INTERNATIONAL JOURNAL OF LEPROSY

Volume 33, Number 3
*Printed in U.S.A.*

# Leprosy Research at the National Institutes of Health: Experience with B.663 in the Treatment of Leprosy

**Temple W. Williams, Jr., M.D., Peter D. Mott, M.D., Paul T. Wertlake, M.D., Jose Barba Rubio, M.D., Richard C. Adler, M.D., George J. Hill II, M.D., Gloria Perez Suarez, M.D. and Vernon Knight, M.D.**[1]

## INTRODUCTION

In 1961 the decision was made to undertake a project in leprosy research, and it seemed appropriate to invite participation from programs where medical supervision before and after periods of study at the National Institutes of Health would be possible. On this basis a visit was made in November 1961 to Centro Pascua in Mexico City, headed by Dr. Fernando Latapí, and to the Dermatologic Institute in Guadalajara, headed by Dr. José Barba Rubio. It was agreed that carefully selected patients would be brought to the Clinical Center, NIH, for studies to be designed by the participating investigators. The program was actually initiated May 6, 1962, with the admission of three young men with unreated leprosy. Two came from Mexico City and one from Guadalajara. A report of this first work was made by Dr. Amado Saúl of Mexico City ([15]).

Subsequently, these three cases were replaced by three others from the Dermatologic Institute in Guadalajara, and these are the basis of the report that follows.

Throughout this program the principle has been followed that therapeutic and research procedures will be planned together by the collaborating investigators, and despite the problem of distance a successful and amicable relationship has been established.

The work in Guadalajara has been assisted by Dr. Gloria Pérez Suarez, assistant to Dr. Barba Rubio, and in Mexico City by Dr. Amado Saúl, previously mentioned. Perhaps less officially, the program has also been beneficially supported by Senora de Latapí. We appreciate her kindly and helpful influence. For a time, also, clinical consultation was provided by Dr. Michel F. Lechat, who is now with the Pan American Health Organization in Mexico City.

The administration of the Clinical Center made a difficult decision when they decided to permit this adventure in medical care. It was recognized that the program might not meet public approval. Nursing and other staff personnel were carefully indoctrinated concerning isolation and other plans for patient care. Lectures were given by the late Dr. James A. Doull, which were both informative and reassuring. With this preparation there was relatively easy acceptance of this potentially explosive situation. Now, after three years, we feel some sense of security about the program. The Clinical Center administration has been represented in all of this work by its Associate Director, Dr. Clifton L. Himmelsbach.

The case reports to follow describe our studies with a new antileprosy drug, B.663. The use of this drug was suggested to us by Dr. Y. T. Chang([10]), who found it to be the most effective drug in mouse leprosy that he has tested to date. B.663 was sup-

[1]The authors are respectively: Clinical Associate, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.; Clinical Associate, NIAID, NIH; Clinical Pathology Dept., Clinical Center, NIH; Director, Dermatologic Institute, Guadaljara, Mexico; Clinical Associate, NIAID, NIH; Clinical Associate, NIAID, NIH; Staff, Dermatologic Institute, Guadalajara, Mexico; and Clinical Director, NIAID, NIH, Bethesda, Md.

plied through the courtesy of J. R. Geigy, S. A. (Basel).

## CASE REPORTS

**1. (J. B.)** This 19-year-old male was admitted to the Clinical Center, NIH, 22 September 1964 with a diagnosis of nodular lepromatous leprosy. The boy's father has lepromatous leprosy. Two and one-half years prior to admission the patient first noted the onset of scattered hypochromic macules with an erythematous halo at the periphery. These lesions progressed to become diffuse erythematous patches and nodular infiltrates, most marked over the face and extremities. During the eight months prior to admission he developed ulcers of feet and legs following minimal trauma and subsequently stopped working because of edema of feet and legs as well as the repeated occurrence of transient febrile episodes accompanied by confluent small, painful, erythematous nodules over arms and legs, marked malaise, anorexia, and weight loss.

Physical examination on admission revealed an emaciated, febrile, acutely and chronically ill male who appeared much younger than his stated age. Skin lesions were generalized, involving face and neck, trunk, and extremities, and excluding only the axillae, antecubital and popliteal fossae, and groin.

Macules were abundant, confluent, with vague borders, and symmetrically distributed over the body surface. The skin was diffusely infiltrated, with nodule formation most marked on ears and face. There was confluent erythema nodosum, with coalescent, elevated, firm, painful, erythematous to violaceous nodules over the arms and thighs. The nasal mucosa was ulcerated. There was partial alopecia of the eyebrows. Multiple nerves were enlarged and painful and there was symmetric hypesthesia, most marked to heat and light touch, over the lower extremities, and anhydrotic areas of both lower legs and feet as well as both forearms and hands. In addition, there were two 2 x 4 cm. punched-out ulcers of the anterior and medial mid-tibial area of the right leg with 1+ pitting edema of both feet and legs.

Laboratory data on admission revealed leprosy bacilli in abundance in skin scrapings as well as in punch biopsies of skin, axillary lymph node, bone marrow, and conjunctiva. The hemoglobin concentration was 10.2 gm./100 ml., the erythrocyte sedimentation rate (Westergren) 100 mm., and the cephalin-cholesterol flocculation 4+, with hypoalbuminemia and hypergammaglobulinemia. Skin tests, including lepromin (Mitsuda reaction) and second strength PPD, were negative. The patient's hospital course is summarized in Figure 1.

During the first two months of hospitalization, the patient had three episodes of transient erythema nodosum leprosum (ENL) with malaise, fever, increased erythrocyte sedimentation rate and confluent erythematous nodules over arms and thighs. Iritis, acute neuritis, and orchitis did not occur, but regional lymphadenitis was present during each episode.

B.663 was started during the third episode of erythema nodosum and seemed to abort this attack. The B.663 dosage was increased at weekly intervals to a maximum of 600 mgm. per day, and this maximum dose was continued for three months. During this period of treatment there was no recurrence of erythema nodosum. There was marked weight gain (9 kgm.), reduction in erythrocyte sedimentation rate, reduction in gamma and beta globulins, as well as reduction in the number of leprosy bacilli in serial skin scrapings and punch biopsies, and, by the third month of treatment, a marked tendency toward fragmentation of the remaining bacilli. During this period the patient developed a ruddy color with a violaceous hue over infiltrated lesions and nodules. The leg ulcers healed rapidly.

B.663 was stopped after four months in order to complete the reticuloendothelial clearance studies (part of the protocol for study of the human pharmacology of the drug). Within two weeks after stopping B.663, painful nodules appeared on the upper arms and thighs and these progressed over a six weeks period to become confluent and quite painful, associated with malaise, anorexia, and swings in the daily temperature to below normal, eleva-

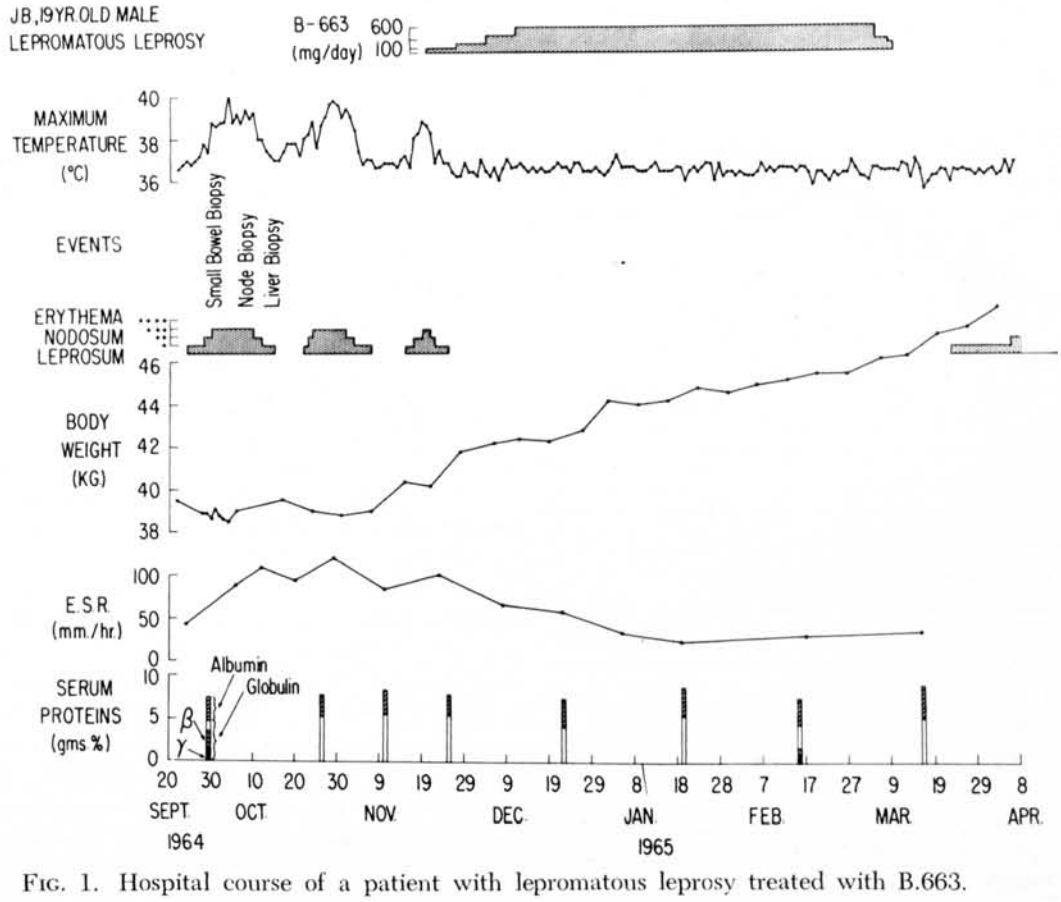


FIG. 1. Hospital course of a patient with lepromatous leprosy treated with B.663.

tion of erythrocyte sedimentation rate to 60 mm., and increase in gamma globulin to 2.7 gm./100 ml. B.663 was re-started 6 May 1965, with prompt regression of the erythema nodosum and symptoms.

**2. (E. F.)** This 23-year-old male was admitted to the Clinical Center 17 December 1962 with a diagnosis of diffuse lepromatous leprosy.

There is no family history of leprosy and no known contact with persons known to have leprosy. Four years prior to admission he first noted the onset of recurrent erythematous nodules over the lower extremities. He was otherwise well and did not consult a physician for three years. During this period the nodules spread to involve arms and thighs as well as the lower legs, and the episodes became more frequent and more severe, lasting up to ten days, and accompanied by fever, malaise, and anorexia. One year prior to admission he noted an erythematous rash over his upper chest and was first seen by a physician. The diagnosis of leprosy was made and he was referred to the Dermatologic Institute in Guadalajara, Mexico, and subsequently to NIH. He received no treatment prior to admission here.

Physical examination on admission revealed a thin but well-developed young male. Skin lesions consisted of extensive, vague hypochromic macules of face, trunk, and extremities. The skin was diffusely infiltrated with ichthyotic changes over the lower legs and violaceous scars over the anterior thighs and extensor surfaces of the upper arms and the volar surfaces of the forearms, with scattered painful erythematous nodules in the latter three areas. The nasal mucosa was ulcerated and edematous. There was alopecia of the lateral eyebrows and visible enlargement of the greater auricular nerves. Rather marked

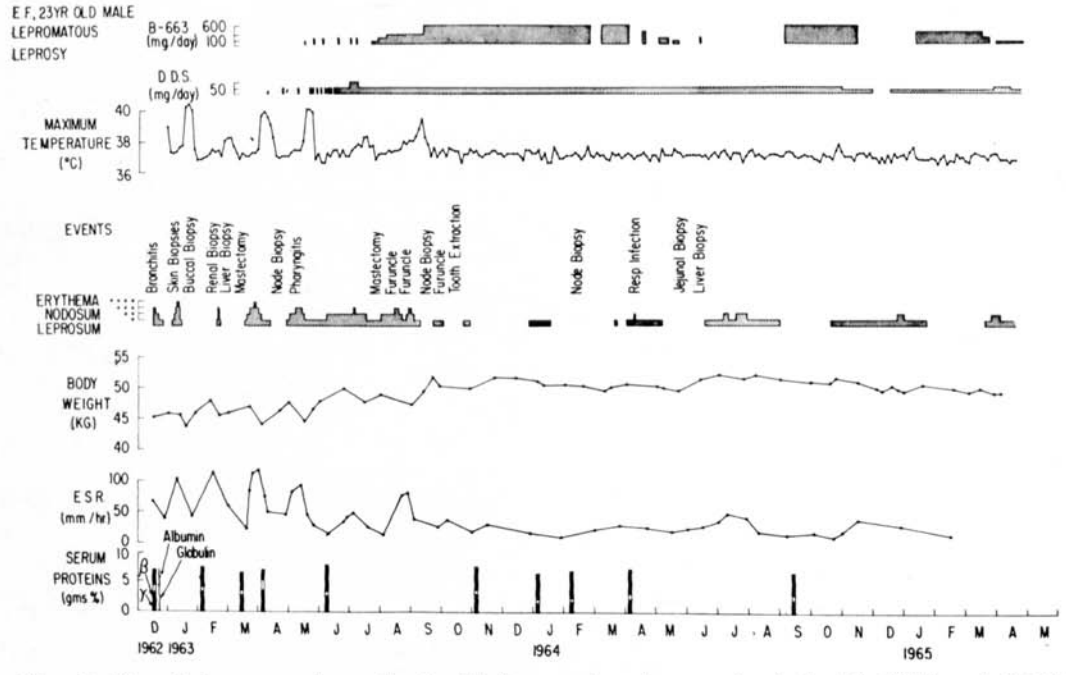


FIG. 2. Hospital course of a patient with lepromatous leprosy treated with DDS and B.663.

gynecomastia was present bilaterally. The ulnar nerves were enlarged and tender bilaterally, and diminished sensation, most marked to heat and light touch, was present in a "stocking-and-glove" distribution over the four extremities.

Laboratory data on admission included the finding of leprosy bacilli in skin scrapings and skin biopsies from multiple sites, lymph node, breast tissue, liver, testis, and bone marrow. The cephalin-cholesterol flocculation was 3+, and there was hypoalbuminemia with hypergammaglobulinemia. First strength PPD skin test was positive. Lepromin skin test (Mitsuda reaction) was negative. The patient's hospital course is summarized in Figure 2.

During the period of initial evaluation he had several bouts of febrile erythema nodosum leprosum accompanied by malaise, increased erythrocyte sedimentation rate, and confluent painful nodules over the anterior thighs and arms. In spite of these reactions, sulfone (DDS) treatment was initiated in March 1963. The reactions became more severe and more persistent with increase in the dosage of DDS to 50 mgm. per day. The DDS dosage was therefore maintained at 25 mgm. per day, and B.663 was started in July 1963. The dosage of B.663 was increased over a two-month period to a maximum of 600 mgm. per day. Once this dosage was reached, and for the subsequent two years of observation, febrile episodes of erythema nodosum leprosum have not occurred. Afebrile episodes, with relatively few systemic symptoms, occurred as noted in Figure 2. These episodes of erythema nodosum, which occurred while the patient was not taking B.663, promptly responded to restart of the drug. Sulfones were given almost constantly during this two-year period and the B.663 treatment was interrupted on numerous occasions for various studies as well as because of gastrointestinal intolerance for B.663 at the higher doses.

The patient has done well during this treatment period, with progressive weight gain during the first year and a relatively stable weight thereafter. The erythrocyte sedimentation rate, which had been elevated during reactions, returned to normal, as did the gamma globulin levels. Bacillus counts from smears made at monthly intervals from eight sites diminished rapidly

during the first six months of sulfone therapy, during the latter three months of which he received B.663 in addition. The smears have been negative since October 1964.

During the period of B.663 treatment he developed a ruddy hue, but never became as highly pigmented as the other two patients.

Toxicity in this patient has been limited to gastrointestinal intolerance in the form of abdominal cramps and diarrhea. Because of the severity of these symptoms, however, B.663 was stopped on several occasions. Following subsidence of the symptoms, B.663 could be restarted at lower doses and given for varying periods of time before symptoms recurred. No systemic symptoms accompany the gastrointestinal complaints and there have been no changes in liver function nor morphology to date. Likewise, no lesion has been demonstrated in x-ray studies of the gastrointestinal tract.

**3. (M. H.)** This 26-year-old male was admitted to the Clinical Center 27 February 1963 with a diagnosis of borderline or dimorphous leprosy. The patient's father died of some form of renal disease and did not have leprosy so far as is known. A younger sister developed leprosy four years after the onset of the patient's symptomatology.

The patient was well until six years prior to admission (age 19) when he first noted dryness and loss of sensation over the exposed areas of the legs and feet. This sensory loss progressed over a four-year period to involve all four extremities in a "stocking-and-glove" distribution. The loss of sensation led to recurrent traumatization of the extremities, with foreshortening of the digits and chronic ulceration of the feet and hands. During the two years prior to admission the macular lesions, which first appeared on buttocks and thighs, spread to involve the entire surface of the trunk, face, and extremities in a generalized roughly symmetric fashion.

Physical examination on admission revealed marked trophic changes of the hands and feet, with resorption of the terminal phalanges of virtually every digit and chronic ulceration of the feet. Skin lesions were extensive, irregular, and elevated centrally, having a bloated aspect and forming a "contour map" configuration. Neuropathy was extensive, with varying degrees of sensory loss of the entirety of all four extremities, with enlargement of ulnar, radial, popliteal, and tibial nerve trunks.

Laboratory findings on admission included an elevated erythrocyte sedimentation rate, cephalin-cholesterol flocculation 4+, hypoalbuminemia and hypergammaglobulinemia. First strength PPD skin test was positive but the lepromin reaction (Mitsuda) was negative. Leprosy bacilli in abundance were found in skin scrapings from multiple sites, as well as in skin biopsies, bone marrow, lymph node, testes, kidney, and liver. This patient's hospital course is summarized in Figure 3.

B.663 treatment was started in July 1963 and the dosage increased over a two-month period to a maximum dose of 600 mgm. per day in late August. The drug was given almost continuously for a total of 11 months, and intermittently thereafter. During the first year of treatment with B.663 the patient improved in general, with a ten kilogram weight gain. Monthly skin scrapings, as well as intermittent skin biopsies, showed progressive increase in the percentage of fragmented bacilli as well as a reduction in the number of bacilli present in some areas. The most striking change was the selective pigmentation of the infiltrated lesions by the rimino dye. These lesions rapidly progressed from their original tan color to a deep ruddy hue and finally a deep blue. The surrounding skin evolved through an orange to red color, but did not pigment as deeply as the infiltrated lesions. A comparison of the appearance of the skin lesions before and after a year's treatment with B.663 is shown in Figures 4a and 4b.

B.663 was discontinued after one year of treatment in order to carry out studies on the delay in excretion of the compound. After about one month without treatment the patient developed vague gastrointestinal symptoms for which no reason could be found on extensive evaluation. Over the

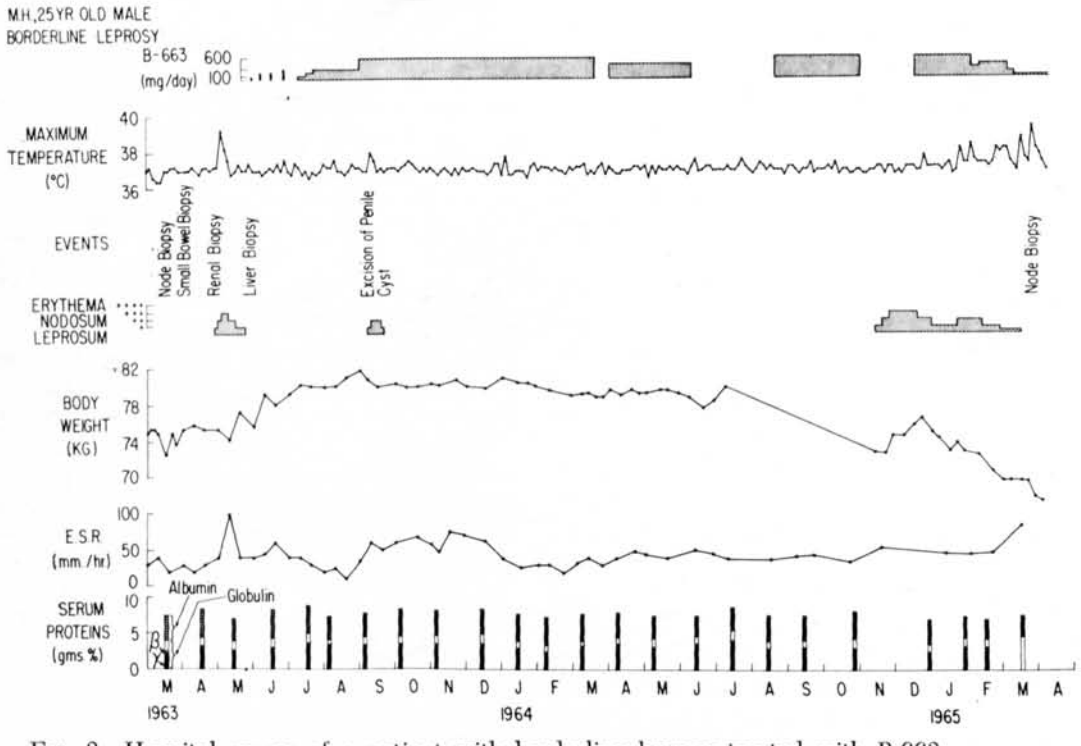


FIG. 3. Hospital course of a patient with borderline leprosy treated with B.663.

next four months the patient developed malaise, anorexia, and weight loss, and this clinical deterioration continued even after B.663 was restarted and continued for three months. There was no evidence of drug toxicity by any of the parameters studied, but serial skin scrapings failed to show an increase in normal-appearing bacilli; so the disease state was attributed to drug toxicity and B.663 was discontinued in November 1964. Within two weeks of stopping B.663 the patient developed a low-grade progressive reactional state, not accompanied by erythema nodosum, but with marked edema of all four extremities, with lymphadenitis and venous stasis. Because of the progressive nature of this reaction, B.663 was restarted on 14 December 1964 with subsequent slow regression of the reaction.

In spite of clinical improvement the patient continued to lose weight and became febrile. In mid-January 1965 cervical lymphadenopathy was noted. Biopsy of an anterior cervical node revealed on microscopic examination multiple caseous and hard granulomata surrounded by epithelial cells and rare foamy histiocytes. A rare Langhans'-type giant cell was seen. No bacilli were seen on Fite stain, but rare acid-fast bacilli were seen on Ziehl-Neelsen stain. Because of the marked change in the histology of this node from that of those examined previously, a workup for tuberculosis was instituted. Sputum specimens taken 16 and 17 March 1965 subsequently yielded cultures of *Mycobacterium tuberculosis*. Chest x-ray examination failed to reveal infiltrative disease, but the cervical lymphadenopathy progressed and the patient continued to lose weight during this period of reevaluation. He was recently started on isoniazid and PAS, and the B.663 was reduced to 100 mgm. per day. As of August 1965, the patient is improved, with loss of swelling in the cervical lymph nodes and gain in weight.

## DISCUSSION

Several findings have become apparent during the study of this limited number of cases. The most recent and possibly the most important is the development of tu-

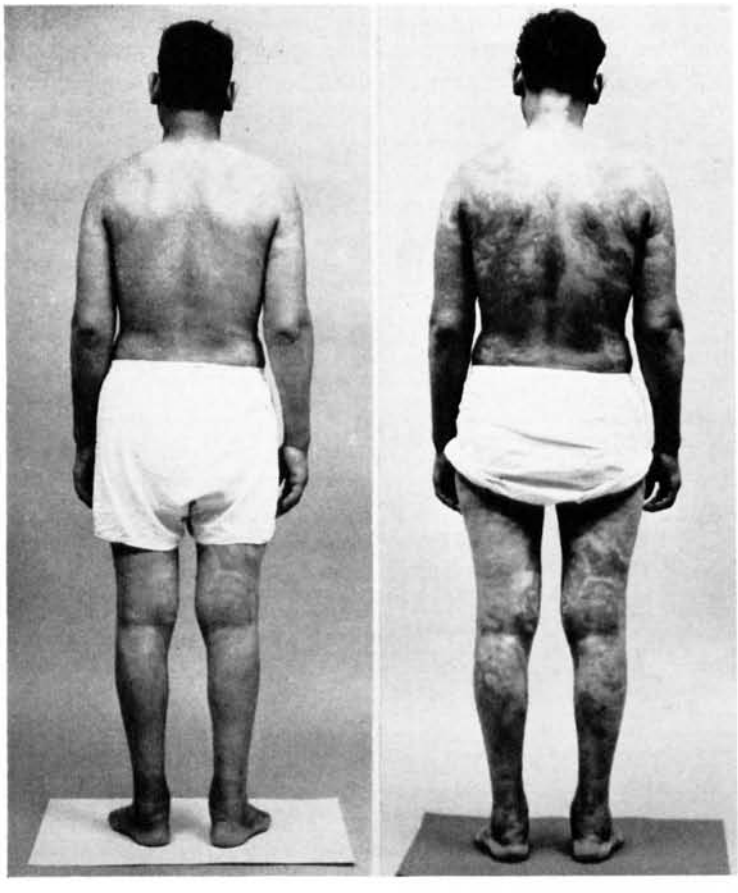

FIG. 4a & 4b. Selective pigmentation of infiltrated lesions by the rimino dye B.663.

berculosis in the third patient. Tuberculosis became clinically apparent at a time when the patient was not taking B.663, but when his tissues were saturated with the drug. Also, the lymphadenopathy, fever, and weight loss progressed after B.663 treatment had been restarted. It seems possible, therefore, that this may represent a drug-resistant infection.

B.663 is known to be quite active *in vitro* against *M. tuberculosis*([1,3,5,11,13,15]), and successive subculturing of bacilli in the presence of subinhibitory concentration of B.663 failed to produce a change in the susceptibility to the drug([3,5,16]). *In vivo* studies in a number of animals confirmed the *in vitro* efficacy of the drug against infection with several varieties of *M. tuberculosis*([1-3,5,11-14]). The development of resistance to B.663 was noted, however, only by Grumbach([11]) and Schmidt([16]). The development of resistance might be avoided by combination treatment utilizing isoniazid or ethionamide in addition to B.663([11]). Barry([4]) was able to document the development of resistance, or "reduced susceptibility," and speculated on the nature of this resistant state. Subsequently, Browne and Hogerzeil([9]) noted the development of apparent resistance of *M. leprae* to B.663 in patients treated with small doses over a twelve month period.

This patient's *M. leprae* appears, from a clinical point of view, to be sensitive to B.663, and the development of resistance by this organism may have been avoided by the high doses used almost continuously throughout the period of treatment.

Another factor that will need further evaluation is the part played in the development of the tuberculous infection by the so-called "anti-inflammatory" effect of B.663. This property of the drug produces amelioration of erythema nodosum leprosum, as was seen in the first and second cases.

It does seem clear that B.663 in some way ameliorated the severity of the reactions that were the presenting complaint in the second case. As we have pointed out previously, and as was noted by Browne([6,7]), this effect of B.663 may allow treatment with sulfones in doses that would otherwise not be tolerated by some patients.

Little can be said of the chemotherapeutic effect of B.663 in the second patient because of the concomitant use of DDS. This is not the case in the first patient, who not only had marked regression of febrile ENL reactions, coterminous with B.663 therapy, but also seemed to be having some chemotherapeutic effect, as evidenced by bacteriologic findings in serial scrapings and biopsies from comparable sites, as well as in the other laboratory parameters of activity of infection. A similar chemotherapeutic effect may have occurred in the third case, although this is more difficult to say with certainty in a case having dimorphous features.

Toxicity from the use of high doses of B.663 was limited to mild gastrointestinal symptoms easily reversed by stopping the drug for a period of time. No functional abnormalities nor anatomic lesions of the gastrointestinal tract could be found by x-ray studies. The skin pigmentation, considered by some to be a disadvantage, did not prove to be a marked psychologic problem in these cases. As noted by Browne([7,8]), the pigmentation diminished when the drug was discontinued for periods of more than one month. The one patient receiving sulfones in addition to B.663 became pigmented to a lesser degree than those receiving B.663 alone.

## SUMMARY

Experience with the use of B.663 in the treatment of leprosy for a period of approximately twenty months in two cases and four months in another is summarized. Except for intermittent bouts of abdominal discomfort and diarrhea, no toxicity was observed. In two cases treatment was associated with near or complete disappearance of erythema nodosum leprosum reactions. In all cases clinical and histopathologic evidence of improvement in the leprosy was observed during treatment. In one case tuberculosis with cervical lymphadenopathy and positive sputum cultures became apparent after 18 months' treatment, suggesting the development of a drug-resistant infection.

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## DISCUSSION

**Dr. Skinsnes.** I wish to thank Dr. Williams and Dr. Knight, and also Dr. Tolentino and Dr. Browne, for their papers in this session and throw them open for as much discussion as time permits. We could start off by asking Dr. Browne to comment on treatment with B.663, since he has had considerable experience with it.

**Dr. Browne.** I have already reported results of B.663 treatment in 28 patients with lepromatous leprosy. Its anti-inflammatory effect was evident and I was convinced that it had a definite therapeutic effect. Since, then, I have put another 44 patients on standard treatment with this drug. I should like to comment, first of all, on the dosage employed. We gave 300 mgm. per day at first. Dr. Williams and Dr. Knight have given 600 mgm. My present regimen is to give only 100 mgm. per day. In one group I started off with a leading dose of 300 mgm. per day for three weeks. So far, the improvement in all these patients, most of whom have severe lepromatous leprosy, has been marked, and there has been no apparent difference as regards speed of diminution of the Morphologic Index and that of the Bacteriologic Index in patients on the low dose of B.663 as compared with those on the dose of 300 mgm. per day. The anti-inflammatory effect, of which I spoke, has been noted equally well in the present series. Two patients only, out of those who had had the drug for more than six weeks, developed erythema nodosum during the entire length of treatment. In the present series only one patient out of 44 has developed subacute erythema nodosum during treatment. These results must be confirmed before they can be considered established. In addition to the treatment of lepromatous leprosy with these doses of B.663, I have nine patients who are receiving the drug for special indications. These include a group with established and chronic reaction, to determine if the drug will have an anti-inflammatory effect on patients who no longer have normal bacillary forms in any of their smears. I have three patients also who are exhibiting very slow clinical and bacteriologic progress under standard treatment with dapsone; and one patient, who, after six years of standard treatment with dapsone, has suddenly developed numerous succulent nodules in the skin, full of normal looking *M. leprae*. We are observing results in these three series of patients on the drug.

Finally, I would like to say how much this kind of chemotherapeutic investigation

depends upon collaboration. Use of the drug was suggested by Dr. Cochrane in conjunction with Dr. Vincent Barry of the Irish Medical Research Council, Dublin. In cooperation with Geigy of Switzerland, we in Nigeria and Dr. Vernon Knight and his colleagues at the National Institutes of Health, Bethesda, are accumulating numerous pathologic, chemotherapeutic and clinical data on B.663. We need this kind of cooperation among the research scientists who develop synthetic drugs, the commercial firms that manufacture them, the scientific institutions that investigate them, and the clinical workers in the field. This kind of collaboration should lead to good results, both for the leprosy patient in the field, and for the establishment of scientific data of value in other departments.

**Dr. Skinsnes.** Dr. Williams, may I ask you one question, of some special interest to me. Since this drug stains the tissues so well clinically is it evident in biopsies or does it disappear in the staining, and if so is it in macrophages or elsewhere?

**Dr. Williams.** The drug is seen eventually in biopsies in the crystalline form. Dr. Knight commented on the fact that the crystals have produced no foreign body reaction whatever. The drug is taken up in those areas where infiltrated lesions of leprosy are most abundant.

**Dr. Skinsnes.** Is it in the cells? It is not sitting outside the cells?

**Dr. Williams.** That question is hard to answer. The crystalline form is sometimes intracellular, and sometimes it appears as if it had ruptured the cell, but with no reaction around it.

**Dr. Latapí.** In Mexico we deeply appreciate the work of Dr. Knight, and are happy to have been invited to give at least modest cooperation. We feel that he and his associates are doing very fine work in Bethesda.

**Dr. Rees.** In our field research unit under Dr. Pettit in Kuala Lumpur we have so far tested B.663 at 300 mgm. a day in nine patients with lepromatous leprosy. Three of these patients were resistant to DDS, and six were previously untreated. The response, as far as their leprosy is concerned, has been good. Their Bacteriologic and Morphologic Indices have responded at least as well as with standard doses of DDS. We have one other trial underway, to determine whether B.663 has any influence on the development of erythema nodosum leprosum. We have no results yet, because this type of trial has to be very carefully controlled. I would make one comment with reference to light-skinned races. Our nine patients are Chinese. The level of 300 mgm. a day of B.663, even if it was far superior to DDS, could not be tolerated because of the discoloration of their skin. In fact so serious was this feature that we have had to abandon further trials until the discoloration in these patients has faded. If I understood Dr. Stanley Browne correctly, he is not seeing discoloration at 100 mgm. a day. I would say that this drug, for very special reasons, still has a great future, if we can find a therapeutic dose that does not discolor the skin. The pharmacologic properties of the drug are such that, at this stage, we do not know how best to test it. Undoubtedly it is a depot type drug, almost certainly deposited in the cells that are most infected with *M. leprae.* Therefore this may well be a suitable drug for intermittent therapy, or for loading patients up and leaving them for considerable lengths of time without further drug treatment.

I have one other comment. I was much interested in Dr. Knight's patient who developed tuberculosis while under treatment with B.663. You may know that all chemotherapeutic trials with B.663 against tuberculosis in man have been unsuccessful, in spite of very strong activity of this drug against experimental tuberculosis in the mouse. And, therefore, on the basis of trials in man, I do not think one can draw the conclusion that this patient's tubercle bacilli will necessarily be resistant to B.663. Undoubtedly the organisms should be tested *in vitro* against B.663, but I would hazard a guess that they are not resistant.

**Dr. Cochrane.** I am particularly inter-

ested in Dr. Browne's remark about dropping down to low dosages. As I said the other day, we are finding that 30 mgm. of DDS a week is more effective than 300 mgm. We may be dealing again with the question of some action on the enzyme system of the macrophages, i.e., making the environment of the macrophage unfavorable to multiplication of the bacillus.

**Dr. Y. T. Chang.** I spent several years on studies of the effect of B.663 in murine leprosy. B.663 has an unusually long-acting activity in the suppression of murine leprosy. Among various antituberculosis drugs that have been tested, B.663 has been the only one to hold the infection in check for as long as 816 days. I have a paper entitled "Effects of B.663, a rimino compound of the phenazine series, in murine leprosy" published in 1962. (Antimicrobial Agents & Chemotherap. (1962) 294-307). It gives some background that leads to the present clinical trial. I have a few reprints of this paper here. Anyone interested please take one.