

INTERNATIONAL JOURNAL OF LEPROSY

VOL. 14

FIFTH SPECIAL WAR NUMBER.

DECEMBER, 1946

INFLUENCE OF TYROTHRICIN IN THE STERILIZATION AND CICATRIZATION OF LEPROUS ULCERS

REPORT OF TREATMENT IN FIFTEEN PATIENTS

BY ARTURO M. MOM, M.D.

Department of Dermatology

University of Buenos Aires, Argentina

Director of the Sanatorio de Las Mercedes, San Jose, Costa Rica

and

SISTER MARIA BERNAL

Technical Assistant, Sanatorio de Las Mercedes, San Jose, Costa Rica

The local treatment of leprous ulcers is one of the most disappointing chapters of leprology. For half a century various methods have been tried but none have proved to be particularly efficacious.

The procedures most commonly used today are the Muir method, local infiltration of chaulmoogra oil, and the topical use of sodium sulfathiazole. The method of Muir which consists of successive applications of gentian violet, tannic acid, and silver nitrate has been used in our Leprosarium with irregular results. The ulcers may appear to be improved. However, this is only temporary and as soon as treatment is stopped all improvement disappears. One of the drawbacks to this technic is that the three products employed in the treatment leave stains which are difficult to remove both from the skin and from the clothing. The method of topical infiltration, around and below the ulcer, with derivatives of chaulmoogra oil is effective in some cases. When there is a heavy secondary infection present, however, it brings no relief. Among the disadvantages in its use are the violent localized reaction to derivatives of chaulmoogra, which sometimes increases the size of the ulcers, the pain which almost all patients experience, and the special technic of administration which requires special training on the part of nurses and other attendants.

Until recently we preferred to use daily applications of a 5 per

cent ointment of sodium sulfathiazole on a hydrosoluble base. In our judgment, this treatment was most easily applied and was most successful in ulcers in which the predominant micro-organism of the secondary infection was staphylococcus, which cases were the most frequent type among the inmates at our Leprosarium. Good results with sulfathiazole depend, however, on a careful removal of the scabs and cleansing of the ulcer with either hot saline solution, or with copper sulfate (1 part per thousand) and zinc sulfate (4 parts per thousand, Alibour Water), or with 4 per cent boric acid solution. The ulcer is rapidly cleaned, but cicatrization is slow in the majority of cases, especially so in old ulcers of the legs with callous borders surrounded by hard sclerous skin.

Since the supply of tyrothricin* available was large enough to make it possible to obtain definite conclusions as to its clinical value, the decision was made to try it on leprosy patients. Originally 25 patients were chosen, but subsequently in 10 of them it was necessary to begin general treatment with promin, diasone, or penicillin so that the number of those treated exclusively with tyrothricin was reduced to 15 patients.

CHARACTERISTICS OF TYROTHRICIN

Tyrothricin is a mixture of two polypeptids: gramicidin and tyrocidin. In 1939 Dubos (1) reported the discovery of a new spore-bearing bacillus (*Bacillus brevis*) isolated from the soil, capable of yielding in autolysates a non-enzymatic alcohol-soluble but water-insoluble substance (gramicidin). This substance is highly bactericidal for gram-positive micro-organisms (staphylococci, streptococci, and pneumococci) but completely ineffective against the gram-negative bacilli (2). In 1941 (3) the addition of tyrocidin, active against both gram-positive and gram-negative micro-organisms, made the mixture tyrothricin a more effective chemotherapeutic agent.**

Unfortunately tyrothricin is highly hemolytic and toxic for

* Courtesy of Parke, Davis & Co.

** In mixed cultures of gram-positive and gram-negative micro-organisms, the antibacterial activity of tyrothricin is reduced by the presence of the latter (2). The scope of activity of tyrothricin shows that the predominant action of gramicidin is exerted mainly on the streptococci (beta hemolytic especially), pneumococci, many strains of staphylococci, *Corynebacterium diphtheriae*, and anaerobic bacilli, but that it is inactive against the *Mycobacterium tuberculosis* (3). Because of the tyrocidin, tyrothricin has some activity *in vitro* against certain gram-negative micro-organisms such as *Hemophilus influenzae*, *Neisseria gonorrhoeae*, *Neisseria catarrhalis*, and *Neisseria intracellularis*. Tyrocidin possesses greater penetrating powers than gramicidin and contributes to the solubility and stability of the latter (2).

the kidneys and liver. For this reason it cannot be used parenterally but is employed only for topical application in the treatment of localized infections of the skin, nose, sinus paranasales, and pleural cavity.

The physical, chemical, and biological properties of tyrothricin give it, *a priori*, a great theoretical advantage over the other treatments mentioned. It was considered of interest, therefore, to try it in the treatment of leprosy ulcers.

METHOD AND PROCEDURE

Tyrothricin, as prepared for topical application, is a 2 per cent alcoholic solution. This must be diluted with sterile distilled water before use, giving a cloudy preparation of the compound in colloidal suspension since it is not soluble in water.

Suspensions are prepared daily. A suspension more than 24 hours old should never be used. For the present test a solution of 33 mg. in 100 cc. (1 cc. of stock solution in 60 cc. of distilled water) was used except in some cases in which solutions of 50 and 66 mg. per 100 cc. were tried with no irritating effect.

For application on the ulcer a large De Vilbiss atomizer was used. Spraying was done daily during the first week, and then every second day over periods of 32 to 138 days. During the first days of treatment, two hours before the spraying, the ulcers were washed with saline solution at a temperature of 37° to remove scabs. The ulcers were sprayed, then covered with a pad of sterilized gauze over which the spray was again applied. When the granulation at the base of the ulcers was so heavy that it hindered epithelization, the area of granulation was touched with 20 per cent silver nitrate on days when tyrothricin was not applied.

The patients with less than 4.0 million red blood corpuscles per cc. and less than 10 Gm. of hemoglobin, were given liver extract orally and ferrous salts (Vertrin, 6 capsules daily). Those having less than 3.5 million were given five to ten anti-anemic units of hepatic extract by injection and ferrous salts orally. This therapy proved to be remarkably efficacious for patients with a very slow cicatrization index. Three patients whose diet was poor in Vitamin C were given daily doses of 500 mgs. of ascorbic acid (Vicetrin) intravenously for 10 days. At the beginning this exerted remarkable acceleration in the cicatrization of the ulcers. This extra therapy should be borne in mind when estimating the clinical results.

from 3 to 20 years. The ulcers had been present for from 1 to 15 years and in the great majority of the cases had not yielded to any local treatment or to general treatment (principally chaulmoogra) prior to the administration of tyrothricin. Several patients gave a history of acquired syphilis with positive serology and had been subjected to treatment with bismuth (bismuth subsalicylate, 0.5 mg. of metallic bismuth per kilogram per day) for months before beginning with tyrothricin without noticing any improvement in the ulcers.

CHARACTERISTICS OF THE ACTION OF TYROTHRIN

Commonly leprosy ulcers are very painful and frequently they are the site of repeated erysipelas which causes (when on the lower limbs) an elephantiasis of both legs and thighs or a secondary dermatosclerosis or both. Tyrothricin exerted a remarkable analgesic effect from the first spray. Localized pain that had not yielded to common analgesics, or to high doses of thiamine hydrochloride or Vitamin B complex, began to decrease and disappear.

In six of seven cases with frequent erysipelas this has not recurred during the period of this experiment (8 months). In the other case, it has recurred only once, before the third week of treatment; it lasted two days and yielded to an injection of 5 cc. of 5 per cent sodium sulfathiazole, and to the spraying which was not interrupted.

With no other method tried has *such a rapid clearing* with subsequent granulation been obtained. This brought as a corollary a *surprisingly quick epithelization* and the formation of a scar which had as its most important characteristics: a geometrical aspect with sharp outline that made it possible to recognize the limit of new epithelial growth, a strong consistency and notable elasticity of the new skin and a lack of retraction, which is so common in large ulcers. In many cases the new skin was marked with dilatations of the veins that disappeared gradually. This telangiectasis occurred more rapidly in two patients with the simultaneous administration of 500 mg. of ascorbic acid (Vicetrin) in intravenous injection and three tablets a day (0.06 Gm.) of citrin (Pevigram).

Bacteriological sterilization of the ulcers, including *Mycobacterium leprae*, was obtained after periods of time ranging from 10 to 35 days but from this no direct bactericidal or bacteriostatic action of tyrothricin on *M. leprae* should be inferred. Next to *M. leprae* the most resistant micro-organisms were the staphylococci, which in one case necessitated the increase in concentration of tyrothricin to 66 mg. per cent, resulting in a rapid disappearance

within 72 hours. Long before the ulcers were completely healed it was impossible to find Hansen bacilli, even by repeated and careful search. The minimum number of applications necessary to obtain this result was 10 (13 days) and the maximum 21 (35 days), except for one case (case 12).

From the first a thin serofibrinous pellicle covering all the granulation surface could be observed which viewed through the microscope was seen to be made up of a little fibrin and an enormous number of normal polymorphonuclear neutrophils and a moderate percentage of large mononuclears that became more numerous as the treatment proceeded. The approximate ratio was 10 neutrophils to 1 mononuclear during the first week gradually changing to 6.5:1 during the 4th week, and remaining constant from this time. In the patient who had to be given a higher concentration of tyrothricin, the ratio of 8:1 (with 33 mg. per cent) dropped to 6.5:1 two days after the spray with double concentration (66 mg. per cent) was started. Although a single experience does not justify a definite conclusion, this seems to suggest that tyrothricin has some promoting action on the development of large mononuclears. Perhaps the sterilization of the granulation surface is an indirect result.

A comparative test was made with a patient who had several ulcers of similar size (approximately 3.5 cm. in diameter). One of the ulcers was treated with daily applications according to the Muir method; another with infiltrations of chaulmoogra oil around and under the ulcer; a third one with 5 per cent sodium sulfathiazole ointment and a fourth one with tyrothricin (33 mg. per cent). Three ulcers were left as controls, one of which was cleansed daily with hot saline solution. Secondary infection with streptococci and staphylococci was present in all ulcers, and they were positive for *M. leprae* though in small numbers. After three weeks the only ulcer cured was the one treated with tyrothricin which was free from all organisms two weeks after beginning of treatment and which showed a 7:1 polymorphonuclear/mononuclear ratio. The ulcer treated with sulfathiazole was positive for *M. leprae*, but had no pyogenic cocci. There was epithelization on only half the original ulcer. There were no marked changes in the other two ulcers, though the lesion treated with gentian-tannic-silver nitrate appeared clean, and no pyogenic cocci were found. The controls had not changed. The ulcer washed with saline solution looked clean, but the same organisms were still present. This comparative trial gives strong support to the observations on different individuals, but nevertheless no decisive conclusion can be drawn.

BACTERIOLOGICAL TRIALS

TESTS OF DIRECT ACTION OF TYROTHRICIN ON *Mycobacterium Leprae*.

The clinical results and the rapid disappearance of bacteria obtained with the topical application of tyrothricin, suggested the possibility of a direct action on *M. leprae*, a gram-positive organism. To test this hypothesis three series of experiments were done.

1. A small leproma of recent formation was extirpated from the forearm of a lepromatous patient. From the skin side of the leproma smears were prepared on sterile slides; these spreads were fixed by heat and when stained by the Ziehl-Neelsen technic revealed an abundance of *M. leprae* in "globi." The epidermis covering the leproma was removed and the remainder ground in a mortar with sterile saline solution. When the solution acquired a homogeneous cloudy aspect, a single drop was placed on each end of eight slides; to one of the drops on each side, a drop of tyrothricin (33 mg. per cent) was immediately added and mixed carefully with the lepromatous solution by means of a sterile toothpick. The slides were kept in Petri boxes to prevent excessive evaporation. Every fifteen minutes a slide was removed and dried by heat, one slide being kept and dried for 24 hours. After staining by the Ziehl-Neelsen method, the two smears on each slide were compared. The preparations showed no differences either morphological or tinctorial between the drop treated by tyrothricin and the control, except for the slide held 24 hours before drying in which lysis of the bacilli appeared to be starting. Some bacilli looked as if they had been pulverized and others had impaired borders and ends. There were no marked changes in the coloration.

2. A small leproma of recent formation was extirpated from the forearm of a lepromatous patient, and the material was prepared as in the preceding experiment. In each of eight centrifuge tubes two drops of the material from the leproma were placed and to seven of them increasing quantities of tyrothricin 33 mg. per cent (2, 4, 6, 8, 10, 12, and 14 drops) were added. All tubes were incubated for an hour at 37° C. Subsequently, at hourly intervals over a period of six hours, then 24 and 48 hours later the material in each of the eight tubes was examined with Ziehl-Neelsen staining. No change could be seen.

3. The second experiment was repeated, but with a leproma from another patient and with tyrothricin of a concentration of 66 to 132 mg. per cent. No morphological or tinctorial changes were observed in the bacilli. These same tubes were re-examined 7, 14, 21, and 28 days later, but no changes in the bacilli were observed.



FIG. 1



FIG. 2



FIG. 3



FIG. 4

- Fig. 1. Case 2 before treatment.
Fig. 2. Case 2 after 37 days of tyrothricin therapy (19 applications).
Fig. 3. Case 12 three weeks after treatment was started.
Fig. 4. Case 12 after 45 days of treatment.



Fig. 5. Case 12 after 45 days of treatment. Detail of cicatrization.
Fig. 6. Case 13 before treatment.
Fig. 7. Case 13 after 35 days of tyrothricin (19 applications).
Fig. 8. Case 14 after 67 days of tyrothricin therapy. The line on the leg shows the original limit of the ulcer.

The results obtained in the first experiment can by no means be considered categorical; they are suggestive of a certain direct action but perhaps are only the consequence of some fault in the technic the reason for which is not known. In this connection it should be remembered that according to Kolmer (*op. cit.* p. 261) "It would appear that lysis is only a secondary process which follows some primary injury of the bacterial cells." Tyrothricin does not destroy the respiration of bacteria and "its antibacterial properties appear to depend upon the inhibition of enzymatic activity, retardation of growth and lysis of bacterial cells" (Kolmer, *op. cit.* p. 260).

CLINICAL OBSERVATIONS

CASE 1.—Female, 36 years of age, with moderately advanced lepromatous leprosy (L_2) of ten years duration and bacteriologically positive examinations since 1939. No history of syphilis, negative serological tests (Eagle, Kahn Standard, and Briceño Rossi flocculation tests). On July 23, 1945, she presented a necrotic ulcer, fetid and very painful, covering a large portion of the right buttock (nearly 20 cm. in diameter). The appearance of this ulcer was associated with an intramuscular injection of chaulmoogra oil (7 cc.) made 9 days previously. A short distance from this large ulcer were three smaller ones (3, 4, and 6 cm. in diameter). Examination revealed abundant Hansen bacilli in "globi," a moderate number of streptococci and only a few staphylococci. After the third day of treatment (10 applications) neither *M. leprae*, streptococci, or staphylococci were found. Complete cicatrization in 39 days (21 applications of tyrothricin).

CASE 2.—Female, 25 years of age, with moderately advanced lepromatous leprosy (L_2) of 8 years duration, positive bacteriology since 1940. No history of syphilis, negative serological tests (Eagle, Kahn Standard, and Briceño Rossi). Since 1940 she has had numerous chronic ulcers of the legs that have never cicatrized, with frequent erysipelas. On July 29, 1945, she had deep ulcers (one 16 cm. in the longer diameter) through which the muscles could be seen; very painful, bleeding, some with necrotic aspect and fetid odor (see Figure 1). The bacteriological examination revealed abundant Hansen bacilli in "globi" and many streptococci. From the first application of tyrothricin pain was eased, fetidness disappeared and the necrotic aspect changed remarkably, and a vivid red granulation tissue followed which rapidly filled the hollows and a rapid epithelization began. From the 28th day on (18 applications) no *M. leprae* or streptococci could be found. Complete cicatrization was obtained in 37 days with 19 applications (see Figure 2). During three months following the cicatrization no erysipelas occurred; in this connection it must be borne in mind that during the last 45 days she has received 38 intravenous injections of promin.

CASE 3.—Male, 32 years of age, with progressive moderately advanced lepromatous leprosy (L_2) of one years duration. Positive bacteriology. No history of syphilis, but Eagle flocculation test intensely positive (+ + + +), Kahn Standard, and Briceño Rossi tests negative. On August 21,

1945, he presented numerous ulcers of the legs, of regular size, very painful, especially those on the malleoli tibiae. Examination showed numerous *M. leprae*, streptococci, and staphylococci. From the 15th day (10 applications) of treatment all micro-organisms disappeared from the ulcers and their complete cicatrization was obtained in 20 days (13 applications). Two weeks later, treatment with intravenous promin was started, and within 18 days, some ulcers reopened with great pain but without micro-organisms. Tyrothricin was applied eight times, resulting in a definitive cicatrization in 18 days. Promin was not discontinued nor did it open the ulcers again.

CASE 4.—Female, 45 years of age, with advanced and progressive lepromatous leprosy (L_3) of 15 years duration. Positive bacteriology since 1940. History of acquired syphilis and positive serological tests (Eagle, Kahn Standard, and Briceño Rossi). Chronic ulcers on the legs, small and deep of more than 10 years duration, and frequent erysipelas. Left plantar ulcers covering the inner border of foot up to the head of the first metatarsal bone. Examination on October 2, 1945, revealed abundant *M. leprae* and staphylococci. No streptococci were found. She was treated with penicillin (4) (25,000 units per day in beeswax-peanut oil, for a total of 1,300,000 units in 53 days). This freed the ulcers from staphylococci and promoted a definite improvement of the appearance of the ulcers with beginning of cicatrization. Ten days after penicillin had been discontinued, the ulcers, especially the one on the plantar surface became extremely painful, and recovered their former appearance, but without staphylococci. Sixteen applications of tyrothricin brought about complete cicatrization. Pain disappeared after the 3rd application. Two months after cicatrization, the new skin had acquired an almost normal aspect. During the last five weeks the patient has received 35 Gm. of diasone.

CASE 5.—Male, 40 years of age, with moderately advanced lepromatous leprosy of seven years duration and history of acquired syphilis and intensely positive serological tests (Eagle, Kahn Standard, and Briceño Rossi). This patient had ecchymotic and necrotic lesions the majority of which became ulcerous and left open lesions from 2 to 6 cm. in diameter. Smears were positive for *M. leprae*, but no streptococci or staphylococci were found. The purpuric character of the lesions required daily intravenous administration of 500 mg. ascorbic acid (Vicetrin) and 0.5 Gm. calcium gluconate, and also 0.06 Gm. per day of Citrin by mouth. This medication was continued for 45 days and though it stopped the progression of the lesions, attenuating their ecchymotic character, it did not cause the cicatrization of the ulcers. Under treatment with tyrothricin cicatrization was complete in 36 days (19 applications). The pain ceased after the 3rd application and there was an early disappearance of micro-organisms (15th day).

CASE 6.—Female, 25 years of age, with moderately advanced lepromatous leprosy of 6 years duration. Positive bacteriology since 1940. Doubtful history of acquired syphilis, positive serological tests (Eagle, Kahn Standard, and Briceño Rossi, June 1945). On August 15, 1945, she presented numerous chronic ulcers of the legs, syphiloid in character, with thick, sharp, clean borders, very painful (necessitating sedatives at night for a whole year), and frequent erysipelas. Bismuth subsalicylate in intramuscular injection had no favorable influence. Smears revealed abun-

dant *M. leprae* with scanty staphylococci on the ulcers. In 62 days (32 applications) all the ulcers were completely cicatrized and bacteriological examination was negative from the 28th day. At present, February 28, 1946, more than 120 injections of promin have been given to this patient and the lesions have not reopened.

CASE 7.—Female, 41 years of age, with progressive lepromatous leprosy (L_2) of 5 years duration. Positive bacteriology since 1942. No history of syphilis, negative serology. Before she entered the Leprosarium Neosalvarsan had been prescribed, possibly because of the macules. On September 17, 1945, she had numerous chronic ulcers on legs and thighs, varying in size from 2 to 6 cm. deeply excavated (both the tibia and several extensor tendons were seen), and intensely painful, especially one on the right heel. Bacteriological examinations were positive for *M. leprae* and staphylococci. During August and September 1945, she was given 300 Gm. sulfathiazole orally, which was poorly tolerated, and which had no influence on the ulcers. Thirty-three applications (75 days) of tyrothricin were necessary to obtain complete cicatrization. Bacteriological examinations were negative after 19 applications. Pain disappeared after the 5th application.

CASE 8.—Female, 40 years of age, with progressive lepromatous leprosy (L_2) of 10 years duration. No clinical or serological proof of syphilis. Positive bacteriology since 1936. Numerous small and painful ulcers on legs and thigh, with bacteriological examinations positive for *M. leprae* and staphylococci. Due to lack of cooperation, only 8 applications were made in 12 days, yet the ulcers cicatrized rapidly in the subsequent fortnight. Bacteriological examinations were not made until the ulcers were completely closed and did not at that time show *M. leprae*.

CASE 9.—Female, 42 years of age, with stationary lepromatous leprosy (L_2) with considerable neural involvement, of 15 years duration. Numerous chronic ulcers on both legs. Positive for *M. leprae*, streptococci, and staphylococci. Frequent erysipelas. As in case 8, owing to lack of cooperation, the treatment was discontinued after 8 applications over a period of 12 days. The ulcers had undergone the change previously described, but the discontinuation of treatment brought the improvement to a standstill. Staphylococci and *M. leprae* persisted. Streptococci disappeared, causing no doubt the disappearance of erysipelas.

CASE 10.—Male, 36 years of age, with advanced lepromatous leprosy (L_3) of 10 years duration. No history of syphilis. For 8 years he had chronic ulcers of medium size (4 and 6 cm. in diameter) on legs and thighs resistant to every treatment, including intravenous and topical penicillin. Frequent erysipelas. Positive for streptococci and *M. leprae*. Unidentified gram-negative bacilli were also present. Complete cicatrization and negative bacteriologically in 19 days (15 applications).

CASE 11.—Female, 30 years of age, with progressive lepromatous leprosy (L_2) of 5 years duration. Positive bacteriology. No clinical or serological history of syphilis. In October 1945 she showed a large ulcer (10 cm. in diameter) and numerous small ulcers of two years standing growing worse with chaulmoogra oil (20 cc. a week). Frequent erysipelas. Total cicatrization with 16 applications over 29 days. Bacteriologically negative (*M. leprae*, streptococci, and staphylococci) after 10th applica-

tion. Erysipelas has not reappeared. During the last five weeks, she has been given 35 Gm. diasone.

CASE 12.—Female, 39 years of age, with moderately advanced lepromatous leprosy (L_2) of 24 years duration much neural involvement (C_2N_3) and positive bacteriology. No history of syphilis. Since her entrance (1930), she has had numerous (23) very large painful ulcers on thighs, knees, legs, ankles and feet, for the most part deep and exposing tendons. In spite of innumerable treatments, they never cicatrized. Frequent erysipelas for the past 15 years. The skin surrounding the ulcers was very hard, as if lacking elastic fibers, and did not slide on the deep planes (dermatosclerosis). Positive bacteriologically for *M. leprae*, streptococci, and staphylococci. Treatment was started on July 18, 1945, with 33 mg. per cent tyrothricin daily for seven days and then every second day. The change on the ulcerated surfaces was evident. Pain was mitigated, the base of the ulcers became cleaner and vivid red granulation tissue started to grow with extraordinary velocity. Figure 3 shows the inferior limb of the patient within the first three weeks of medication, and figures 4 and 5, 45 days after the beginning of treatment. In figure 5 details of cicatrization can be seen within 45 days of the treatment; 16 ulcers had wholly cicatrized. The other seven appeared stationary. Streptococci had disappeared but not *M. leprae* and staphylococci. Concentration of tyrothricin was increased to 66 mg. per cent, in daily application, resulting in the disappearance of staphylococci within 72 hours and a new reaction in the growth of tissues. This lasted for only one week, subsequent to which therapy with ferrous salts, liver extract (16 units per week) by injection, and Vicetrin (500 mg. per day) was started because the red blood cell count had fallen to 3.2 million with 9 Gm. hemoglobin. This treatment plus the continuation of tyrothricin for two more weeks, brought about a third tissular reaction, closing two of the seven ulcers and improving the appearance of the others. The red blood cell count increased to 4.3 million and the hemoglobin to 12 Gm. This lasted only a short time. Friction with 20 per cent silver nitrate and treatment with Peruvian balsam were not successful. The ulcers were still positive for *M. leprae*. Therefore, the following medication was tried. Daily treatment with intravenous promin was added. The largest ulcer was treated with promin jelly*; another with penicillin ointment (1000 units per Gm.); a third one with 5 per cent sodium sulfathiazole in hydrosoluble base, and the remaining two with tyrothricin 66 mg. per cent. Despite its larger size, the ulcer treated with penicillin ointment cicatrized rapidly in three weeks with disappearance of *M. leprae* within 10 days of treatment. Next, the one treated with sulfathiazole (26 days) closed and, last, within 35 and 37 days, those treated with promin jelly and tyrothricin respectively. This curiously tardy resistance to treatment after an excellent early response is at present the object of a special study. We consider that in this case the intervening factors do not depend exclusively on micro-organisms or antibiotics. The resistant zones were in the majority anesthetic and probably this trophic factor was responsible for the resistance. The improvement of the general condition by therapy with liver, ferrum, vitamins (59 mg. of thiamin were added daily over a period of 20 days, when promin was started, following

* (Courtesy of Parke, Davis & Co.)

Higgin's (5) suggestion) and promin itself, was reflected in a better response to topical treatment.

CASE 13.—Male, 32 years of age, with moderately advanced lepromatous leprosy of 15 years duration. No history of acquired syphilis, negative serology. Large, painful ulcers on the legs and thighs since entrance (1938) that never cicatrized and did not improve under any treatment tried. Frequent erysipelas brought about an edema of legs and feet that produced an elephantiasis deformity (see Figure 6). Examination showed abundant *M. leprae*, streptococci, and staphylococci. From August 29 to October 3, 1945, nineteen applications of tyrothricin (33 mg. per cent) were made with marked improvement that can be appreciated by comparing figures 6 and 7. On October 4 (after 24 days of treatment) *M. leprae*, streptococci, and staphylococci had disappeared from the ulcers. The lesions showed a remarkable improvement, with normal granulation, disinfiltration of borders, almost complete disappearance of edema of the legs and gradual healing of the ulcers. On October 29 when the legs were as shown in figure 7 treatment with promin by intravenous injection was started, tyrothricin being continued. Towards the end of December cicatrization was complete.

CASE 14.—Male, 28 years of age, with moderately advanced lepromatous leprosy (L_2) of 10 years duration. No history of syphilis. Since entrance (1939) he showed large, deep ulcers on both thighs and both legs, resisting all treatment. Repeated erysipelas. Examination showed moderate numbers of *M. leprae* and many staphylococci and streptococci. Cicatrization was obtained after 32 applications in 67 days to the extent shown in figure 8. All bacteria had disappeared. On October 29 intravenous promin was started, and the topical treatment with tyrothricin was continued. By the end of December cicatrization was complete.

CASE 15.—Male, 50 years of age, with moderately advanced lepromatous leprosy (L_2) of 8 years duration. Since entrance (1940) he has had numerous painful ulcers on the legs of medium size (4 to 6 cm. in diameter). Examination showed moderate numbers of *M. leprae* and streptococci. Eighteen applications were made in 32 days with complete cicatrization, bacteriologically negative after the 10th application.

SUMMARY

Topical treatment with tyrothricin of ulcers on the lower limbs of 15 patients with lepromatous leprosy was successful in 14 cases with a relative failure in one case.

The relative failure was due to resistance offered by one third of the ulcers on the lower limbs of a woman after the others had become cicatrized. The resistant ulcers were situated in anesthetic zones. Cicatrization occurred after abundant administration of liver extract, ferrum, vitamins B and C, and general treatment with promin.

For the 14 successful cases a maximum of 75 days and a minimum of 19 days was necessary to obtain cicatrization of ulcers, with a maximum of 33 and a minimum of 13 applications of tyro-

thricin. In all instances the ulcers became bacteriologically negative before complete cicatrization.

In the experience of the authors, topical treatment with tyrothricin has proved to be the best treatment for leprous ulcers.

REFERENCES

1. DUBOS, R. J.—*J. Exper. Med.* **70** (1939) **1**, 11, 249.
2. KOLMER, J. A.—*Penicillin Therapy, including Tyrothricin and Other Antibiotic Therapy.* D. Appleton-Century Co., New York-London, 1945.
3. HOTCHKISS, R. D. and DUBOS, R. J.—*J. Biol. Chem.* **136** (1940) 803; **141** (1941) 155.
4. MOM, A. M., and BERNAL, M.—*Internat. J. Leprosy.* **14** (1946) 37.
5. HIGGINS, M.—*Proc. Staff Meet. Mayo Clin.* **19** (1944) 202.