INTERNATIONAL JOURNAL OF LEPROSY Volume 35, Number 1
Printed in U.S.A.

Chemotherapeutic Trials in Leprosy

3. Pilot Trial of a Riminophenazine Derivative, B.663, in the Treatment of Lepromatous Leprosy[1]

John H. S. Pettit, R. J. W. Rees and D. S. Ridley[2]

The riminophenazine derivative B.663, one of an entirely new series of antimycobacterial drugs (1), has recently been reported to be active in leprosy (3, 4, 8, 17). In this paper we present the results of a pilot trial of B.663 in the treatment of lepromatous leprosy at the Leprosy Research Unit, Sungei Buloh, Malaysia. The opportunity to test this new drug allowed us to find out if we could assess its antileprosy activity within a period of only four and a half to six months, using a small number of patients, on the basis of experience gained from studies in the conduct of larger and more prolonged controlled trials undertaken at the Unit during the previous six years. These studies were based on controlled trials of Macrocyclon (15) and Ditophal (16) in the treatment of patients with lepromatous or near-lepromatous leprosy, which incorporated nearly one hundred cases.

There is now overwhelming experimental evidence that irregularly stained bacilli are dead and that the majority of solid-staining bacilli are alive (9, 10, 14). It was thought, therefore, that this feature might provide the most sensitive indirect method for determining the rate at which a drug kills leprosy bacilli, and so be used to assess a new drug in patients with lepromatous leprosy who had not received previous treatment. The importance and value of the morphologic index (MI), i.e., the percentage of solidly staining acid-fast bacilli determined from routinely stained skin smears, became increasingly clear, since without exception all cases showed a significant fall within a period of six months.

SELECTION OF CASES

As in our previous studies, patients had to be suffering clinically and histologically from pure lepromatous (LL) or near-lepromatous (BL) leprosy in which there were only a few atypical features. Continued experience has given no cause to revise the 5-group TT-LL classification (12) that we have always used. The histologic grading of pure leproma (LL) is given to the type of granuloma which, in the most active phase of the condition, is composed of macrophages heavily loaded with acid-fast bacilli but having a minimal fat content, and in which, with maturation, the cells undergo foamy change and eventually, as the fat accumulates, globus formation. Multinucleate globus cells, if present, are diagnostic of this group. Lymphocytes are scanty or diffuse, except

[1]Received for publication 28 June 1966.

[2]J. H. S. Pettit, M.R.C.P., The Research Unit, Sungei Buloh Leprosarium, Selangor, Malaysia, and National Institute for Medical Research, Mill Hill, London, N.W. 7, England; R. J. W. Rees, F.C. Path., National Institute for Medical Research; D. S. Ridley, M.D. The Hospital for Tropical Diseases, 4 St. Pancras Way, London, N.W. 1. Requests for reprints should be addressed to Dr. R. J. W. Rees.

perhaps in reacting lesions, and, although nerves may be damaged, they are not infiltrated or cuffed by cells. The borderline-leproma (BL) group is marked either by a heavy infiltration of lymphocytes into a granuloma of the LL type, in which case the histiocytes usually show some degree of foamy degeneration, though globi never occur, or, alternatively, by a tendency for histiocytes to resemble epithelioid cells, although true epithelioid cells are not seen. In the latter case there is no foamy change, and usually only a few lymphocytes are present. There may be a slight cellular infiltration of nerves or perineural cuffs of lymphocytes. Both in the LL and the BL groups bacilli are numerous and the subepidermal zone is usually clear of infiltrate. In Malaysia the proportion of LL to BL cases is approximately 2 to 1 and there is a tendency for patients to fall between the two groups. Despite this problem the majority of patients can be classified without much difficulty and we find good agreement between clinical and histologic assessments.

Adult males only were studied and no patients who had received chemotherapy of any sort for leprosy were admitted to the trial, nor were those who suffered from any other significant organic disease.

METHOD OF STUDY

The investigations performed and the methods of assessing progress were based on protocols used previously ([15, 16]), with some modifications. Prior to the start of treatment a clinical examination was performed, which included x-ray observation of the chest and urine and blood examinations. The leprosy condition was carefully recorded, with charting of the lesions, color photographs, regular skin smears from the same six sites (including both ear lobes), estimation of the bacteriologic (BI) and the morphologic (MI) indices, and biopsies of skin from two recorded sites. Lepromin (Mitsuda type) and tuberculin (PPD, RT23) tests were also performed.

The trial was originally planned to last four and a half months, but, because of administrative difficulties, it was extended to five months. During this period the blood and urine were checked regularly. Smears for assessing the BI and MI were taken after one and half and three months of treatment and again at the end of the trial, at which time two further biopsies were made. A complete clinical examination was also made, including color photographs and charting of the lesions. To ensure that the clinical assessments were as unbiased as possible, we had the services of an independent assessor who was kept in ignorance throughout the study as to the method of treatment the patients were receiving. He was shown each patient and the clinical photographs at the beginning and the end of the five month period and made detailed notes, including drawings of his own. On his second examination he was asked to answer the following questions:

In comparison with the first examination, is the leprosy (a) markedly improved, (b) moderately improved, (c) slightly improved, (d) unchanged, (e) slightly worse, (f) moderately worse, or, finally, (g) markedly worse? This question was answered by the assessor through the use of his own records and with no assistance from the clinical worker who was in charge of the cases. It had been decided at the beginning of the study that the assessor would have been told if any of the patients were receiving steroids at the time of the follow-up, but this proved to be unnecessary, as no patients were receiving such treatment at that time.

Treatment. One 100 mgm. capsule of B.663 was given by mouth three times a day for six days per week to each patient for the first five-month trial period, and this treatment was extended for an additional four weeks and then changed to DDS therapy, with 50 mgm. by mouth twice weekly.

CASE HISTORIES

Case 1. (No. 15055) Chinese male, age 23. This patient was admitted to the hospital with a one-year history. The disease had started with a patch of hypopigmentation over the left ankle. Similar lesions

slowly appeared elsewhere. He complained of a feeling of numbness in nearly all areas of the body, and on examination showed diffuse infiltration of the face and ears and a number of infiltrated hypopigmented patches on the limbs and back. Clinically the diagnosis was of a near-lepromatous case. The lepromin test was negative for the Mitsuda reading. The smears on admission showed an average BI of 4.5 and an average MI of 43. The histology was reported as BL and the average biopsy index was 1.7. The hemoglobin on admission was 12.0 gm. per cent. The tuberculin test was at first negative to 1 TU, but later led to reaction of 15 mm. to 20 TU.

Case 2. (No. 15057) Chinese male, age 21. On admission to the hospital he gave a one-year history of an anesthetic patch on the posterior aspect of the left elbow. At the same time the ears had become markedly thickened and later the nose and the rest of the body became infiltrated and somewhat hypoesthetic. The great auricular and ulnar nerves were palpable and hard. Clinically the patient showed a diffuse lepromatous leprosy all over the body, with very thick ears and general hypopigmentation. The lepromin test was negative for the Mitsuda reading. On admission the smears gave an average BI of 4.8 and MI of 16. The histology showed active lepromatous leprosy (LL), and the biopsy index was 1.4. The hemoglobin on admission was 14.5 gm. per cent. The tuberculin test was negative to 1 TU, but gave a reaction 18 mm. in diameter to 20 TU.

Case 3. (No. 15058) Chinese male, age 38. On admission this patient stated that nine years previously he had noticed anesthesia on the left arm and that later a red patch appeared at the same site. A few years after this, a similar patch was found on the left forearm, and shortly afterward wasting had appeared between the left thumb and the left index finger. About a year prior to admission there was a general spread of the disease. On his admission the face showed diffuse infiltration of lepromatous type with early loss of eyebrows and very large ears. The clinical diagnosis was diffuse lepromatous leprosy. At the time of admission the Mitsuda reaction measured 4 mm. Smears on admission gave an average BI of 4.8 and MI of 15. The biopsies were reported as showing active lepromatous leprosy (LL) and the biopsy index was 1.25. On admission the hemoglobin was 10.9 gm. per cent. The tuberculin test was negative to both 1 and 20 TU.

Case 4. (No. 15059) Chinese male, age 24. Eight years prior to admission this patient noticed numbness on the dorsal aspect of the right foot. Later a similar numbness appeared at the elbows. About seven months before admission a number of thick red patches had appeared on the body. Clinically the patient appeared to be nearer to borderline leprosy than to lepromatous leprosy and he was diagnosed by the clinical worker as BB to BL. The Mitsuda reading at this time was negative. Smears taken from both ears were negative, but lesions on the body were strongly positive, giving for all sites an average BI of 3.2 and MI of 26. Histologically this patient was close to LL, but, in view of the discrepancy between this and the clinical appearance, he was given a final classification of BL. The biopsy index was 1.2. On admission to hospital his hemoglobin was 14.5 gm. per cent. The tuberculin test was negative to 1 TU, but gave a reading of 30 mm. to 20 TU.

Case 5. (No. 15075) Malay-aborigine male, age 46. Two years before coming to the hospital this patient noticed a patch on the left loin that was somewhat redder than the rest of the body but was not anesthetic. Other lesions appeared on the left and right limbs, and about one month before admission the face was noticed to be somewhat thicker than previously. On clinical examination he was found to have typical lepromatous leprosy with enlarged ears, thickened alae nasi, diminished eyebrows, and somewhat hypopigmented infiltration all over the body. The lepromin reaction showed a negative Mitsuda reading. Smears taken on admission showed a BI of 4.3 and an MI of 30. The biopsies showed typical lepromatous leprosy (LL), and the biopsy index was 2.25. On admission his hemoglobin was 14.1 gm. per cent. The tuberculin test gave a reading of 17 mm. to 1 TU.

Case 6. (No. 15077) Malay-aborigine male, age 42. This patient had noticed some diminished sensation on his feet for about a year after his attention was drawn to the fact that he had an ulcer on the left sole. The diminished sensation gradually spread over the body, and seven months prior to his admission a large number of hard nodules appeared on the ears, forehead, nose, chest, forearms, and hands. Clinically he had severe nodular lepromatous leprosy and the lepromin reading was negative. His smears when first seen had a BI of 4.5 and an MI of 38. The histology showed typical lepromatous leprosy (LL) with a biopsy index of 1.25. His hemoglobin was 10.8 gm. per cent. on admission. The tuberculin test gave a reading of 6 mm. to 1 TU.

ANALYSIS OF RESULTS

Clinical. At the end of the five month period the independent assessor rated the improvement as "slight" in all cases. None of the patients had developed any form of reaction, nor did they do so during the additional four weeks on B.663. During the next six months of observation, three of the patients (Cases 3, 5 and 6) developed attacks of erythema nodosum leprosum (ENL). One patient (Case 3) showed lesions of ENL 18 days after stopping B.663, but a BCG vaccination had been given only a few days prior to the attack and it is possible that it precipitated the reaction. Two patients (Cases 5 and 6) each developed ENL four months after the beginning of treatment with DDS, i.e., some ten months after the treatment was started originally.

Bacteriology. The figures for the BI and MI are summarized in Table 1. The details of the MI for each patient, together with indices after an additional month on B.663 and then three months after the treatment was changed to DDS, are shown in Figure 1. There was a significant fall in the MI's of all patients. In fact only the patient in Case 6 had an MI above 3 at five months and it is interesting to note that with the continuation of B.663 for an additional month this patient's MI fell to 3. These results were consistent with our previous experience, and indicated that B.663 was an active antileprosy drug capable of killing leprosy bacilli.

Histology. Improvement as indicated by histologic changes during treatment, was assessed from the biopsy index ([11]), and the overall fall in index of each patient from the beginning to the completion of five months of treatment is shown in Table 1. The average fall was 40 per cent. There was considerable variation in the individual results, although all patients showed some improvement. This index, however, is designed primarily to assess the progress of a group, and individual results are not accurate unless there are at least two six-month periods of observation. The mean fall of 40 per cent is almost exactly the figure that would have been expected with DDS in a group comprising one BL case for every two LL cases, which was the proportion in this trial.

TABLE 1. *Bacteriologic and histologic response of six patients treated for five months with B.663 (300 mgm. daily for six days/week).*

Case No.	Period of treatment				Percentage fall in biopsy index
	At start	After 5 months	At start	After 5 months	
	Bacteriologic index		Morphologic index		
1	4.5	4.2	43	1	65
2	4.8	4.3	16	3	50
3	4.8	4.3	15	1	8
4	3.2	2.7	26	1	36
5	4.3	4.3	30	1	69
6	4.5	3.8	38	8	12
Average	4.3	3.9	26	2.5	40

Reactions to B.663. Perhaps the most important finding in the four Chinese patients studied was the development of a deep and persistent pigmentation of the skin. Redness appeared within one to four weeks after the start of treatment and increased steadily during the period of study. The normal skin acquired a marked and rather unpleasing color, and the leprosy lesions themselves became hypermelanotic as well as red. There was dramatic sparing of the axillae in all cases (Fig. 2a & b), even when the condition was diffusely spread throughout the rest of the skin. The two dark-skinned aborigine patients did not manifest significant pigmentary changes.

The histology of all six patients showed an appreciable increase in the melanin content of the basal layer of the epidermis at five months, on comparison with the initial pretreatment biopsies. This increase was due to greater density of pigment in the pigment-bearing cells, and in five of the six patients this was the only factor. The other patient (Case 3) showed in addition an increase in the number of pigment-bearing cells, with reduplication of the basal layer, and also incontinence of pigment into the dermal papillae. The findings in this patient agree with those reported by Browne [2].

No evidence of the drug could be found in any section either as colored deposits or as crystals. This is not surprising, because the skin specimens were dispatched to London in 70 per cent alcohol, which had dissolved out the drug and become pink. Although ghosts of crystals have been reported in animals [5, 13] and man [17], after large doses of B.663, we have been unable to demonstrate them in our cases.

Our experience with B.663 in these and other cases [7, 8] has confirmed previous reports [6, 17] that occasionally patients re-

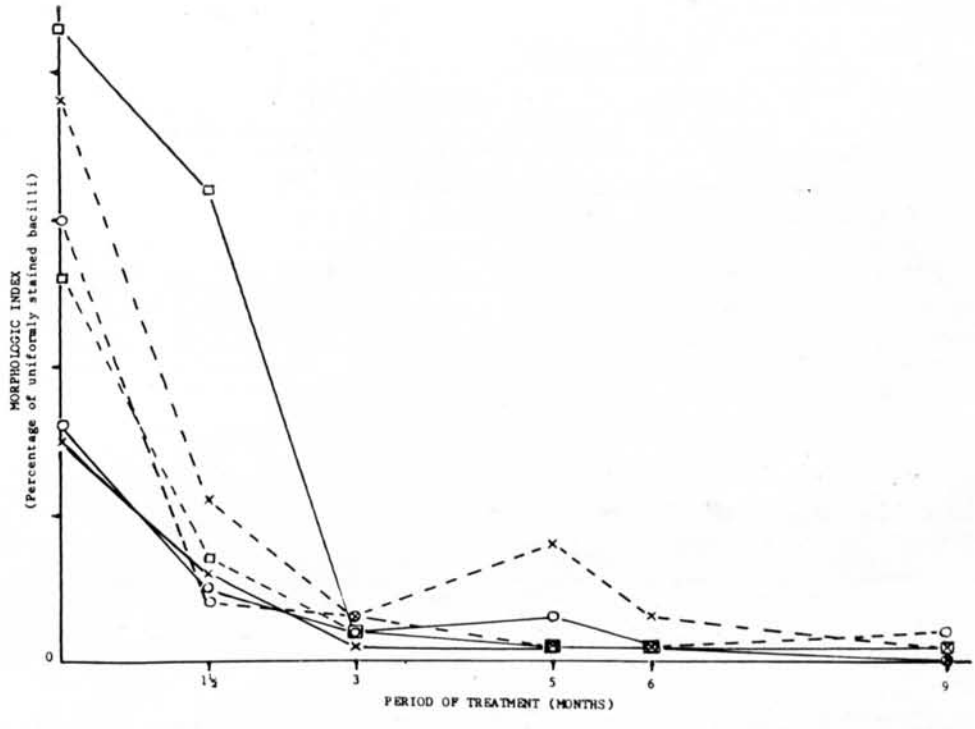

FIG. 1. The effect of treatment with B.663 (300 mgm. daily 6 days/week) on the morphologic index (MI) in 6 previously untreated lepromatous patients. Treatment was changed to DDS (50 mgm. twice weekly) at 6 months. Case 1, □ ——— □; Case 2, 0 ——— 0; Case 3, X ——— X; Case 4, □ - - - - - - - □; Case 5, 0 - - - - - - - 0; Case 6, X - - - - - - X.

ceiving B.663 develop diarrhea, which, however, is usually not incapacitating. The present series of patients were on the whole satisfactorily free from this complaint, but one patient (Case 1) complained intermittently of loose stools and upper abdominal pain during the third and fourth months of his treatment. These symptoms responded to simple medication and the B.663 therapy was not interrupted.

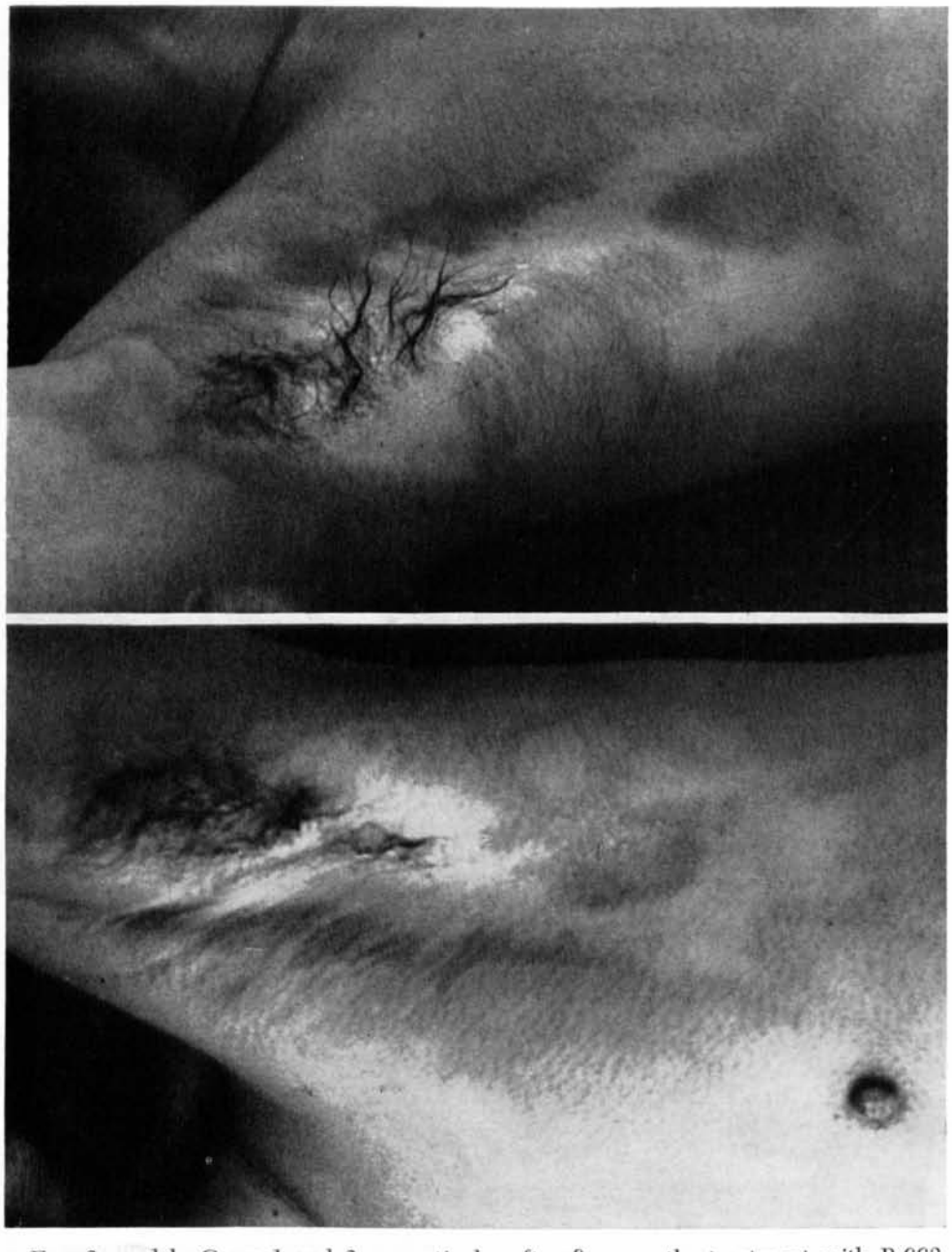

FIG. 2a and b. Cases 1 and 2 respectively, after five months treatment with B.663, showing hyperpigmentation of skin with sparing of the axilla.

DISCUSSION

The rigorous clinical, bacteriologic and histologic assessment applied in this pilot trial of B.663 all indicated that the drug is active in lepromatous leprosy. In particular, the fall in the MI, which is considered to be the most sensitive method for assessing therapeutic activity of a drug as a measure of its ability to kill leprosy bacilli, confirmed previous evidence that B.663 is an active drug producing changes in the first five to six months of treatment comparable with DDS therapy.

Contrary to the implications in Browne's work ([3]), and confirming our own work on B.663 treatment of sulfone-resistant leprosy ([8]), the drug was not found acceptable to the majority of the paler-skinned peoples in the presently used dosage. During this trial, for the first time in the history of our Unit, cooperation with patients in the Settlement was less than whole-hearted, and this was attributable directly to the fact that most patients were afraid that they too would be treated with B.663 and turn an extremely unpleasant color. It is suggested that, in the future, studies should be made on the therapeutic success and acceptability of lower dosages than have been used heretofore in leprosy.

Although controlled studies are essential for an exact comparison of different treatments in leprosy, as in the case of any disease, such trials are complicated, difficult and lengthy, especially in view of the diminishing supply of suitable patients. It was important, therefore, to investigate the design of pilot trials, to see if these could provide a rapid and reasonably accurate assessment of the value of a new drug, using a minimal number of patients. The present trial, using the new antileprosy drug B.663, was our first attempt to evolve a methodology for such a trial. The results, based on only six previously untreated patients with lepromatous leprosy, indicate that the antileprosy activity of a drug can be detected within a relatively short period by applying the clinical, bacteriologic and histologic methods of assessing progress that have been developed in our larger controlled trials.

The first attempt confirmed the particular value and importance of the MI. In retrospect it must be admitted that the present trial was not based upon a perfect protocol. If, as we believe, the fall in the MI is of primary importance in the assessment of therapeutic activity of a drug, it was unjustifiable to use cases in which the original MI was already low (Cases 2 and 3). It is also of dubious scientific value to include even "near lepromatous (BL)" cases in a study of this type; our experience suggests that the greater clinical and bacterial lability known to be associated with the borderline type disease extends to such BL cases that have only minimal evidence of a borderline tendency. It is therefore emphasized that in future pilot trials it is imperative that the histology be fully lepromatous (LL) and that the average MI, when calculated from smears of six different sites, should be at least 25. Four and a half months is probably an adequate time for a pilot trial, although it may be shown later that this suggestion is slightly optimistic and that a period of six months may be preferable.

SUMMARY

On the basis of experience in previous clinical trials a pilot trial was devised for study of the effect of B.663 in patients with previously untreated lepromatous leprosy.

B.663, in a dosage of 100 mgm. three times a day for six days a week, produces therapeutic results, after five months, that are comparable to those with sulfone therapy. Several patients objected to the pigmentation produced.

Experience during this study has led us to recommend modifications in future pilot trials of a similar nature.

RESUMEN

Basado en experiencias de ensayos clínicos previos un ensayo piloto fué planeado para estudiar el efecto de B.663 en pacientes con lepra lepromatosa sin tratamiento previo.

B.663, en dosis de 100 mgm. tres veces al día por seis días a la semana, produce re-

sultados terapeúticos, después de cinco meses, que son comparables a aquellos con tratamiento con sulfonas. Varios pacientes reclamaron de la pigmentación producida.

Experiencia adquirida durante este estudio nos ha llevado a recomendar modificaciones en ensayos pilotos futuros de naturaleza similar.

RÉSUMÉ

Sur la base de l'expérience acquise au cours d'essais cliniques antérieurs, on a organisé un essai pilote pour étudier l'effet du B.663 chez des malades atteints de lèpre lépromateuse non traitée antérieurement.

Administré à la dose de 100 mgm. trois fois par jour, six jours par semaine, le B.663 a produit, après 5 mois, des résultats thérapeutiques comparables à ceux obtenus par la thérapeutique sulfonée. Plusieurs malades se sont plaints de la pigmentation qui en résulte.

L'expérience acquise au cours de cette étude a conduit les auteurs à recommender des modifications à apporter lors des essais pilotes similaires qui seront menés à l'avenir.

Acknowledgments. The Leprosy Research Unit is jointly administered by the Malaysian Ministry of Health and the British Medical Research Council. Our thanks are due to the staff and patients of the Sungei Buloh Leprosarium, without whose assistance this work could not have been carried out. We are particularly grateful also to Dr. K. M. Reddy who acted as the independent assessor for our studies and to Dr. P. D. Fowler of Geigy (Manchester), for the supply of B.663 used.

REFERENCES

1. Barry, V. C. and Conalty, M. L. The antimycobacterial activity of B.663, Leprosy Rev. **36** (1965) 3-7.
2. Browne, S. G. Red and black pigmentation developing during treatment of leprosy with B.663. Leprosy Rev. **36** (1965 17-20.
3. Browne, S. G. and Hogerzeil, L. M. "B.663" in the treatment of leprosy. Preliminary report of a pilot trial. Leprosy Rev. **33** (1962) 6-10.
4. Browne, S. G. and Hogerzeil, L. M. "B.663" in the treatment of leprosy. Supplementary report of the pilot trial. Leprosy Rev. **33** (1962) 182-184.
5. Conalty, M. L. and Jackson, R. D. Uptake by reticulo-endothelial cells of the riminophenazine B.663 (2-*P*-chloroanilino-5-*P*-chlorophenyl-3: 3-dihydro-3-isopropyliminophenazine). British J. Exper. Path. **43** (1962) 650-654.
6. Lunn, H. F. and Rees, R. J. W. Treatment of mycobacterial skin ulcers in Uganda with a riminophenazine derivative (B.663). Lancet **1** (1964) 247-249.
7. Pettit, J. H. S., Marchette, N. J. and Rees, R. J. W. *Mycobacterium ulcerans* infection. Clinical and bacteriological study of the first cases recognized in South East Asia British J. Dermatol. **78** (1966) 187-197.
8. Pettit, J. H. S. and Rees, R. J. W. Studies on sulfone resistance in leprosy. 2. Treatment with a riminophenazine derivative (B.663). Internat. J. Leprosy **34** (1966) 391-397.
9. Rees, R. J. W. Recent bacteriologic, immunologic and pathologic studies on experimental human leprosy in the mouse foot pad. Internat. J. Leprosy **33** (1965) 646-655 (Part 2).
10. Rees, R. J. W. and Valentine, R. C. Application of quantitative electron microscopy to the study of *Mycobacterium lepraemurium* and *M. leprae*. *In* Leprosy in Theory and Practice, R. G. Cochrane and T. F. Davey, Eds. Bristol, John Wright & Sons Ltd., and Baltimore, Williams and Wilkins Co., 2nd ed., 1964, pp. 26-35.
11. Ridley, D. S. Therapeutic trials in leprosy using serial biopsies. Leprosy Rev. **29** (1958) 45-52.
12. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity. A five-group system. Internat. J. Leprosy **34** (1966) 255-273.
13. Shepard, C. C. and Chang, Y. T. Activity of antituberculosis drugs against *Mycobacterium leprae*. Studies with experimental infection of mouse foot pads. Internat. J. Leprosy **32** (1964) 260-271.
14. Shepard, C. C. and McRae, D. H. *Mycobacterium leprae* in mice; minimal infectious dose, relationship between staining quality and infectivity, and effect of cortisone. J. Bact. **89** (1965) 365-372.
15. Waters, M. F. R. Chemotherapeutic trials in leprosy. 1. Comparative trial of Macrocyclon plus dapsone and dapsone alone in the treatment of lepromatous leprosy. Leprosy Rev. **34** (1963) 173-192.

16. WATERS, M. F. R. and PETTIT, J. H. S. Chemotherapeutic trials in leprosy. 2. Comparative trial of dapsone plus ditophal (Etisul) and dapsone alone in the treatment of lepromatous leprosy. Internat. J. Leprosy **33** (1965) 280-296.
17. WILLIAMS, T. W., MOTT, P. D., WERTLAKE, P. T., BARBA RUBIO, J., ADLER, R. C., HILL, G. J., PEREZ SUAREZ, G. and KNIGHT, V. Leprosy research at the National Institutes of Health: Experience with B.663 in the treatment of leprosy. Internat. J. Leprosy **33** (1965) 767-775. (Part 2)