COMMITTEE 4: WORKSHOP ON EXPERIMENTAL CHEMOTHERAPY

Chairman: R. J. W. Rees

Rapporteur: G. A. Ellard

Participants: E. J. Ambrose
M. J. Colston
L. Levy
N. E. Morrison
S. R. Pattyn
J. M. H. Pearson
J. H. Peters
C. C. Shepard

Progress in the last five years ([14]) has largely resulted from exploitation of mouse models for the further evaluation of drugs and the demonstration of both drug-resistant and persisting drug-sensitive *M. leprae* in patients.

ANIMAL MODELS

In view of the continued failure to cultivate *M. leprae in vitro*, the activity of antileprosy drugs must be assessed using animal models. The two established animal models are:

a. **Normal mouse.** This, the standard experimental model, enables the minimal inhibitory concentrations (MICs) of drugs against *M. leprae* to be determined, the nature of their antibacterial activity to be assessed, the rate at which viable leprosy bacilli are eliminated in patients during treatment to be measured, and the occurrence of drug-resistant organisms to be detected. Whether drugs have significant antileprosy activity is determined by ascertaining whether continuous administration in the diet prevents multiplication of *M. leprae* in the mouse foot pad. Administration of drugs in graded doses allows the minimal effective dose (MED) to be measured. Following the growth of bacilli after administering a drug for a limited period reveals whether or not its activity is purely bacteriostatic (kinetic method) ([81]), whereas the degree of bacterial killing engendered by drugs displaying potential bactericidal activity can be quantified using the proportional bactericidal test method ([11]). The normal mouse can be used to monitor the loss of viability of leprosy bacilli in lepromatous patients early in chemotherapy ([82]); when inocula from biopsies are no longer infective at least 99% of the original viable population must have been killed. The level of drug resistance of strains of *M. leprae* can be determined from their ability to multiply in mice fed graded doses of drugs that are normally inhibitory ([76, 77]).

b. **Thymectomized-irradiated mouse (T/R mouse).** This immunologically suppressed mouse enables the killing of *M. leprae* to be followed in treated patients to the time when the proportion of viable bacilli has been reduced to less than 0.1% of its original value ([78]).

PHARMACOKINETICS

Measurement of serum and tissue levels of drugs in mice fed with the MED enables their MICs to be determined. Pharmacokinetic studies in man indicate by how many fold the peak serum/tissue concentrations are likely to exceed the MIC, the duration after a single dose that inhibitory levels will be maintained, and whether or not treatment will be actively bactericidal.

CLINICAL TRIALS

Studies of the treatment of lepromatous leprosy extending from six months to ten years have been monitored in the mouse to assess the rapidity with which viable bacilli are initially killed either by single drugs or by combinations of drugs, and whether drug-sensitive survivors ("persisters") can be eliminated by continued treatment.

DRUG RESISTANCE SURVEYS

Because of the importance of dapsone resistance, investigations have been undertaken in various parts of the world to estimate the

prevalence of dapsone-resistant strains of *M. leprae* among patients who have relapsed during continuing treatment (secondary resistance) ([47, 61, 63, 75]), and in those with previously untreated leprosy (primary resistance) ([62]).

Dapsone. The exceptional sensitivity of *M. leprae* to inhibition by dapsone is indicated by its MIC of only 0.003 μg/ml ([46, 68]). At concentrations near to its MIC, dapsone is essentially bacteriostatic; but at concentrations in excess of 100 times this value it is weakly bactericidal ([11, 17, 41]). Studies in both experimental animals and man show that it penetrates readily into all tissues including nerves ([1, 54, 71, 72, 73]). A dose of 100 mg dapsone results in peak concentrations that exceed the MIC by a factor of about 500-fold and maintains inhibitory levels for about 10 days ([15, 25, 27, 65-67, 74, 75]).

Several studies have shown that 30% or more of leprosy outpatients are grossly irregular in self-administering their dapsone treatment ([5, 15, 31, 51]). A reliable method of maintaining inhibitory levels of dapsone is to treat patients with acedapsone, the repository form of dapsone, because intramuscular injections of 225 mg of acedapsone maintain dapsone concentrations well in excess of the MIC for over three months ([27, 57, 74]).

Viable dapsone-sensitive leprosy bacilli ("persisters") can be recovered from up to 50% of lepromatous patients after as many as ten years of continuous dapsone monotherapy ([90]). Estimates of the prevalence of dapsone-resistant strains of *M. leprae* among lepromatous patients have ranged from 3% to 20% ([47, 61, 63, 75]). In the worst situation new dapsone-resistant cases were occurring at a rate of about 3% per annum of those at risk ([61]). Dapsone-resistance is a stable characteristic of *M. leprae* ([32, 87]), and resistant strains are clearly infectious for man since dapsone-resistant strains of *M. leprae* have been isolated from previously untreated patients ([33, 62]). Although dapsone is a weak carcinogen in the male rat ([7, 56, 64]), epidemiologic studies indicate that it is probably not carcinogenic in man ([38]). Furthermore, neither dapsone nor any of its known metabolites displays mutagenic activity *in vitro* ([69, 70]).

Rifampicin. The extremely powerful bactericidal activity of rifampicin against *M. leprae* has been demonstrated in the mouse by both the kinetic ([29, 30, 83, 85]) and proportional bactericidal test methods ([11]). Rifampicin is fully active against dapsone-resistant strains ([78, 79]), and experimental studies indicate that it penetrates excellently into nerves ([1, 35, 52]). In man it reduces the levels of dapsone in the body ([22, 25, 71, 73]), but this is probably without therapeutic significance. In clinical treatment the bactericidal activity of rifampicin is so powerful that single doses of 1200 mg or as few as four consecutive daily doses of 600 mg of the drug killed over 99% of the viable bacilli ([49, 78, 83, 84]). However, even five years of continuous treatment with rifampicin plus thiambutosine failed to eliminate the remaining persisters ([79, 91]). Drug-sensitive persisters were also isolated after combined treatment with daily dapsone plus rifampicin ([26]). Patients have relapsed with rifampicin monotherapy ([32]), but no such relapses have occurred among patients treated up to five years with rifampicin plus thiambutosine ([79, 91]).

Clofazimine. Because of marked tissue accumulation, it is impossible to determine the MIC of clofazimine against *M. leprae* ([6, 39]). Clofazimine treatment is less effective when doses are given at intervals of a week or more ([8]). Patients with dapsone-resistant leprosy have been treated for up to ten years with daily or thrice weekly clofazimine monotherapy without relapses occurring ([87, 89]), although the relatively small number of patients treated in this way does not exclude the possibility that clofazimine resistance might eventually emerge in a pattern similar to that originally observed with dapsone. Persisters can also be isolated after many years of continuous clofazimine treatment ([87]).

Ethionamide/Prothionamide/Thiacetazone/Thiambutosine. The most important results of studies of the antileprosy activities of these drugs in the normal mouse and investigations of their pharmacology in man ([9, 12, 34]) are summarized in the Table. The corresponding data for rifampicin and dapsone are included for comparison. Strains of *M. leprae* exhibit cross-resistance between ethionamide, prothionamide, thiacetazone, and thiambutosine ([59, 77]) but are not cross-resistant with dapsone or rifampicin ([32, 58, 76, 77]).

Streptomycin/Sulphamethoxypyridazine. A pilot clinical trial has shown that the antileprosy activity of streptomycin is inferior to that of dapsone ([88]). Experimental and pharmacological data relevant to the antileprosy activity of sulphamethoxypyridazine ([12]) are given in the Table.

Potential new drugs. The antileprosy activities of a series of dihydrofolate reductase inhibitors ([23, 24]), antithyroid compounds ([44, 50]), interferon inducers ([43, 45]), chaulmoogric- ([40]), clofazimine- ([42]) and long-acting rifampicin-analogs ([60]) have been investigated experimentally. The rifampicin derivatives appeared the most promising for further investigation.

FUTURE STUDIES

In vitro models. *M. leprae* has been shown to incorporate radioactive thymidine and DOPA in a cell-free system and in human macrophage culture ([2-4, 36, 86]). These systems may provide more rapid means of testing the activity of new drugs and identifying drug-resistant strains.

Animal models. The neonatally-thymectomized rat ([18]) and the athymic "nude" mouse ([10, 37]) may be useful for detecting *M. leprae* and for studies of drug combinations and microbial persistence. A newly-discovered nude rat ([13, 16]) may also be useful for these studies. The armadillo is currently the only source of the large numbers of bacilli required for enzymatic studies of drug action and represents the only model in which the development of drug resistance can be studied. The pharmacokinetics of dapsone, rifampicin and prothionamide have been studied in the armadillo ([55]), but more developmental work will be required before the armadillo can be evaluated as a model for long-term chemotherapeutic studies.

Drug development. Employing new methods for drug screening, computer-assisted technics for studies of quantitative structure-action relations and new analytical methods for studies of enzyme activity, it may be possible to develop new drugs specifically active against *M. leprae*. Employing two experimental models of ENL, it may now be possible to develop an active thalidomide derivative that is nonteratogenic ([28, 53]).

Clinical and field studies. Surveys of the point-prevalence of secondary and primary dapsone resistance must be conducted in various parts of the world. Surveillance programs should be initiated immediately for rifampicin

TABLE 1. *Minimal inhibitory concentration against* M. leprae *(MICs), peak serum concentrations, durations of coverage and bactericidal activities of antileprosy drugs.*

Drug	MIC (μg/ml)	Dosage (mg)	Ratio peak serum[a] MIC	Duration for which serum concs. exceed[b] MIC (days)	Bactericidal activity[c]
Rifampicin	0.3	600	30	1	+++
Dapsone	0.003	100	500	10	+
Acedapsone	0.003[d]	225	15	200	-
Ethionamide	0.05	500	60	1	++
Prothionamide	0.05	500	60	1	++
Thiacetazone	0.2	150	8	2	-
Sulphamethoxypyridazine[e]	30	1,000	3	3	-
Thiambutosine[f]	0.5	1,500	1	< 1	-

[a]Ratio of peak serum concentration in man after a single dose to MIC determined in the mouse.
[b]Serum concentrations in man after a single dose.
[c]Purely bacteriostatic (-); relative degrees of bactericidal activity (+, ++, +++).
[d]Acedapsone is inactive against *M. leprae* but is converted to dapsone — the figures for MIC and peak serum concentration refer to the values for dapsone.
[e]Cross-resistant with dapsone.
[f]Manufacture discontinued.

resistance, in view of the increasing use of rifampicin, which is often irregular and unsupervised. The efficacy of rifampicin administered intermittently and of ethionamide, prothionamide and thiacetazone must be established in short-term trials monitored by inoculation of normal mice. Combined regimens of drugs in dosage schedules already shown to be effective in short-term monotherapy trials must be tested among patients with lepromatous leprosy in formal clinical trials monitored by inoculation of immunosuppressed rodents. Finally, those combined regimens that appear most promising in formal clinical trials must be tested in the field to determine their acceptability to patients, the ease of their application to leprosy control programs and, most importantly, their ability to interrupt the transmission of *M. leprae*.

IMPLICATIONS FOR PRESENT TREATMENT

The widespread emergence of dapsone resistance has emphasized the necessity of using combinations of at least two antileprosy drugs for the treatment of lepromatous leprosy. The experimentally determined antileprosy activity and pharmacological characteristics of the available drugs are shown in the Table. For previously untreated patients, dapsone administered at a dosage of 50-100 mg daily must remain the primary drug, and the maintenance of inhibitory levels of dapsone could be guaranteed by the administration of acedapsone in addition to daily doses of dapsone. Of the drugs available for use in combination with dapsone, rifampicin with its rapid bactericidal activity is the first choice. Clofazimine is less costly than rifampicin, and its antileprosy activity is of the same order as that of dapsone. Thiacetazone might be a suitable drug for inclusion in drug combinations ([12]), although the experimental data suggest that one of the thioamides, ethionamide or prothionamide, would be more effective ([9]). However, the antileprosy activity of the thioamides in patients has yet to be fully evaluated. The cross-resistance demonstrated between the thioamides and thiacetazone ([59]) indicates that only one drug from this group should be used in combination with other antileprosy drugs.

The role of combinations of drugs for eliminating or decreasing the number of persisting bacilli has yet to be determined. The current "THELEP" trials should establish the best possible combinations.

REFERENCES

1. Allen, B. W., Ellard, G. A., Gammon, P. T., King, R. C., McDougall, A. C., Rees, R. J. W. and Weddell, A. G. M. The penetration of dapsone, rifampicin, isoniazid and pyrazinamide into peripheral nerves. Br. J. Pharmacol. **55** (1975) 151-155.
2. Ambrose, E. J., Khanolkar, S. R., Antia, N. H., Chulawalla, R. G. and Koticha, K. K. Rapid test for drug resistance in leprosy. Lancet **2** (1977) 1036.
3. Ambrose, E. J., Khanolkar, S. R., Antia, N. H., Chulawala, R. and Koticha, K. K. An *in vitro* test for drug response and drug resistance of *Mycobacterium leprae* using labeled metabolites. XI Int. Lepr. Congress, Mexico City, Mexico, November 13-18, 1978. Excerpta Medica, in press.
4. Ambrose, E. J., Khanolkar, S. R. and Chulawalla, R. G. A rapid test for bacillary resistance to dapsone. Lepr. India **50** (1978) 131-141.
5. Balakrishnan, S. Monitoring self administration of dapsone by patients. Lepr. India **49** (1977) 364-371.
6. Banerjee, D. K., Ellard, G. A., Gammon, P. T. and Waters, M. F. R. Some observations on the pharmacology of clofazimine (B663). Am. J. Trop. Med. Hyg. **23** (1974) 1110-1115.
7. Bergel, M. Actividad cancerigena de la diaminodifenilsolfona (DDS). Publ. Cent. Est. Leprol. **13** (1973) 30.
8. Collaborative Effort Of The U.S. Leprosy Panel and The Leonard Wood Memorial. Spaced clofazimine therapy of lepromatous leprosy. Am. J. Trop. Med. Hyg. **25** (1976) 437-444.
9. Colston, M. J., Ellard, G. A. and Gammon, P. T. Drugs for combined therapy: Experimental studies on the antileprosy activity of ethionamide and prothionamide, and a general review. Lepr. Rev. **49** (1978) 115-126.
10. Colston, M. J. and Hilson, G. R. F. Growth of *Mycobacterium leprae* and *M. marinum* in congenitally athymic (nude) mice. Nature **262** (1976) 399-401.
11. Colston, M. J., Hilson, G. R. F. and Banerjee, D. K. The "proportional bactericidal test": A method for assessing bactericidal activity of drugs against *Mycobacterium leprae* in mice. Lepr. Rev. **49** (1978) 7-15.
12. Colston, M. J., Hilson, G. R. F., Ellard, G. A., Gammon, P. T. and Rees, R. J. W. The activity of thiacetazone, thiambutosine, thiocarlide and sulphamethoxypyridazine against *Mycobacterium leprae* in mice. Lepr. Rev. **49** (1978) 101-113.

13. COLSTON, M. J., LANCASTER, R. D. and HILSON, G. R. F. Unpublished results.
14. COMMITTEE ON EXPERIMENTAL CHEMOTHERAPY. Experimental chemotherapy of leprosy. Bull. WHO **53** (1976) 425-433.
15. ELLARD, G. A., GAMMON, P. T. and HARRIS, J. M. The application of urine tests to monitor the regularity of dapsone self-administrations. Lepr. Rev. **45** (1975) 224-234.
16. FESTING, M. F. W., MAY, D., CONNORS, T. A., LOVELL, D. and SPARROW, S. An athymic nude mutation in the rat. Nature **274** (1978) 365-366.
17. FIELDSTEEL, A. H. and LEVY, L. Dapsone chemotherapy of *Mycobacterium leprae* infection of the neonatally thymectomized Lewis rat. Am. J. Trop. Med. Hyg. **25** (1976) 854-859.
18. FIELDSTEEL, A. H. and LEVY, L. Neonatally thymectomized Lewis rats infected with *Mycobacterium leprae*: Response to primary infection, secondary challenge and large inocula. Infect. Immun. **14** (1976) 736-741.
19. FREERKSEN, E. and ROSENFELD, M. Leprosy erradication project of Malta: first published report after 5 years running. Chemotherapy **23** (1977) 356-386.
20. FREERKSEN, E., ROSENFELD, M., BONNICI, E., DE PASQUALE, G. and KRUGER-THIEMER, M. Combined therapy of leprosy: Background and findings. Chemotherapy **24** (1978) 187-201.
21. GATTI, J. C., CARDAMA, J. E., BALINA, L. M., GABRIELLI, M., OCAMPO, J. C., PIZZARIELLO, G. E. A., LUPPI, M., DE HERRERA, M. P., BIANCHI, O. and SANTABAYA, E. Rifampicin in the treatment of lepromatous leprosy. XI Int. Lepr. Congress, Mexico City, Mexico, November 13-18, 1978. Excerpta Medica, in press.
22. GELBER, R. H., GOOI, H. C. and REES, R. J. W. The effect of rifampicin on dapsone metabolism. Proc. West. Pharmacol Soc. **18** (1975) 330-334.
23. GELBER, R. H. and LEVY, L. The effect of dihydrofolate reductase inhibitors on *Mycobacterium leprae* in the mouse foot pad. Int. J. Lepr. **44** (1976) 124-132.
24. GELBER, R. and LEVY, L. Further studies of dihydrofolate reductase inhibitor activity on the multiplication of *M. leprae*. Int. J. Lepr. **46** (1978) 111-112.
25. GELBER, R. H. and REES, R. J. W. Dapsone metabolism in patients with dapsone-resistant leprosy. Am. J. Trop. Med. Hyg. **24** (1975) 963-967.
26. GELBER, R. H., WATERS, M. F. R., PEARSON, J. M. H., REES, R. J. W. and MCDOUGALL, A. C. Dapsone alone compared with dapsone plus rifampicin in short-term therapy of lepromatous leprosy. Lepr. Rev. **48** (1977) 223-229.
27. GLAZKO, A. J., DILL, W. A., MONTALBO, R. G. and HOLMES, E. L. A new analytical procedure for dapsone. Application to blood-level and urinary-excretion studies in normal men. Am. J. Trop. Med. Hyg. **17** (1968) 465-473.
28. HASTINGS, R. C. Personal communication (1978).
29. HOLMES, I. B. and HILSON, G. R. F. The effect of rifampicin and dapsone on experimental *Mycobacterium leprae* infections: minimum inhibitory concentrations and bactericidal action. J. Med. Microbiol. **5** (1972) 251-261.
30. HOLMES, I. B. and HILSON, G. R. F. The rate of bactericidal action of rifampicin on *Mycobacterium leprae* in the mouse foot pad. Proc. Soc. Exp. Biol. Med. **145** (1974) 1395-1400.
31. HUIKESHOVEN, H. C. J., HONHOFF, C., VAN EYS, G. J. J. M., ANTEN, J. G. F., MAYER, J. M. A. and VAN HELDEN, H. P. T. Weekly self-medication of leprosy patients monitored by DDS/creatinine ratios in urines. Lepr. Rev. **47** (1976) 201-209.
32. JACOBSON, R. R. and HASTINGS, R. C. Rifampicin-resistant leprosy. Lancet **2** (1976) 1304-1305.
33. JACOBSON, R. R. and HASTINGS, R. C. Primary sulfone resistant leprosy. Int. J. Lepr. **46** (1978) 116.
34. JENNER, P. J. and ELLARD, G. A. Unpublished results.
35. KEBERLE, H., SCHMID, K. and MEYER-BRUNOT, E. The metabolic fate of rimactane in the animal and in man. *In:* A Symposium on Rimactane, Basle: CIBA, 1968, pp 20-27.
36. KHANOLKAR, S. R., AMBROSE, E. J., CHULAWALA, R. G. and BAPAT, C. V. Autoradiographic and metabolic studies of *Mycobacterium leprae*. Lepr. Rev. **49** (1978) 187-198.
37. KOHSAKA, K., MORI, T. and ITO, T. Lepromatoid lesion developed in nude mouse inoculated with *M. leprae*. La Lepro **45** (1976) 177-183.
38. KOLONEL, L. N. and HIROHATA, T. Leprosy and cancer: a retrospective cohort study in Hawaii. J. Natl. Cancer Inst. **58** (1977) 1577-1582.
39. LEVY, L. Pharmacologic studies of clofazimine. Am J. Trop. Med. Hyg. **23** (1974) 1097-1109.
40. LEVY, L. The activity of chaulmoogra acids against *Mycobacterium leprae*. Am. Rev. Respir. Dis. **111** (1975) 703-705.
41. LEVY, L. Bactericidal action of dapsone against *Mycobacterium leprae* in mice. Antimicrob. Agents Chemother. **9** (1976) 614-617.
42. LEVY, L. Unpublished results.
43. LEVY, L., AIZER, F., NG, H. and WELCH, T. M. The effects of tilorone on mycobacterial infections in mice. Lepr. Rev. **49** (1978) 215-222.
44. LEVY, L. and ANANDAN, J. A. Further studies of the action of antithyroid drugs on *Mycobacterium leprae*. Proc. Soc. Exp. Biol. Med. **158** (1978) 582-585.

45. LEVY, L. and MERIGAN, T. C. Inhibition of multiplication of *Mycobacterium leprae* by polyinosinic-polycytidylic acid. Antimicrob. Agents Chemother. **11** (1977) 122-125.
46. LEVY, L. and PETERS, J. H. Susceptibility of *Mycobacterium leprae* to dapsone as a determinant of patient response to acedapsone. Antimicrob. Agents Chemother. **9** (1976) 102-112.
47. LEVY, L., RUBIN, G. S. and SHESKIN, J. The prevalence of dapsone-resistant leprosy in Israel. Lepr. Rev. **48** (1977) 107-112.
48. LEVY, L., SHEPARD, C. C. and FASAL, P. Clofazimine therapy of lepromatous leprosy caused by dapsone-resistant *Mycobacterium leprae*. Amer. J. Trop. Med. Hyg. **21** (1972) 315-321.
49. LEVY, L., SHEPARD, C. C. and FASAL, P. The bactericidal effect of rifampicin on *M. leprae* in man. Int. J. Lepr. **44** (1976) 183-187.
50. LEVY, L. and ULLMANN, N. M. Inhibition of multiplication of *Mycobacterium leprae* by several antithyroid drugs. Am. Rev. Respir. Dis. **111** (1975) 651-655.
51. LOW, S. J. M. and PEARSON, J. M. H. Do leprosy patients take dapsone regularly? Lepr. Rev. **45** (1974) 218-223.
52. MCDOUGALL, A. C., ROSE, J. A. and GRAHAME-SMITH, D. G. Penetration of C^{14}-labeled rifampicin into primate peripheral nerve. Experientia **31** (1975) 1068-1069.
53. MIRANDA, R. O., MORALES, M. J. and HASTINGS, R. C. Thalidomide's effect on antibody formation. Fed. Proc. **36** (1977) 1231.
54. MURRAY, J. F., JR., GORDON, G. R. and PETERS, J. H. Tissue levels of dapsone and monoacetyldapsone in Lewis rats receiving dietary dapsone. Proc. West. Pharmacol. Soc. **17** (1974) 150-154.
55. MURRAY, J. F., JR., GORDON, G. R., PETERS, J. H., GROVE, G. R. and WALSH, G. P. Metabolic disposition of dapsone (DDS) and rifampicin (RFM) in nine-banded armadillos *(Dasypus novemcinctus)*. XI Int. Lepr. Congress, Mexico City, Mexico, November 13-18, 1978. Excerpta Medica, in press.
56. NATIONAL CANCER INSTITUTE. Bioassay of dapsone for possible carcinogenicity. CAS No. 80-08-0, NCI-CG-TR-20 (1977).
57. OZAWA, T., SHEPARD, C. C. and KARAT, A. B. A. Application of spectrophotofluorometric procedures to some problems of *Mycobacterium leprae* infections in mice and man treated with dapsone (DDS), diacetyl-DDS (DADDS), and di-formyl-DDS (DFD). Am. J. Trop. Med. Hyg. **20** (1971) 274-281.
58. PATTYN, S. R. Unpublished results.
59. PATTYN, S. R. and COLSTON, M. J. Cross-resistance amongst thiambutosine, thiacetazone, ethionamide, and prothionamide with *Mycobacterium leprae*. Lepr. Rev. **49** (1978) 324-326.
60. PATTYN, S. R. and SAERENS, E. J. Activity of three new rifamycin derivates on the experimental infection by *Mycobacterium leprae*. Ann. Soc. Belge Med. Trop. **57** (1977) 169-173.
61. PEARSON, J. M. H., CAP, J. A., HAILE, G. S. and REES, R. J. W. Dapsone-resistant leprosy and its implications for leprosy control programs. Lepr. Rev. **48** (1977) 83-94.
62. PEARSON, J. M. H., HAILE, G. S. and REES, R. J. W. Primary dapsone-resistant leprosy. Lepr. Rev. **48** (1977) 129-132.
63. PEARSON, J. M. H., REES, R. J. W. and WATERS, M. F. R. Sulfone resistance in leprosy. A review of 100 proven clinical cases. Lancet **2** (1975) 69-72.
64. PETERS, J. H. Carcinogenic activity of dapsone. Int. J. Lepr. **44** (1976) 383-384.
65. PETERS, J. H., GORDON, G. R., GHOUL, D. C., TOLENTINO, J. G., WALSH, G. P. and LEVY, L. The disposition of the antileprotic drug dapsone (DDS) in Philippine subjects. Am. J. Trop. Med. Hyg. **21** (1972) 450-457.
66. PETERS, J. H., GORDON, G. R. and KARAT, A. B. A. Polymorphic acetylation of the antibacterials, sulfamethazine and dapsone, in South Indian subjects. Am. J. Trop. Med. Hyg. **24** (1975) 641-648.
67. PETERS, J. H., GORDON, G. R., LEVY, L., STORKAN, M. A., JACOBSON, R. R., ENNA, C. D. and KIRCHHEIMER, W. F. Metabolic disposition of dapsone in patients with dapsone-resistant leprosy. Am. J. Trop. Med. Hyg. **23** (1974) 222-230.
68. PETERS, J. H., GORDON, G. R., MURRAY, J. F., JR., FIELDSTEEL, A. H. and LEVY, L. Minimal inhibitory concentration of dapsone for *Mycobacterium leprae* in rats. Antimicrob. Agents Chemother. **8** (1975) 551-557.
69. PETERS, J. H., GORDON, G. R., SIMMON, V. F. and TANAKA, W. Mutagenesis of dapsone and its derivatives in *Salmonella typhimurium*. Fed. Proc. **37** (1978) 450.
70. PETERS, J. H., GORDON, G. R., SIMMON, V. F. and TANAKA, W. Mutagenesis-carcinogenesis of antileprosy drugs in animal systems: pertinence to man. XI Int. Lepr. Congress, Mexico City, Mexico, November 13-18, 1978. Excerpta Medica, in press.
71. PETERS, J. H., MURRAY, J. F., JR., GORDON, G. R., GELBER, R. H., LAING, A. B. G. and WATERS, M. F. R. Effect of rifampin on the disposition of dapsone in Malaysian leprosy patients. Fed. Proc. **36** (1977) 996.
72. PETERS, J. H., MURRAY, J. F., JR., GORDON, G. R., GELBER, R. H., LEVY, L., LAING, A. B. G. and WATERS, M. F. R. Tissue levels of dapsone in mice, rats and man. Int. J. Lepr. **44** (1976) 545-546.
73. PETERS, J. H., MURRAY, J. F., JR., GORDON, G. R. and JACOBSON, R. R. Metabolic-bacteriologic relationships in the chemotherapy of

lepromatous patients with dapsone or dapsone-rifampin. Int. J. Lepr. **46** (1978) 115-116.

74. PETERS, J. H., MURRAY, J. F., JR., GORDON, G. R., LEVY, L., RUSSELL, D. A., SCOTT, G. C., VINCIN, D. R. and SHEPARD, C. C. Acedapsone treatment of leprosy patients: response versus drug disposition. Am. J. Trop. Med. Hyg. **26** (1977) 127-136.
75. PETERS, J. H., SHEPARD, C. C., GORDON, G. R., ROJAS, A. V. and ELIZONDO, D. S. The incidence of DDS resistance in lepromatous patients in Costa Rica: their metabolic disposition of DDS. Int. J. Lepr. **44** (1976) 143-151.
76. REES, R. J. W. Drug resistance of *Mycobacterium leprae* particularly to DDS. Int. J. Lepr. **35** (1967) 625-636.
77. REES, R. J. W. A preliminary review of the experimental evaluation of drugs for the treatment of leprosy. Trans. R. Soc. Trop. Med. Hyg. **61** (1967) 581-595.
78. REES, R. J. W., PEARSON, J. M. H. and WATERS, M. F. R. Experimental and clinical studies on rifampicin in treatment of leprosy. Brit. Med. J. **1** (1970) 89-92.
79. REES, R. J. W., WATERS, M. F. R., PEARSON, J. M. H., HELMY, H. S. and LAING, A. B. A. Long-term treatment of dapsone-resistant leprosy with rifampicin: Clinical and bacteriological studies. Int. J. Lepr. **44** (1976) 159-169.
80. ROSENFELD, M., FREERKSEN, E., BONNICI, E. and DEPASQUALE, G. Practical experience with combined therapy in leprosy. XI Int. Lepr. Congress, Mexico City, Mexico, November 13-18, 1978. Excerpta Medica, in press.
81. SHEPARD, C. C. A kinetic method for the study of activity of drugs against *Mycobacterium leprae* in mice. Int. J. Lepr. **35** (1967) 429-435.
82. SHEPARD, C. C., LEVY, L. and FASAL, P. The death of *Mycobacterium leprae* during treatment with 4,4′-diaminodiphenylsulfone (DDS). Am. J. Trop. Med. Hyg. **17** (1968) 769-775.
83. SHEPARD, C. C., LEVY, L. and FASAL, P. Rapid bactericidal effect of rifampin on *Mycobacterium leprae*. Am. J. Trop. Med. Hyg. **21** (1972) 446-449.
84. SHEPARD, C. C., LEVY, L. and FASAL, P. Further experience with the rapid bactericidal effect of rifampin on *Mycobacterium leprae*. Am. J. Trop. Med. Hyg. **23** (1974) 1120-1124.
85. SHEPARD, C. C., WALKER, L. L., VAN LANDINGHAM, R. M. and REDUS, M. A. Kinetic testing of drugs against *Mycobacterium leprae* in mice. Activity of cephaloridine, rifampin, streptovaricin, vadrine and viomycin. Am. J. Trop. Med. Hyg. **20** (1971) 616-620.
86. TALWAR, G. P., KRISHNAN, A. D. and GUPTA, P. D. Quantitative evaluation of the progress of intracellular infection *in vitro*: incorporation of ^{3}H-thymidine into deoxyribonucleic acid by *Mycobacterium leprae* in cultivated blood monocytes. Infect. Immun. **9** (1974) 187-191.
87. WATERS, M. F. R. The diagnosis and management of dapsone-resistant leprosy. Lepr. Rev. **48** (1977) 95-105.
88. WATERS, M. F. R. and REES, R. J. W. Unpublished results.
89. WATERS, M. F. R., REES, R. J. W. and MCDOUGALL, A. C. and LAING, A. B. G. The problem of microbial persistence in leprosy. XI Int. Lepr. Congress, Mexico City, Mexico, November 13-18, 1978. Excerpta Medica, in press.
90. WATERS, M. F. R., REES, R. J. W., MCDOUGALL, A. C. and WEDDELL, A. G. M. Ten years of dapsone in lepromatous leprosy: clinical, bacteriological and histological assessment and finding of viable leprosy bacilli. Lepr. Rev. **45** (1974) 288-298.
91. WATERS, M. F. R., REES, R. J. W., PEARSON, J. M. H., LAING, A. B. G., HELMY, H. S. and GELBER, R. H. Rifampicin for lepromatous leprosy: nine years' experience. Brit. Med. J. **1** (1978) 133-136.