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Evaluation of B.663 in Human Leprosy[1, 2]

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In a previous report ([13]), observations were presented on three patients with leprosy treated with B.663, a rimino compound of the phenazine series. One patient with borderline leprosy, previously untreated, showed pronounced improvement during 18 months of B.663 treatment. A patient with lepromatous leprosy improved following treatment for a similar period with B.663 and dapsone. Also described was the initial favorable response to eight months of B.663 therapy of a third patient with previously untreated lepromatous leprosy.

This patient has now received B.663 for two years and has shown continued clinical and bacteriologic improvement despite recent gastrointestinal symptoms and weight loss probably representing drug intolerance. The present report will describe the completed study of this patient and review our experience with the use of B.663 in the treatment of leprosy.

CASE REPORT

J.B. (NIH No. 05-81-51). A 19 year old Mexican male was admitted to the National Institute of Allergy and Infectious Diseases on 22 September 1964 for treatment of lepromatous leprosy. For two and one-half years the patient had noted pale cutaneous patches with reddened borders and nodular skin thickening, most marked over his face and extremities. For one year he had had recurrent episodes of fever associated with the eruption of painful nodules over his extremities, face and ears. Subsequently he developed lower extremity ulcers and edema, which led to complete disability and hospitalization at the Dermatologic Institute in Guadalajara, Mexico, from which he was transferred without treatment to the National Institutes of Health.

On admission he was small and appeared younger than his age. There were early leonine facies and generalized, hyperpigmented cutaneous infiltration with ichthyosis, particularly marked over the shins. There was pedal edema, and over the right shin were two 2 x 3 cm. ulcers. Over the face, trunk, and extremities were discrete nodules, which were either erythematous and tender or hyperpigmented and nontender. Lateral eyebrow alopecia and nasal and palatal mucosal ulceration were present. There was moderate gynecomastia. The ulnar and posterior cervical nerves were enlarged and painful; and thenar and hypothenar wasting was accompanied by hypesthesia and anhydrosis in a stocking-glove distribution.

Admission laboratory findings included a hemoglobin of 10.2 gm./100 ml., white blood count of 8,400 cells/cmm. and an erythrocyte sedimentation rate of 43 mm./hr. (Westergren method). Urinalysis gave normal values and the creatinine clearance was 41 ml./min. The serum albumin was 3.3 gm./100 ml., globulin 4.1 gm./100 ml.,

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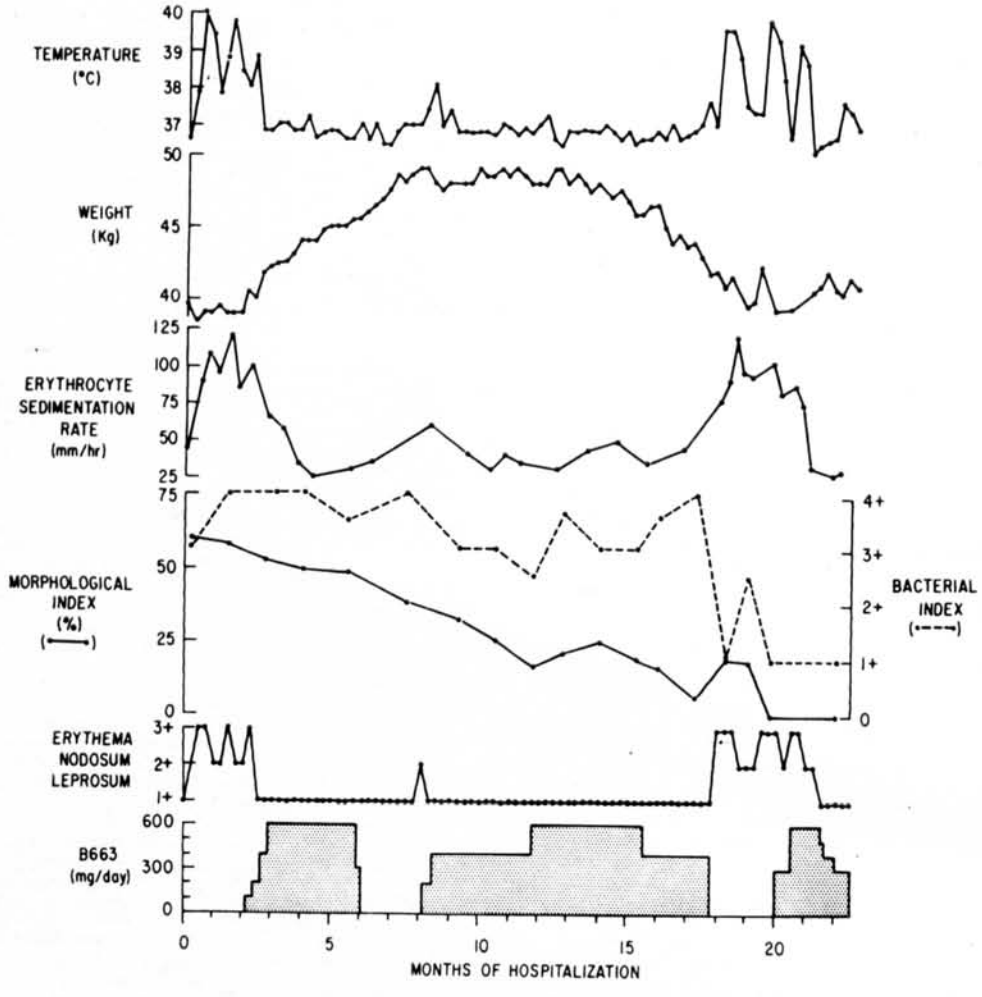

FIG. 1. Clinical course of J. B., patient with lepromatous leprosy described in the text: response to treatment with B.663 alone.

and cholesterol 167 mgm./100 ml. The serum iron was 20 μgm./100 ml., with a total iron binding capacity of 330 μgm./100 ml. Roentgenograms of the hands were normal, but there was slight squaring of the distal ends of the terminal phalanges of both great and second toes. The tibial bone beneath the right leg ulcers showed cortical lytic changes suggesting early osteomyelitis. Roentgenographic examination of the small intestine showed no abnormalities.

Skin scrapings, smears of blood plasma, bone marrow aspirate, and sputum, biopsies of skin, lymph nodes and liver, and conjunctival washings, all contained numerous bacilli and globi, which were acid-fast by the Fite staining technic. A bacterial index (BI) of 3 and a morphologic index (MI) of 59 per cent were calculated from skin scrapings (see METHODS AND SPECIAL TECHNICS below). Microscopic examination of a peroral jejunal biopsy specimen revealed plasmacytosis of the lamina propria and a normal villous pattern of the mucosa; leprosy bacilli were not seen. Rectal and gingival biopsy specimens did not contain amyloid material. Lepromin (Mitsuda) and second strength purified protein derivative tuberculin tests were negative.

During a two month period of observation without specific treatment for leprosy,

the leg ulcers completely healed, but the patient failed to gain weight. Repeat determinations of the BI and MI were unchanged (Fig. 1). On three occasions there were grade 3+ (see METHODS AND SPECIAL TECHNICS) exacerbations of erythema nodosum leprosum (ENL), and with these attacks the white blood count and erythrocyte sedimentation rate rose as high as 18,200 cells/cmm. and 120 mm./hr.

With the onset of the third attack of ENL, B.663 was begun at a dose of 100 mgm. daily and was increased over the following three weeks to 600 mgm. daily given in divided doses. The ENL subsided promptly, and over the next three and one-half months the patient became fully ambulatory and gained 10 kgm. in weight. The skin developed a violaceous hue, but became less thickened; in addition, only an occasional fresh lesion of ENL developed. Although the ulnar and posterior cervical nerves remained enlarged, they were not tender; manual muscle strength and stocking-glove hypesthesia improved. Therapy was discontinued after three and one-half months and within two weeks there was an increase in the cutaneous manifestations of the patient's ENL, although the violaceous color of his skin persisted and the MI continued to fall. Fever reappeared after two months; therapy was resumed, and again the ENL improved promptly.

Two months later the patient began to complain of anorexia and periodic epigastric distress with occasional vomiting unrelated to meals. The following month he began to lose weight and the episodes of abdominal pain became more severe. This pain was prostrating and was accompanied by generalized abdominal and rebound tenderness without localization, but not by fever or leukocytosis. Despite bacteriologic improvement during the next seven months of therapy, the patient lost 7 kgm. in weight, and stools, which had been examined weekly, were found to contain occult blood. The serum albumin was 2.6 gm./100 ml., globulin 4.1 gm./100 ml., and the cholesterol had fallen to 120 mgm./100 ml. The serum carotene was 52 μgm./100 ml. (borderline to low normal) and D-xylose excretion was 5.6 gm. in 5 hours (normal). A stool specimen contained microscopic fat and undigested muscle fibers. Roentgenographic examination revealed coarsening of the mucosal pattern and segmentation of barium in the ileum and distal jejunum. A specimen of jejunum was obtained by biopsy and again showed a normal mucosal villous pattern and moderate numbers of plasma cells in the lamina propria. In addition, red crystals in the lamina propria (Fig. 2) were seen, resembling those described by Wertlake in other tissues (Fig. 3) and identified by ultraviolet spectroscopy as B.663 ([12]).

After high doses of B.663 had been administered for 14 months the medication was stopped because of the suspected gastrointestinal toxicity. There was a marked improvement in the patient's appetite and caloric intake, but four days later a grade 3+ exacerbation of ENL was accompanied by further weight loss. This episode subsided within three weeks, but was followed shortly by a second severe attack during which B.663 therapy was resumed. A daily dose of 300 mgm. did not prevent the occurrence of a third attack, and increasing the dose to 600 mgm. led to recurrence of the gastrointestinal symptoms. Mild abdominal symptoms persisted despite again lowering of the dose of B.663 to 300 mgm. daily. The patient's appetite improved, however; his weight remained stable, and he had no further exacerbations of ENL for the last six weeks of hospitalization.

At the time of discharge only scattered, nonnecrotic cutaneous nodules were present over the face and extremities. Induration of the skin was improved over that present on admission. Moderate nontender ulnar nerve enlargement remained, but hypesthesia was confined to the areas of cutaneous induration and was not present in a neuropathic distribution. There was no muscle weakness. The hemoglobin was 11.3 gm./100 ml., and serum iron and total iron binding capacity were 58 and 296 μgm./100 ml., respectively. The creatinine clearance was 50 ml./min. The serum albumin was 3.4 gm./100 ml., globulin 3.6 gm./100 ml., and the cholesterol had risen to 168 mgm./100 ml. Chemical tests of liver function gave normal results. Fewer acid-fast bacilli were present in skin scrapings and globi were rarely seen. The BI

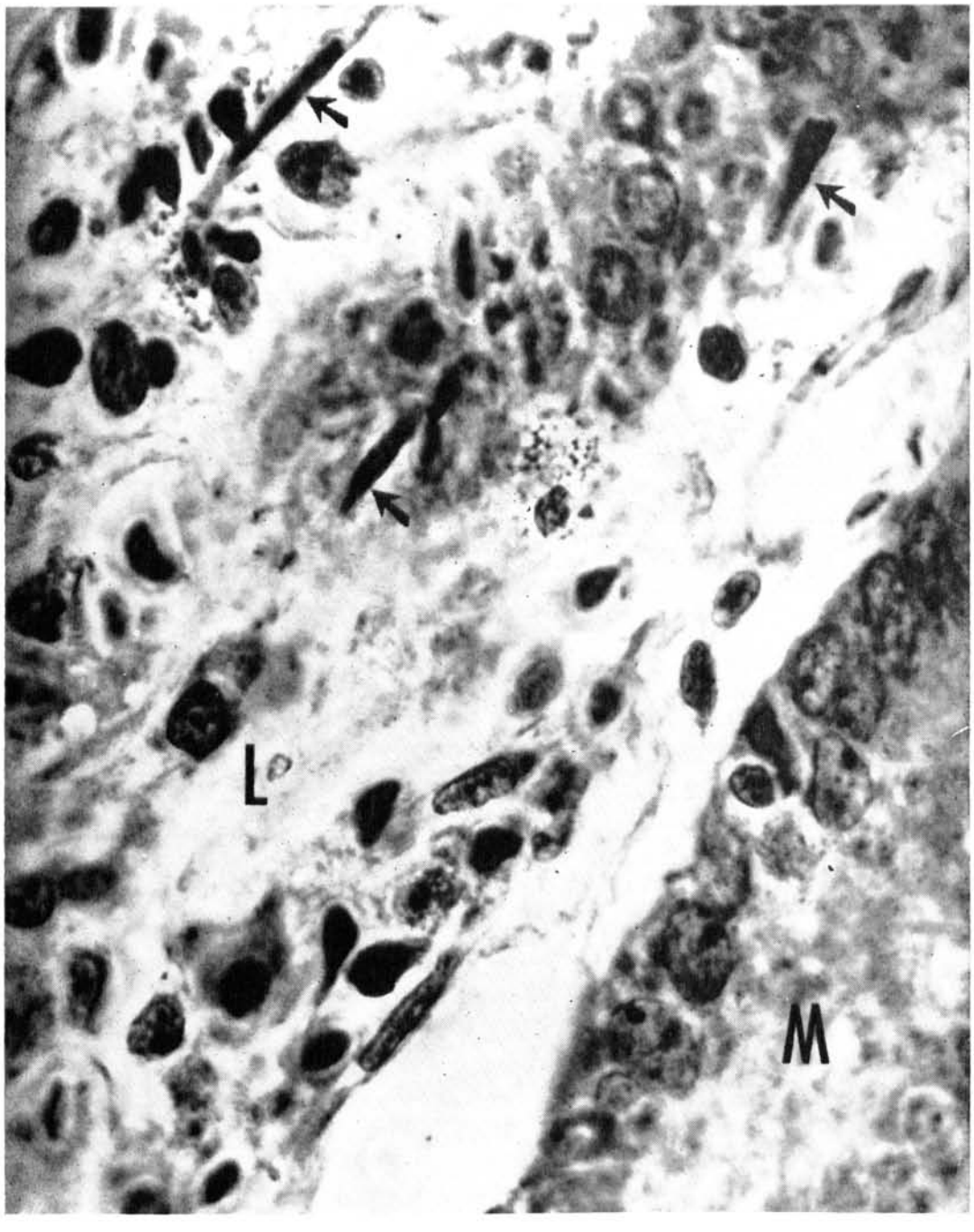

FIG. 2. Patient J. B. Phase contrast photomicrograph of a jejunal biopsy specimen showing the lamina propria (**L**) containing B.663 crystals (arrows). The mucosa (**M**) is to the right. (B-5 fixative, hematoxylin and eosin stain, original magnification X2,000.)

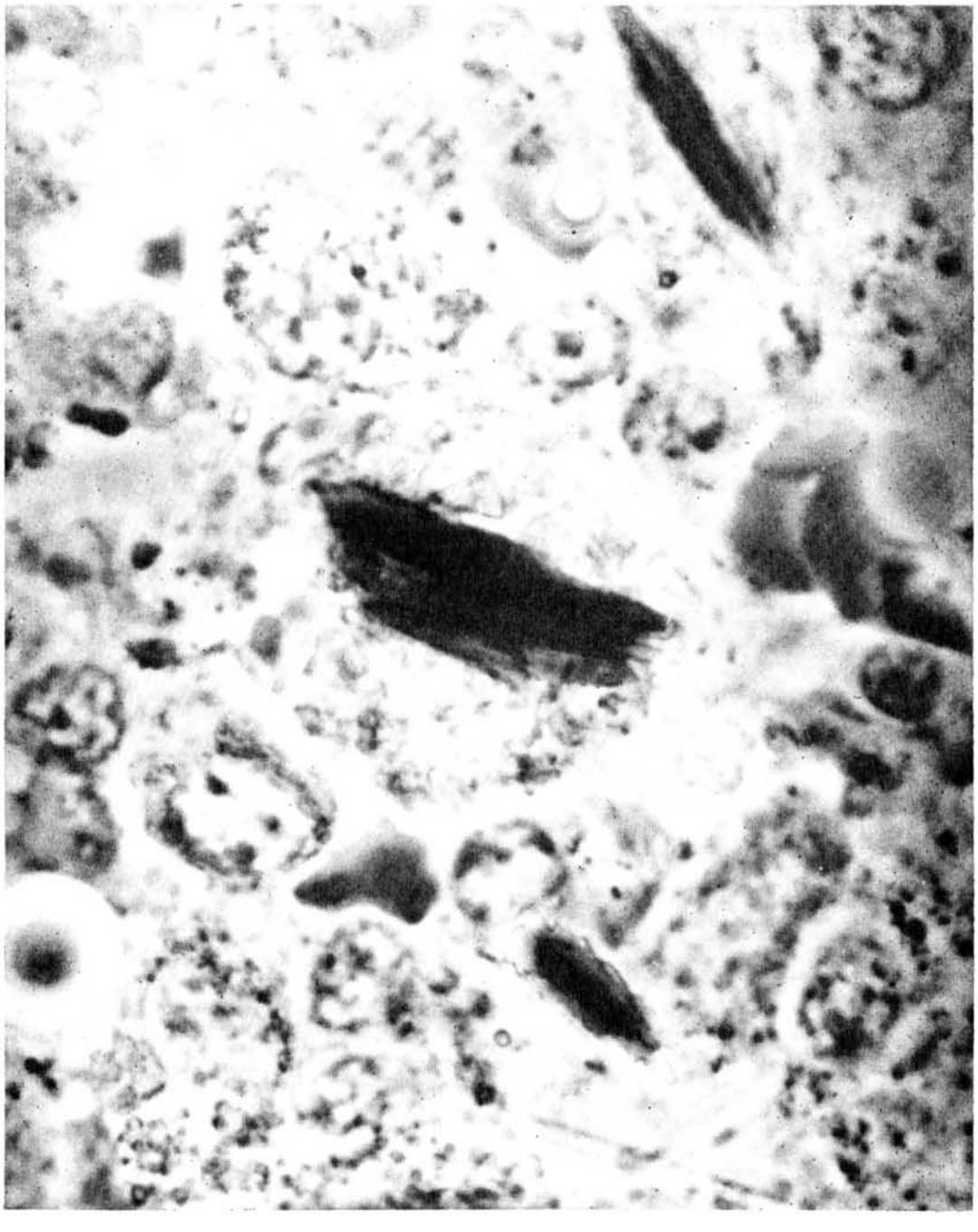

FIG. 3. Patient M. H. Phase contrast photomicrograph of an aspirate of bone marrow demonstrating B.663 crystals. (original magnification X4,000, photomicrograph courtesy of Dr. Paul T. Wertlake).

was 1+ and the MI zero for three months prior to discharge. The lepromin test remained negative.

METHODS AND SPECIAL TECHNICS

The severity of ENL was graded as outlined by Jopling (7), 1+ corresponding to mild ENL without systemic symptoms, with only a few erythematous nodules present at any given time, 2+ or moderately severe reactions consisting of mild constitutional symptoms with more numerous, but nonnecrotic nodular lesions, and 3+ being reserved for prostrating reactions associated with numerous, occasionally necrotic lesions.

Smears were made routinely from both legs, arms, and ear lobes, and from the right and left surfaces of the nasal septum, and stained by Fite's method. Smears from the legs consistently provided the greatest number of bacilli for microscopic examination and the final BI and MI values were obtained by averaging the results from these two sites. The BI for each site was determined as outlined by Ridley (10), each unit representing roughly a ten-fold difference in bacillary concentration. The MI consists of the percentage of solid-staining forms present (3). One hundred to 200 bacilli were examined from each site, except near the end of this patient's hospitalization, when the fall in BI necessitated using smaller numbers. The standard error of the MI, determined by comparing the results from the right and left legs, averaged ± 3 per cent.

DISCUSSION

Evaluation of the chemotherapeutic efficacy of B.663. Evaluation of chemotherapy in leprosy is hindered by the fluctuating course of the untreated disease, by the tendency of the disease to improve symptomatically with the institution of nonspecific measures, and by the length of time required for detectable improvement even with effective antimicrobial therapy. Although clinical evaluation may be complicated by symptomatic deterioration due to episodes of ENL, the relentless progression of untreated lepromatous leprosy makes this form of the disease the most suitable for therapeutic studies.

Because of the lack of cultural methods, the leprologist has had to rely chiefly on the appearance and number of leprosy bacilli in accessible tissues to bolster his clinical impressions. In the experience of several investigators (8) effective antimicrobial therapy is accompanied by the appearance of increasing proportions of irregularly stained bacilli. Working with *Mycobacterium lepraemurium*, Rees *et al* (9) found that irregularly stained bacilli were dead, whereas the solid staining forms were viable. Thus, the rate of fall of the MI, measured by the per cent of solid-staining bacilli, provides one useful measure of response to therapy. Another index is the BI, which presumably represents the total load of both viable and nonviable bacilli with which the patient must contend. Even with effective therapy the BI declines much more slowly than the MI. There is evidence that bacterial clearance, measured by the fall in BI, proceeds more rapidly in patients with borderline leprosy than in those with pure lepromatous disease (11), and may depend on host factors unrelated to drug potency (5).

Waters and Rees (11) used these indices to evaluate the effectiveness of drug therapy in patients with lepromatous and borderline leprosy, in their study of the effect of dapsone, either alone or in combination with another agent. The MI fell from an average of 54 per cent to 4 per cent after 6 to 9 months of treatment, but during the same period the BI fell only slightly. Browne (3) evaluated B.663 in doses of 100 to 300 mgm. daily, and with similar patients obtained results comparable to those of Waters and Rees.

Our limited experience utilizing these indices also indicates that B.663 is therapeutically effective, although the response of the present patient, even with high doses, was not as rapid as that described by Browne. Two other patients have been treated at this Institute and previously reported (13), one (M.H.) with borderline leprosy treated with B.663 alone, and

another (E.F.) with diffuse lepromatous leprosy treated with B.663 in combination with dapsone. In both, the BI became negative after 20 months of therapy. These two patients have returned to Mexico and are outpatients at the Dermatologic Institute in Guadalajara. Both are being maintained on dapsone therapy. M.H. was examined eight months after cessation of B.663 therapy and his leprosy had remained well controlled. He still exhibited considerable pigmentation of his skin lesions, which had heavily concentrated the blue color of the B.663. Tuberculosis had become apparent during his course of B.663 therapy, but this complication was responding satisfactorily to isoniazid and para-aminosalicylic acid. E. F. was examined four months after B.663 therapy was stopped and his disease was continuing under good control. ENL reactions had occurred whenever B.663 had been discontinued previously, but did not accompany final withdrawal of the drug, three years after treatment of his disease had been initiated.

B.663 suppression of ENL. The clinical picture of ENL has been well described (1). Whereas the acute lepra reaction indicates deterioration of the disease with bacterial proliferation and exacerbation of the underlying lepromatous process, ENL commonly afflicts the patient whose bacteriologic disease is responding satisfactorily to therapy. ENL reactions are thought to represent hypersensitivity to bacterial products, but their etiology remains poorly understood. Antimony compounds and corticosteroids have been used with some success to ameliorate these reactions, but the overall results with such drugs have not been entirely satisfactory.

Browne (2) observed that patients receiving B.663 developed appreciably less ENL than a control group receiving dapsone. Previous experience at this Institute (13) is in agreement with that observation, and has suggested that the apparent success in suppression of ENL by B.663 may be dose-related, as much as 600 mgm. daily being required to control the more stubborn attacks. Browne (6) has extended these observations to an additional group of 10 patients with lepromatous leprosy, all suffering from persistent exacerbations of ENL that could not be controlled by corticosteroids. He was able to suppress ENL with B.663 in a dose of 100 mgm. daily in all but four patients, and these patients were successfully controlled when that dose was administered twice daily. Corticosteroids could be withdrawn without exacerbation in all patients while they were receiving B.663.

The patient described in the present report was treated with B.663 for prolonged periods, during which an adequate bacteriologic response occurred, as indicated by the fall in the MI, without a concomitant attack of ENL. On two occasions initiation of B.663 therapy was associated with prompt improvement in ENL, which had worsened when the patient was off therapy. On a third occasion the response was less dramatic and B.663 in a daily dose of 300 mgm. was apparently ineffective in suppressing an exacerbation. However, once the dose was raised to 600 mgm. daily, further reactions were suppressed and the patient remained free from exacerbations of ENL for the six weeks prior to discharge. The final series of attacks of ENL was accompanied by a rapid and unexplained fall in the BI, which had not changed significantly for the previous 18 months, suggesting a possible relationship between ENL and mechanisms of bacterial clearance.

The spontaneously remitting character of ENL makes it hazardous to draw conclusions from our limited experience, and further clinical studies are needed to document the anti-inflammatory properties of B.663, as well as to elucidate the pharmacologic mechanism of this action. However, it does not seem premature at this point to recommend further study of the addition of B.663 to dapsone therapy in those patients in whom ENL reactions have been troublesome.

Toxicity of B.663. Toxicity of B.663 has been limited to skin pigmentation and gastrointestinal symptoms. The cutaneous changes have been described by Browne (4). The skin initially becomes ruddy, and then progressively darker and violaceous as treatment is continued. The pigmentation is most marked in the infiltrated areas and does not diminish appreciably until the second month after therapy is discontinued.

On occasion this pigmentation has limited patient acceptance of the drug ([2]), but this did not prove to be a serious problem with the patients studied at this Institute.

Browne has not reported gastrointestinal toxicity in his experience with B.663, although he used daily doses of 300 mgm. or less. Our earlier patients manifested symptoms of mild gastrointestinal pain and anorexia with B.663 administered in this dose for several months, but no abnormalities were detected on roentgenographic examination ([13]) or on biopsy ([12]) of the gastrointestinal tract. The present patient received 600 mgm. of B.663 daily for eight months, and during his entire course of therapy seldom received a dose less than 300 mgm. daily. His symptoms of abdominal pain, with occasional vomiting and severe anorexia with weight loss, were more marked than those of the patients previously described. In addition, there was definite roentgenographic evidence of lower small bowel irritation, and peroral jejunal biopsy demonstrated B.663 crystals in the lamina propria of the intestinal wall. Unequivocal malabsorption was not demonstrated, and weight loss was consonant with the documented reduction in caloric intake. The symptoms of anorexia and abdominal pain subsided promptly when the medication was discontinued, although the patient preferred his gastrointestinal symptoms to the severe attacks of ENL, which he felt were prevented by B.663.

Extensive studies in all of our patients have not demonstrated renal, hepatic or hematologic toxicity following high doses of B.663 administered for periods of almost two years.

SUMMARY

Evidence has been presented which suggests that B.663 is effective in the treatment of leprosy. Follow-up studies of two of the patients described previously have revealed that improvement noted after treatment with B.663 alone or in conjunction with dapsone has been sustained. A third patient with lepromatous leprosy has improved clinically and bacteriologically while receiving B.663 alone for two years. It is suggested that this drug has been of additional benefit in diminishing the severity of associated ENL reactions.

Violaceous pigmentation of the skin occurred in all patients, but did not interfere with treatment. Abdominal pain, anorexia and weight loss, apparently related to irritation of the gastrointestinal tract by B.663, limited the prolonged use of the drug in high doses, but appeared to be fully reversible. No other manifestations of drug toxicity have been demonstrated.

RESUMEN

Evidencia ha sido presentada que sugiere que el B.663 es efectivo en el tratamiento de la lepra. Estudios continuados (follow-up) de dos de los enfermos descritos anteriormente han revelado que la mejoría observada después del tratamiento con B.663 solo o combinado con dapsona ha sido mantenida. Un tercer enfermo con lepra lepromatosa ha mejorado clínica y bacteriologicamente mientras recibía B.663 solo por dos años. Se sugiere que esta droga ha sido de beneficio adicional en disminuir la severidad de las reaciones ENL asociadas.

Pigmentación violacea de la piel se presentó en todos los enfermos, pero no interfirió con el tratamiento. Dolor abdominal, anorexia y pérdida de peso, aparentemente debida a la irritación del tracto gastrointestinal por B.663, limitó el uso prolongado de la droga en altas dosis, pero estos efectos eran reversibles. No hubo otras manifestaciones de toxicidad de la droga.

RÉSUMÉ

On a ici présenté des données qui suggèrent que le B.663 est actif dans le traitement de la lèpre. Les observations poursuivies chez deux des malades décrits précédemment ont révélé que l'amélioration notée apres le traitement par le B.663 seul ou administré avec de la dapsone a été maintenue. Un troisième malade atteint de lèpre lépromateuse a témoigné d'une amélioration clinique et bactériologique à la suite de l'administration de B.663 seul pendant deux ans. On suggère que ce médicament a présenté un avantage supplémentaire en diminuant la gravité des rèactions d'ENL associées.

Une pigmentation violacée de la peau est survenue chez tous les malades, mais n'a pas interféré avec le traitement. Des douleurs abdominales, de l'anorexie et une perte de poids, qui paraissent reliées à l'irritation du tractus gastro-intestinal par le B.663, n'ont pas permis que le médicament soit utilisé à hautes doses pendant une période prolongée. Cette action, cependant, est apparue entièrement réversible. Aucune autre manifestation de toxicité médicamenteuse n'a été observé.

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REFERENCES

1. Barrera, F. De P. and Chavarría, A. P. The acute exanthem of leprosy. Bull. Johns Hopkins Hosp. **35** (1928) 147-158.
2. Browne, S. G. B.663: possible anti-inflammatory action in lepromatous leprosy. Leprosy Rev. **36** (1965) 9-11.
3. Browne, S. G. Treatment of leprosy with B.663: appraisal of the pilot trial after three years. Leprosy Rev. **36** (1965) 13-15.
4. Browne, S. G. Red and black pigmentation developing during treatment of leprosy with B.663. Leprosy Rev. **36** (1965) 17-20.
5. Browne, S. G. Bacterial clearance in patients with lepromatous leprosy not receiving treatment. Leprosy Rev. **36** (1965) 161-162.
6. Browne, S. G. B.663 (Geigy). Further observations on its suspected anti-inflamatory action. Leprosy Rev. **37** (1966) 141-145.
7. Jopling, W. H. Treatment of acute phases (reactional states) in lepromatous leprosy. *In* Leprosy in Theory and Practice. Cochrane, R. G. and Davey, T. F. Eds. Bristol, John Wright & Sons, Ltd. and Baltimore, Williams & Wilkins Co., 2nd ed., 1964, pp. 418-424.
8. Rees, R. J. W., and Valentine, R. C. The appearance of dead leprosy bacilli by light and electron microscopy. Internat. J. Leprosy **30** (1963) 1-9.
9. Rees, R. J. W., Valentine, R. C., and Wong, P. C. Application of quantitative electron microscopy to the study of *Mycobacterium lepraemurium* and *M. leprae*. J. Gen. Microbiol. **22** (1960) 443-457.
10. Ridley, D. S. Bacterial indices, Appendix III. *In* Leprosy in Theory and Practice. Cochrane, R. G. and Davey, T. F., Eds. Bristol, John Wright & Sons, Ltd. and Baltimore, Williams & Wilkins Co., 2nd ed, 1964, pp. 620-622.
11. Waters, M. F. R., and Rees, R. J. W. Changes in the morphology of *Mycobacterium leprae* in patients under treatment. Internat. J. Leprosy **30** (1962) 266-277.
12. Wertlake, P. T. Pathologic and chemical studies of B.663 in the therapy of leprosy. *In preparation.*
13. Williams, T. W., Jr., Mott, P. D., Wertlake, P. T., Barba Rubio, J., Adler, R. C., Hill, G. J. II, Pérez Súarez, 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)