqueous polymer system (polyethylene glycol:dextrose) was not detectably harmful. The results further suggested that purification of the bacilli could improve their immunogenicity.

In another experiment we compared strain 100616 (from Nakamura), *M. tuberculosis* (H37 Ra), *M. habana*, *M. simiae*, *M. nonchromogenicum*, *M. vaccae*, BCG, and *M. leprae*. *M. leprae* was heat-killed; the others were not. All the mycobacteria caused local reactions and enlargement of the regional lymph nodes. With *M. leprae* as challenge antigen, significant FPE was seen with all vaccines but *M. vaccae*; that with BCG and *M. leprae* vaccines was significantly greater. Significant protection against infection was seen only with BCG- and *M. leprae*-vaccinated mice.

In another experiment we compared Talwar's *M.w* culture (a member of the avium-intracellulare-scrofulaceum complex) with BCG and *M. leprae*. *M.w*, even when heat-killed, caused local reaction and regional lymph node enlargement. It also sensitized mice to give FPE on challenge with heat-killed *M.w*. Significant FPE with *M. leprae* challenge was not seen, however. *M.w* did not provide protection against infection although, as usual, BCG and *M. leprae* did.

From these experiments it appears that the mere sharing of antigens with *M. leprae* does not insure that a culture will provide protection against infection with *M. leprae*. Instead, particular determinants seem to be critical, and searches for vaccine candidates need to take this into account.—[Leprosy and Rickettsial Diseases Branch, Virology Division, Center for Disease Control, Atlanta, Georgia]

Lefford, M. J. Interaction of *M. lepraemurium* and BCG infections in mice.

In an earlier report, it was shown that concurrent or pre-immunization of mice with heat-killed *M. lepraemurium* (HK-MLM) intravenously (IV) enhanced footpad infection with MLM due to suppression of cell-mediated immunity (CMI). Pretreatment of such mice with 10^7 BCG IV appeared to block the suppressive effect of HK-MLM IV.

Two possible modes of action of BCG were considered. Firstly, BCG might be acting as a nonspecific immunopotentiator, much as it does in the sheep red blood cell system. Alternatively, BCG might generate a CMI response against itself which also acted against MLM, since these mycobacterial species possess common antigens.

It has been shown by others that the role of BCG as a nonspecific immunopotentiator is critically dependent upon its dose, route of administration, and interval between BCG and antigen injections. By contrast, specific immunity is induced by BCG after administration by various routes and doses, and immunity persists for long periods after immunization. Advantage was taken of these differences to design experiments that would elucidate the effects of BCG in undisturbed and enhanced MLM infections.

It was found that BCG immunization by various routes protected mice against footpad infection with MLM. The reciprocal relationship was also found, namely, that infecting mice with MLM, could confer adoptive immunity to BCG.

The interaction of BCG and enhanced MLM infection was complex. In mice that received BCG, followed by HK-MLM IV and footpad infection with MLM, the MLM infection was less severe than that observed in mice not given BCG. However, mice receiving BCG, HK-MLM IV, and MLM footpad infection had more severe disease than mice given only BCG and footpad infection with MLM. Thus, BCG ame-

liorated the infection-enhancing effects of HK-MLM IV, but HK-MLM IV blunted the protective effect of BCG. In these experiments, the route of BCG administration and the interval between BCG immunization and MLM infection were not critical.

It is concluded that the beneficial effect of BCG upon MLM infection is due to cross-reactive CMI between BCG and MLM. These experiments also suggest that the therapeutic role of BCG in lepromatous leprosy may be very limited.—[Trudeau Institute, Inc., Saranac Lake, New York]

Patel, P. J. Systemic macrophage activation in *M. leprae* immunized mice.

Immunization of mice with 100 μg of armadillo derived irradiation-killed *M. leprae* (I-ML) in aqueous suspension produces a state of cell-mediated immunity (CMI) to *M. leprae* and related antigens. Along with various established criteria for CMI, it was shown that there was increased resistance to *Listeria monocytogenes*, indicative of macrophage activation, at the immunization site. In spite of the high level of localized macrophage activation, no such activity could be demonstrated systemically in these mice, as evidenced by the absence of increased nonspecific resistance to an intravenous challenge with *L. monocytogenes*. However, systemic nonspecific resistance could be generated in I-ML immunized mice by an intravenous injection of either disrupted I-ML or related mycobacterial antigens. This has provided a simple and highly sensitive assay system to study the taxonomic relationship of *M. leprae* to other mycobacteria.—[Trudeau Institute, Inc., Saranac Lake, New York]

Colston, M. J., Fieldsteel, A. H., Lancaster, R. D. and Dawson, P. J. The athymic rat: immunological status and infection with *Mycobacterium leprae*.

The neonatally thymectomized Lewis rat (NTLR) is currently being used in our laboratory to study microbial persistence—the phenomenon by which viable drug-sensitive *Mycobacterium leprae* can remain in patient tissue for many years despite apparently adequate therapy. An animal model for the study of persistence is required from two aspects: as a model of the lepromatous patient for screening chemotherapeutic regimens and as a means of detecting small numbers of viable organisms against a background of large numbers of dead bacilli in the tissues of patients undergoing treatment. In this paper we report preliminary evidence that the recently described "nude" rat may be superior to other animal models for this type of work.

Evidence of immunological incapacity of the nude rat will be presented in terms of its ability to accept skin heterografts (Wistar Firth rat skin) and xenografts (CBA mouse tail skin) and xenografts of human tumor tissue. The *in vitro* response of splenic lymphocytes from the nude rat will be compared with that of the NTLR and the normal Lewis rat; these experiments demonstrate that the nude rat shows a greater degree and uniformity of immunosuppression than the NTLR.

Finally, the infection resulting from the inoculation of nude rats with *M. leprae* will be described. Growth curves of *M. leprae* in the footpad of the nude rat show enhancement of the infection compared to normal control rats, and dissemination of the infection to superficial tissue and internal organs. Bacillary growth in the footpads of nude rats and NTLR resulting from the inoculation of large numbers of *M. leprae* will be described.—[Life Sciences Division, SRI International, Menlo Park, California; St. George's Hospital and Medical School, London, United Kingdom; Department of Pathology, University of Chicago, Chicago, Illinois]

Mehra, V., Mason, L. H., Wells, L. M. and Bloom, B. R. Lepromin induced suppressor cells in leprosy patients: evidence for mediation by TH_2^+ T-cell subset.

The possible involvement of a suppressor subset of T-cells (TH_2^+) in the unresponsiveness of leprosy patients to *M. leprae* antigens has been assessed by sorting for this subset using a fluoresence activated cell sorter. TH_2^+ T-cells from lepromatous patients were mixed with mononuclear cells from normal donors and their ability to induce suppression of mitogenic response of normal mononuclear cells to Con A in the presence of lepromin was examined. When TH_2^+ cells from lepromatous or

borderline leprosy patients were admixed with mononuclear cells from normal donors, significant suppression of Con A response was induced by lepromin. In contrast, no suppression was seen on mixing TH_2^+ cells from tuberculoid patients and normal subjects with normal mononuclear cells.—[Albert Einstein College of Medicine, Bronx, New York; USPHS Hospital, Staten Island, New York]

Artz, R. P., Bullock, W. E. and Jacobson, R. R. Decreased suppressor cell activity in leprosy and other disseminated granulomatous infections.

A subclass of T lymphocytes exists within the population of human peripheral blood mononuclear cells (PBMC) that can be induced by concanavalin A (Con A) to manifest suppressor functions. The hypothesis that certain infections may alter this suppressor activity was explored in 11 anergic patients with disseminated mycobacterial or mycotic infections. Six of these had lepromatous leprosy. PBMC from patients or normal controls were pre-incubated with and without Con A (60 μg/ml) for 72 hr, washed ×3, and 2×10^5 cells co-cultured with equal numbers of allogeneic PBMC freshly drawn from healthy donors sensitive to histoplasmin. DNA systhesis was measured and compared in co-cultures stimulated either by Con A, histoplasmin, or by the mixed lymphocyte reaction (MLR) alone. There was marked suppression of the co-culture response to Con A by Con A-pre-incubated cells (versus cells pre-incubated without Con A) from 10 of 12 normal donors (median, −31%; range, +35% to −73%). Conversely, the response was augmented in 6 of 11 co-cultures of PBMC from healthy donors to which Con A-pre-incubated PBMC from patients had been added (median, +5%; range, +640% to −21%). These differences were significant at $p < 0.01$. In addition, there was significantly less suppression by the patients' pre-incubated cells of the responses to histoplasmin ($p < 0.05$) and the MLR ($p < 0.02$) as compared with controls.

There were no consistent differences between the suppressor activities of Con A-pre-incubated PBMC from patients with localized granulomatous infections and normal donors.

These results suggest that dysfunction of or diminution of the Con A-inducible T-suppressor cell subpopulation in peripheral blood may be one of the immunoregulatory disturbances associated with lepromatous leprosy and other disseminated granulomatous infections.—[Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky; USPHS Hospital, Carville, Louisiana; this work was supported by USPHS Grant AI-10094]

Rea, T. H. and Terasaki, P. I. HLA-DRW antigens in Mexican patients with leprosy.

Twelve studies of HLA-A and HLA-B antigen frequencies in patients with leprosy have provided no consistent support for an association of leprosy with an antigen of the HLA-A or HLA-B loci. However, investigations of HLA-A and HLA-B haplotype inheritance in families with tuberculoid leprosy have provided evidence that susceptibility to, and expression of, leprosy is linked to the major histocompatability complex. Furthermore, study of HLA-DRW antigens, a locus putatively near the hypothetical immune response gene, has provided evidence that susceptibility to tuberculoid leprosy is associated with HLA-DRW2 (de Vries, *et al.*, Lancet **2** [1976] 1328–1330). We have studied HLA-DRW antigens in Mexican-born patients with leprosy.

Thirty-nine patients with polar lepromatous leprosy, 19 with tuberculoid leprosy (mostly BT by Ridley's histological criteria) and Mexican or Mexican-American blood bank and tissue transplant donors were typed for workshop antigens of the HLA-DR locus by a serologic, microlymphocytotoxic technique (HLA-DRW6 was sought but was excluded from this report because it probably does not exist).

In polar lepromatous leprosy the HLA-DRW antigen frequencies showed not even a suggestion of a significant difference from those of the controls. However, in tuberculoid leprosy, HLA-DRW2 was present in 31.6% of patients and in 14.9% of controls (chi-square = 2.32, not significant).

No evidence of an association between an HLA-DRW antigen and leprosy was found. However, a higher incidence of HLA-DRW2 in tuberculoid patients than in controls was in accord with the findings of de Vries, *et al.* Differences between our findings and those of de Vries, *et al.* might be explained by differences in patient classification or in linkage disequilibrium in the populations studied. The latter is particularly attractive because it is consistent with a genetic explanation for the high prevalence rate of tuberculoid leprosy in populations studied by de Vries but the low prevalence rate of tuberculoid leprosy in Mexicans.

This study and the studies of de Vries, *et al.* and Stoner, *et al.* (Lancet **2** [1978] 543–547) have provided no evidence for an association between HLA-DR and HLA-D antigens and leprosy. These negative findings do not undo the substantial evidence that susceptibility to lepromatous leprosy is under genetic control but suggest that a marker for the gene(s) will not be found in the major histocompatability complex.—[Department of Medicine, University of Southern California School of Medicine, and the Department of Dermatology, Los Angeles County, University of Southern California Medical Center, Los Angeles, California]

Gordon, G. R., Murray, J. F., Jr., Peters, J. H., Gelber, R. H. and Jacobson, R. R. Studies on the urinary metabolites of dapsone in man.

Earlier reports that dapsone (DDS) is a weak carcinogen in male rats and that its analog, 4,4′-diaminodiphenyl sulfide (DDSF), is a potent carcinogen in both sexes of mice and rats receiving maximally tolerated doses of these compounds for their lifetimes raise questions of the iatrogenic risk in leprosy patients of long-term chemotherapy with DDS. We had previously found that neither DDS nor any of its known urinary metabolites in man were mutagenic in the Ames *Salmonella*/microsome technique. However, such tests of DDSF and 4,4′-diaminodiphenyl sulfoxide (DDSO) showed that the former was a weak direct mutagen and that both were strong promutagens, i.e., required metabolic activation.

To determine if DDSF or DDSO were present in pharmaceutical preparations of DDS or in urine from volunteers or patients receiving DDS, we developed highly sensitive and selective high pressure liquid chromatographic (HPLC) procedures capable of detecting nanogram quantities of DDSF and DDSO. Neither derivative could be detected in DDS tablets (Ayerst or Winthrop). We estimate that patients receiving 100 mg tablets would ingest <10 μg of either analog of DDS. Also, neither DDSF nor DDSO could be detected in aliquots of 24-hour urine collections before or after acid hydrolysis (2 N HCl, 100°C, 60 min) from 6 volunteers or 9 patients receiving single or daily doses of 50 or 100 mg of DDS. From the limits of sensitivity of detection, we estimate that DDSF or DDSO or their potential conjugates were present at $\leq 2 \times 10^{-2}\%$ of the dose of DDS in these urine collections. It seems clear from these results that these analogs of DDS or their conjugates are not urinary metabolites in man. Thus, man is not at risk due to conversion of DDS to the mutagenic and/or carcinogenic DDSF and/or DDSO.

Also, in our continuing efforts to define quantitatively the various groups of metabolites in urine of man receiving DDS, we developed an HPLC method for the measurement of 4-amino-4′-hydroxyaminodiphenyl sulfone (DDS-NOH). In 48-hour urine collections from two normal volunteers, we found that unconjugated DDS-NOH comprised only 1.6 and 1.7% of the dose. After incubation of urine aliquots with a commercial enzyme preparation containing β-glucuronidase and sulfatase, we found only slight increases of urinary DDS-NOH that were 3.4 and 1.6% of the dose. However, subjecting urine aliquots to weak acid hydrolysis (0.3 N HCl, 22°C, 60 min) yielded large amounts of DDS-NOH (an additional 32.3 and 52.0% of the dose). We interpret these results to indicate that the major urinary DDS-NOH conjugate is the amino-N-glucuronide of DDS-NOH. In 24-hour urine collections from six patients receiving 100 mg DDS daily, we found that unconjugated DDS-NOH comprised 0.6 to 7.7% and acid-labile DDS-NOH, 13.7 to

43.6% of the dose. Urine from one patient receiving 100 mg DDS combined with 300 mg rifampin daily contained amounts of unconjugated and acid-labile DDS-NOH that did not differ from those receiving only DDS. Proof that the HPLC peak from urine was DDS-NOH was obtained by comparative mass spectrometry. By combining these new techniques with earlier procedures, we are now defining the total pattern of urinary metabolites in man.—[SRI International, Menlo Park, California; USPHS Hospital, San Francisco, California; USPHS Hospital, Carville, Louisiana; supported in part by Grant AI-08214]

Peters, J. H., Murray, J. F., Jr., Gordon, G. R., Tatsukawa, H. and Matsuo, Y. Thioamides and thioamide-S-oxides for leprosy chemotherapy.

Ethionamide (ETH) and prothionamide (PTH) are now well established as highly bactericidal drugs against *Mycobacterium leprae* in animals and man. In addition, ETH is active against *M. leprae* resistant to dapsone (DDS) and rifampin (RFM). Thus, the thioamides are promising adjunctive drugs for combination chemotherapy with DDS or RFM. Earlier, it was found that the S-oxide metabolite of ETH was equipotent against *M. tuberculosis* H37Rv *in vitro*. In recent tests using this organism, we found that PTH and PTH-S-oxide (PTHSO) also exhibited equal antimycobacterial activity and that the metabolite, 2-propylisonicotinamide (PINA), was inactive. Thus, it is reasonable to presume that the antimycobacterial activity of the thioamides *in vivo* results from a summation of the activity of both the parent drugs and their S-oxide metabolites.

Following the development of a selective, sensitive high pressure liquid chromatographic technique for PTH, PTHSO, and PINA, we measured plasma levels of PTH and PTHSO in Lewis rats receiving oral and intravenous doses of PTH and PTHSO equivalent to 500 mg PTH per 70-kg man. The results after PTH administration indicated that PTH was rapidly metabolized to PTHSO in both sexes but that males exhibited approximately twofold greater activity for this oxidation than females. In both sexes, levels of PTH declined more rapidly than levels of PTHSO and $T\frac{1}{2}$ values of the latter were about twofold longer than those of PTH. After PTHSO administration, we found that PTH was formed by reduction of PTHSO, but in this case males were less active than females. Again, PTHSO plasma levels declined more slowly than PTH levels. Thus, PTH and PTHSO are metabolically interconvertible, and prolonged antibacterial activity is more a result of PTHSO than of PTH. However, the sum of PTH and PTHSO by either route in either sex after PTHSO administration was about 50% of the sum after PTH. Subsequent studies in armadillos following intravenous PTH or PTHSO yielded similar conclusions.

Finally, tests of equal oral doses of PTH and PTHSO (10 mg/kg) in mice infected with *M. leprae* showed that, using the kinetic method, the two drugs produced approximately equal bacterial growth delays, 86 and 78 days, respectively. Thus, PTHSO appears to exhibit equal antileprosy activity to PTH.—[SRI International, Menlo Park, California; USPHS Hospital, San Francisco, California; Hiroshima University School of Medicine, Hiroshima, Japan; supported in part by Grant AI-08214 and by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases]

Jacobson, R. R. Rifampin for the treatment of lepromatous leprosy.

Rifampin has now been used for over 8 years both alone and in combination regimens to treat cases of lepromatous leprosy at Carville. Resistance to it may develop within 4 years if it is used as monotherapy, but this result is not inevitable and some patients may attain an inactive status receiving rifampin alone. Why such significant variability in response occurs is uncertain although several possible explanations exist. No evidence of resistance has appeared in those receiving combination regimens with the possible exception of one patient who may have been infected with primary sulfone resistant bacilli prior to the start of therapy. Toxicity has been a serious problem only in some of those receiving the drug with ethionamide. In three instances the drug had to be dis-

continued, and one patient died of liver failure. ENL occurs with about the same frequency in those on rifampin as with other regimens. Clearance of bacilli on the other hand appears to be somewhat slower though the difference has not yet achieved statistical significance.

Although it is not an FDA approved indication, the drug is now widely employed in the United States to treat leprosy but has often been inappropriately given as monotherapy in patients referred to us. Rifampin has proven to be an important addition to our armamentarium of drugs to treat leprosy as a part of combination drug regimens. It is, however, for a variety of reasons less useful than clofazimine.—[USPHS Hospital, Carville, Louisiana]

Collins, F. M., Klayman, D. L. and Morrison, N. E. Antimycobacterial activity of thiosemicarbazone derivatives *in vitro* and in chronically infected mice.

Thiosemicarbazones have been tested for antituberculous activity both against experimental and human infections. A number of substituted thiosemicarbazones with potential antimalarial (as well as antibacterial) activity were screened for their ability to inhibit the growth of *M. smegmatis* 607, which has a drug sensitivity profile similar to that of *M. leprae* with respect to a number of proven antileprosy drugs. Thiosemicarbazone derivatives with activity against strain 607 were tested against a number of mycobacteria including *M. kansasii* (2 strains), *M. marinum*, *M. simiae*, *M. avium* (4 strains), *M. intracellulare* (2 strains), *M. nonchromogenicum*, *M. vaccae*, and *M. fortuitum*. Control drugs included isoniazid, rifampin, amakacin, dapsone, and clofazimine. The MIC values for the thiosemicarbazone derivatives varied extensively for the different test organisms. The best inhibition was observed for rifampin, clofazimine, and the thiosemicarbazone derivatives designated L, H, R and Y. Improved effectiveness was observed when a mixture of rifampin, clofazimine and thiosemicarbazone L or Y was used *in vitro* at concentrations of 1-5 μg of each per ml. Some evidence of additivity and, possibly, synergism were observed, and the mixed drugs were active against all of the test strains *in vitro*.

Groups of ICR mice were infected with 10^8 viable BCG, *M. kansasii*, or *M. simiae*, and after 30 days incubation, half of each group was placed on a regimen of 100 μg of rifampin, 20 μg of clofazimine and 20 μg of compound L per ml of drinking water. The antibacterial effect of the combined drug regimen was compared with that seen when each drug was tested separately over a 3 month period. The utilization of drinking water was checked daily for each group to insure that the mice were receiving adequate amounts of drug. The number of viable bacilli in the lungs, liver, and spleen was determined at weekly intervals, comparing the survival of the 3 organisms *in vivo* with that for the mice receiving drug-free drinking water. Rifampin, clofazimine, and compound L treatment resulted in a 1,000-fold drop in the number of *M. simiae* present in the liver and spleen after 2 months of treatment. The drop by the lung population was only about 50-fold during this time but was still statistically significant ($p < 0.01$). The drug sensitive BCG population was eliminated by rifampin alone as well as by the triple regimen within 14–21 days. Studies of the triple drug regimen on the survival of the other drug resistant atypical mycobacteria in the treated mice is in progress. Preliminary data indicate that several of the thiosemicarbazone derivatives have sufficient activity against the atypical mycobacteria to justify further study of their activity against both chronic lung infections and against *M. leprae* in the mouse infection model.—[Trudeau Institute, Saranac Lake, New York; WRAIR, Washington, D.C.; Johns Hopkins School of Public Health and Hygiene, Baltimore, Maryland]

Meyers, W. M., Heggie, C. D., Kay, T. L., Staple, E. M. and Kvernes, S. The Ridley-Jopling five-group classification of leprosy—correlations of parameters of the classification in 1,429 leprosy patients.

The Ridley-Jopling classification of leprosy (Int. J. Lepr. **20** [1966] 255–273) is useful to the clinician and histopathologist for the treatment and prognosis of the patient. This system, however, has had remarkably

few independent evaluations. We have compared the clinical, histopathologic, bacteriologic, and lepromin reaction data on 1,429 leprosy patients treated at Kimpese, Zaïre. All but 30 of the patients were Kikongo speaking.

There was complete agreement between the clinical and histopathologic diagnosis in 77.2% of the patients, and an additional 21.4% of the patients differed by only one group in the Ridley-Jopling system (contingency coefficient = 0.705). The Mitsuda reactions ($\bar{x}$ in mm) versus clinical diagnoses were as follows: LL—1.8; BL—3.6; BB—6.8; BT—9.6, and TT—10.9. The Mitsuda reactions versus histopathologic diagnoses were similar and all groups differed significantly at the 5% level or less. The Bacterial Indexes ($\bar{x}$) versus histopathologic diagnoses were: TT—0.01; BT—0.04; BB—0.11; BL—1.18, and LL—2.73. Thus we observed that the primary parameters of the Ridley-Jopling system correlate well with the anticipated findings.

We further compared the Fernandez reactions and Mitsuda reactions with the class of leprosy and found the following correlations (Pearson's correlation coefficient): TT—0.60; BT—0.52; BB—0.14; BL—0.12, and LL—0.29. Thus the Fernandez reactions vary increasingly independently of the Mitsuda reaction toward the lepromatous end of the spectrum of leprosy, and this independence is most pronounced in the BB-BL area. This observation suggests that the Fernandez reaction is the result of sensitization to mycobacterial antigens that cross react with *M. leprae*. This cross reactivity apparently is not nearly so strikingly suppressed in BB-LL patients as is the Mitsuda reaction.—[Armed Forces Institute of Pathology, Washington, D.C.; Institut Médical Evangélique, Kimpese via Kinshasa, Republic of Zaïre]

Gelber, R. and Gibson, J. The killing potential of various aminoglycoside antibiotics for *M. leprae*.

Although there is a large amount of data on the effect of aminoglycoside antibiotics on *Mycobacterium tuberculosis*, there is a paucity of similar studies on *Mycobacterium leprae*. Previously, Shepard reported treating *M. leprae* infected mice with 2 mg of streptomycin subcutaneously three times weekly from day 30 to day 86 after footpad infection. In these studies growth of the organisms was inhibited during the period of drug administration but resumed, without delay, once administration was terminated. These studies were interpreted as demonstrating that streptomycin was purely bacteriostatic for *M. leprae*. Earlier, Gaugas had screened gentamicin against *M. leprae* footpad infections by administering 165 mg/kg to mice by daily injection and found it "partially active." A small pilot trial in previously untreated lepromatous leprosy conducted in Malaysia utilizing streptomycin intramuscularly in a daily dose of 0.75 to 1 gm resulted in clinical improvement comparable to dapsone, a fall in the morphological index similar to that observed with dapsone monotherapy, and a loss of mouse footpad infectivity of skin biopsy specimens that was somewhat faster than with dapsone. This clinical study suggested that streptomycin might have a greater killing potential for *M. leprae* than animal studies had implied. Hence, we further investigated the ability of various aminoglycoside antibiotics to kill *M. leprae* in the mouse footpad by utilizing the "proportional bactericide" technique. In these studies mice were inoculated with 10^1, 10^2, and 10^3 *M. leprae* in both hind feet and treated as controls or with daily intraperitoneal injections of aminoglycoside antibiotics for the next sixty days. The dose of aminoglycosides chosen for study was the highest dose previously found to be nontoxic and was adjusted weekly on the basis of animal weight. Mice were treated with daily doses of streptomycin 150 mg/kg (approximately 3.1 mg), kanamycin 100 mg/kg, tobramycin 20 mg/kg, gentamicin 20 mg/kg, and amikacin 100 mg/kg. One year after the completion of therapy 5 mice were sacrificed, and the 10 footpads counted individually. Percent bactericide was calculated by a most probable number calculation. Certain of the aminoglycosides had impressive killing potential—kanamycin 99.8% bactericidal, streptomycin 97% bactericidal, and amikacin 97% bactericidal. Others did not kill *M. leprae* significantly—gentamicin 58% bactericidal and tobramycin 27% bactericidal. From these studies a structure-activity relationship for amino-

glycosides against *M. leprae* was suggested. Furthermore, when 0.0001% dapsone in mouse chow was given for the 60 day treatment period in addition to intraperitoneal streptomycin, no additive effect could be demonstrated. These studies resulted in very impressive killing potential for certain aminoglycoside antibiotics against *M. leprae* that should stimulate a reconsideration of the possible clinical role for these agents.—[Leprosy Research Unit, USPHS Hospital, San Francisco, California]

CLOSING REMARKS

This conference was the most magnificent we have yet had. The recent progress of the research is quite splendid and is approaching our goal of control of leprosy. Nevertheless, the disease is still a major problem in many areas of the world, and the number of persons afflicted with leprosy is increasing in certain areas. More intensive work than ever is required from everyone in the field to attain the goal of total control of the disease by the year 2000 as the World Health Assembly has resolved (Int. J. Lepr. **47** [1979] 525–526).

Although the marked decrease of new patients in Japan has resulted in a decline of the zest of young researchers, new cooperation is emerging between leprologists and neurologists who are concerned about the nature of nerve involvement and tissue reactivity; this is aiding in the treatment and prevention of neural involvement and its sequelae. I commend all young researchers in my own country to continue their efforts to control this most serious disease.

I wish to extend my thanks to Dr. Bloom, the U.S. Leprosy Panel Chairman, to Drs. Gwinn and Beck, and to the USPHS Hospital staff for your kindness and care in arranging this conference.

I wish also to add that this charming city of New Orleans is familiar to aged Japanese people. The famous New Orleans novelist Lafcadio Hearn (Yakumo Koizumi in Japanese) lived here until 1890 and was Editor of the *Times Democrat*. After moving to Japan, he wrote many stories about Japan, lectured at Waseda University, and is buried in Ikebukuro, Tokyo. Next to his grave is that of the notorious and mysterious thief of Ido (who was nicknamed "Oni Azami" or "Horse Thistle" in English), a character very similar to some of Hearn's. Oni Azami was famous for the ability to obtain anything he tried for, a Robin Hood type in the time of the Tokugawa shogunate, who even broke into the Castle of Shogun and was at last captured. There seems a strange kind of karma in the fact that both of these persons are buried together. I hope that many of you here will be in Japan next year so that I may show the place to you.

I thank you.

—Masashi Namba, *Chairman*
Japanese Leprosy Panel