

STUDY OF THE MORPHOLOGICAL MODIFICATIONS OF  
*MYCOBACTERIUM LEPRAE* DURING CHEMOTHERAPY

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In furtherance of our investigations on the effects of chemotherapeutic agents on the morphology of *Mycobacterium leprae*, in patients under treatment with sulfones, thiosemicarbazone and isonicotinic acid hydrazide, we have complemented the direct electron microscope observation used in our earlier study with the "shadow-casting" technique. This method offers advantages when one wishes to ascertain the surface changes occurring in the germs subjected to treatment. In our earlier reports (6, 7) we dealt with the morphology of *M. leprae* from untreated patients and from 6 patients under the effects, in different degrees, of sulfones. Those reports are now complemented with findings in new cases selected from more than 30 patients studied, using particularly the shadow-casting technique which has given us interesting results.

We are aware that many investigators have studied, by classical optics, the bacterial lysis of *M. leprae* as a result of treatment with various kinds of sulfones. Thus in our earlier reports we cited the work of Chaussinand (2), Gomes (4), Fernandez and Carboni (3), and Cochrane (1) bearing on this matter. As had others, Lowe and Smith (5) asserted in 1949 that chemotherapy causes the bacillary body to become thinner and to stain weakly, even though frequently one can find large-sized, intensely-stained granules, these changes appearing after a few months of treatment. Later the bacilli show a fragmented appearance, and finally they become undetectable.

In 1951, Wheate (8) noted two fundamental facts under similar conditions: (1) There is a marked tendency of the globi and bacillus masses to disintegrate, the latter seeming less clear-cut, less compact, and of smaller size, these changes appearing within nine months of sulphetrone treatment. Furthermore, the isolated bacilli all showed bipolar staining and were granular and dispersed due, the author believed, to the disintegration of the globi or bacillary conglomerations. (2) In addition, there is an increase of intracellular bacilli.

## TECHNIQUE AND MATERIAL

For many months the senior author (M.) made attempts to complement the direct technique with the shadow-casting procedure, with the valued collaboration of Prof. Ing. E. Gallini in the construction of a simple vacuum apparatus which would permit the vaporization of metal and achieve a good deposition of it. Excellent results having been obtained, we proceeded to apply this technique to the study of the leprosy bacillus from untreated and treated cases.

The material was obtained from patients in the Sanatorio Baldomero Sommer, in General Rodriguez near Buenos Aires, by the same technique as in our first investigations. Small incisions were made in lepromas located on various parts of the body, to obtain leprous exudate and lymph without blood.

The material so obtained was systematically distributed to four stainless steel screens. These were first examined by the direct technique, and then after that control examination one screen was shadowed with gold or aluminum. The examinations were made at magnifications of from 3,700 to 10,000 diameters.

## RESULTS

First of all we wish to say that the shadow-casting technique reveals structural details that are practically impossible to visualize by any other means. The observations have seemed to confirm once more that chemotherapy is effective in leprosy, and that it constitutes the best promise for people suffering from that disease.

The observations were mainly made on patients under treatment with sulfones, although there were included one receiving thiosemicarbazone and two receiving isonicotinic acid hydrazide. These were included for the purpose of complementing our investigations and to determine by means of the electron microscope the degree of therapeutic efficacy of these different drugs employed in the treatment of the disease we are concerned with. The following are the reports of the cases we have selected, starting with those treated for the shortest periods of time in order to ascertain at what stage of the treatment the first modifications occur.

CASE 1 (No. 1780). M. N., female, Argentinian, 30 years old, with L2 leprosy, admitted May 1952 with 2+ nasal smears and 3+ skin smears. She was treated from the date of admission up to September with disulone, 183 tablets (18.3 gm.), and the bacillus count was brought down to 1+ and 2+.

After the short period of four months of treatment with disulone the electron micrographs, exemplified by Fig. 1, revealed evident disturbance of the morphology of the bacilli, which must be attributed to the effect of treatment. Actually, isolated bacillary forms were very scarce, and the globi appeared

to be constituted by evidently deformed bacilli which showed polar cytoplasmic condensations while the central parts of the cells were swollen and on the way to disintegration. We believe that the treatment had been very effective, in view of these changes and the evident clinical improvement of the patient that was observed.

It should be pointed out that the morphological modifications demonstratable by the electron microscope must precede clinical improvement of the patients. As the bacilli deteriorate and progress to total disintegration, the granulomatous lesion slowly regresses, until the bacillary remains are removed and the pathologic process is absorbed. Thus clinical improvement must follow, as the shadow follows the body, after the lapse of a certain period of time from the death of the bacilli. The enormous amount of germs present is naturally an obstacle to rapid recovery of the tissue. We believe that interruption of the treatment is a very unfavorable thing, for it permits exuberant multiplication of those bacilli which still retain vital capacity.

CASE 2 (No. 1375). E. O., female, Argentinian, 40 years old, an L2 case, admitted January 1949, bacteriology 2+ and 3+. Treated with 1,523 tablets (505 gm.) of diasone up to May 1951, and from then to September 1952 with 1,000 tablets (500 gm.) of sulphetrone, a total of 3 years and 9 months under sulfone. The bacilli were reduced to 1+.

Fig. 2 is of a globus from this patient which showed well the marked modifications which the bacilli have undergone. They present irregular cytoplasmic condensations, with their central parts swollen and on the way to lysis and disintegration. There is no doubt that these bacillary forms have been affected by the treatment, and their vitality seems to have been so seriously depressed that some of them cannot be considered as viable. Since the granulations probably remain acid-fast, by ordinary microscopy they would be considered as simple granular forms, because by that method it is impossible to detect the extraordinary structural details which are shown by the electron microscope.

CASE 3 (No. 1319). M. E. P., female, Argentinian, 36 years old, an L2 case, admitted August 1948, bacilli 3+. Treated until October 1951 with promine, total dose of 3,800 cc. (1,535 gm.), and from then to September 1952 with disulone, 380 tablets (38 gm.), her present bacteriological status being 1+ and 2+.

For this case there are presented two electron micrographs, Figs. 3 and 4, which show the marked disturbance of the bacilli after four years of treatment. Fig. 3 is of a large, disintegrating globus, with evidently altered bacilli. In Fig. 4 are several

germs remaining from a globus in full process of lysis, some of them being cell remnants which can hardly be recognized as representing bacillary entities.

Leprosy is so slow in its evolution, as well as in its response to treatment, that although these forms in lysis testify to the efficacy of the treatment, their presence after four years of it indicates the extraordinary resistance of the leprosy bacillus once it is established as a real parasite in the human organism. If any of these forms should turn out to be viable and capable of regenerating new bacillary forms, there would result a difficult problem that only time could make clear.

CASE 4 (No. 253). F. V., female, Argentinian, 81 years old, an L1 case, admitted in 1942. Treated until 1949 with chaulmoogra ethyl esters, in spite of which she became worse, the bacteriological status changing from the original 1+ and 2+ to 2+ and 3+. Diasone treatment was started in 1950, and to the present time a total of 1,180 tablets have been taken (650 of diasone and 534 of diamidin).

The effect of this treatment on the bacilli is shown in Figs. 5 and 6. The bacilli are seen to be on their way to total disintegration.

CASE 5 (No. 1774). J. Z., male, Argentinian, 42 years old, an L2 case, which had shown no improvement after three years of treatment with chaulmoogra esters. In September 1951 thiosemicarbazone treatment was started, and to June 1952—the time of observation—a total dose of 878 tablets had been given. Smears, which were 1+ and 3+ at the time of admission, had become 1+ and 2+. There had been distinct although slight clinical improvement.

The electron microscope examination was made by the direct technique because at the time our shadow-casting equipment was not in working condition. Fig. 7 shows bacillary elements which have evidently been injured.

The last two cases to be presented were treated with isonicotinic acid hydrazide.

CASE 6 (No. 1960). M. P., male, 24 years old, Argentinian, an L2 case, bacteriological status 2+ and 3+. This patient had received no treatment until, from November 18 to December 5 (17 days), he was given isoniazid (Cotinazin) by mouth in an average daily dose of 317 mgm.

The electron microscope examination made before the treatment was begun revealed numerous isolated bacilli of the solid type and many globi composed of solid forms, indicating high virulence. In such cases the bacilli and globi always show an outer covering which we attribute to the glœa. After 17 days of treatment we found that, although solid forms still persisted, yet there could be observed an increase in the number of granular forms, some of which seemed to present the first modifications due to the action of the drug. Clinically the condition

was stationary, but, as has been said, even if the treatment were effective it would be impossible in so short a time to observe evident clinical changes. The electron micrographs in Figs. 8 and 9 illustrate the bacillary modifications referred to.

CASE 7 (No. 1967). J. Z., male, Argentinian, 59 years old, an L2 case, bacilli on admission 2+ and 3+. This patient was given the isonicotinic acid hydrazide (Cotinazin) by intramuscular injection, the average daily dose over the 17 days being 250 mgm.

Before treatment the examination showed numerous bacilli, solid and "toscan" forms, and globi of large size filled with active bacillary forms. After 17 days of treatment the fields examined showed no definite bacillary changes ascribable to the action of the drug. The forms seen are illustrated by Fig. 10.

Final observations were made on these two patients after 47 days of isoniazid treatment. The preparations from the second of them, J. Z., who by that time had had a total of 8 gm. of the drug, did not show any distinct modifications of the bacilli that could unquestionably be attributed to the effect of the chemotherapy. Those of the patient M.P., however, who had been given 20 gm. of the drug, showed visible bacillary disturbance manifested by numerous forms that were granular and on the way to disintegration, although, naturally, there still existed virulent bacillary units which were without notable structural modifications. These findings are illustrated by Figs. 11 and 12. The fact that, clinically, the condition of these patients was stationary is simply in keeping with our belief that the changes in the bacilli occur earlier than clinical regression.

#### DISCUSSION

It should be noted that the modifications of the bacilli are sometimes difficult to detect by ocular observation of the screen of the electron microscope. They are much more evident in the electron micrographs. In routine observations with the ordinary microscope, where the difficulties are greater, it is practically impossible for the ordinary observer to determine whether he is dealing with normal granular forms or with bacillary units at the beginning of disintegration which are provided with granules. We believe that in the latter case the investigator is faced with two serious pitfalls—the modifications resulting from the staining technique, and the limitations of the resolving power of the classical optics.

Our observations of the effects of modern chemotherapy on leprosy lead to the following conclusions: (1) The peripheral envelope surrounding the isolated bacillary units and the globi,



which is always observed in preparations from untreated patients, is an index of cellular vitality and consequently of the virulence of the leprosy bacillus. (2) Lowering of the bacterial vitality, or death of the cells, results in the disappearance of the peripheral envelope, since this depends upon the normal bacterial metabolism. (3) The lack of the peripheral envelope increases with the degree to which the germs show morphological disturbances, this being an index of their injury which increases in keeping with increased duration of treatment. (4) With the death of the bacteria the outer envelope disappears and the germs appear to be free in the medium, as cellular remains. (5) The rupture of the isolating blockade by the action of the drugs, caused by their bacteriostatic and modifying effect on the cellular metabolism, inhibits the production of the substance which is the source of this envelope. This permits direct contact of the germs with the medium, which facilitates the action and production of natural antibodies derived from the reticuloendothelial system. (6) Thus are initiated the processes of reabsorption of the bacterial remnants by increasing the phagocytized material, resulting in the regression of the granulomatous processes of the lepromas, which coincides with the beginning of the clinical improvement.

#### SUMMARY AND CONCLUSIONS

The authors have studied with the electron microscope, using the direct and shadow-casting techniques, the modifications occurring in *M. leprae* under the action of the sulfones, thiosemicarbazone and isonicotinic acid hydrazide.

On the basis of the greater number of observations and the longer duration of treatment, the sulfones are the drug with which the authors have most experience.

Swelling of the cytoplasm and the granular state are signs of the first modifications of morphology. Also notable is the disappearance of the peripheral halo, which is apparently dependent upon the normal bacilli metabolism.

It can be gathered from our investigations that chemotherapy is the most promising hope in the present treatment of leprosy.

#### SUMARIO Y CONCLUSIONES

Los autores estudian las modificaciones que sufre el *M. leprae* sometido a la acción de sulfonas, tiosemicarbazona e hidracida del ácido isonicotínico mediante el microscopio electrónico y con las técnicas directa y del "shadow casting."

En atención al mayor número de observaciones y al mayor plazo de tratamiento son las sulfonas las drogas sobre las cuales los autores tienen mayor experiencia.

La hinchazón del citoplasma y el estado granuloso son testigos de las primeras modificaciones de la morfología siendo digno de señalar la desaparición del halo periférico vinculado al parecer al metabolismo bacilar normal.

De nuestras investigaciones se deduciría que la quimioterapia constituye la más firme promesa en el tratamiento actual de la lepra.

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## DESCRIPTION OF PLATES

(All pictures are enlargements of electron micrographs taken at 3,700 diameters, somewhat reduced in reproduction for printing. All shadow-cast pictures were metallized with aluminum.)

## PLATE (6)

FIG. 1. An electron micrograph showing a cluster of bacilli from a globus with no covering envelope, the bacillary units swollen and with polar condensations, evidence of damage. Case 1, after four months of sulfone treatment. Shadow-casting technique.

FIG. 2. Showing a globus-mass of bacillary units in different degrees of disintegration, some remaining as veritable shells, with granules of cytoplasmic condensation, the "ultimum moriens" of bacillary lysis. The peripheral gloea has practically disappeared. Case 2, treated with sulfones for 3 years and 8 months. Shadow-casting technique.

FIG. 3. Showing a large globus undergoing disintegration. Case 3, treated with sulfone for 4 years. Shadow-casting technique.

FIG. 4. Bacillary remnants with polar granules the central parts swollen and in lysis. Same patient as Fig. 3.

FIG. 5. Showing damaged bacilli which are in lysis. Case 4, under sulfone treatment for 2 years. Shadow-casting technique.

FIG. 6. Showing almost unrecognizable bacillary remnants of granular aspect. Same patient as Fig. 5. Shadow-casting technique.



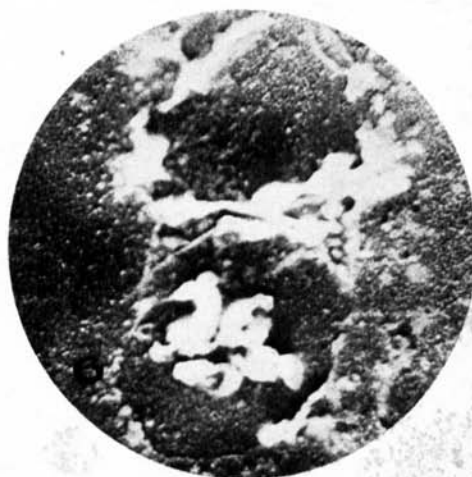
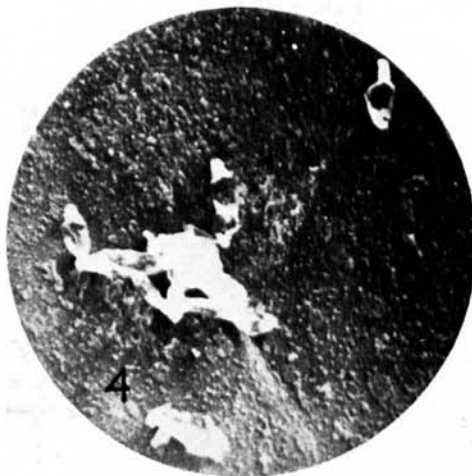
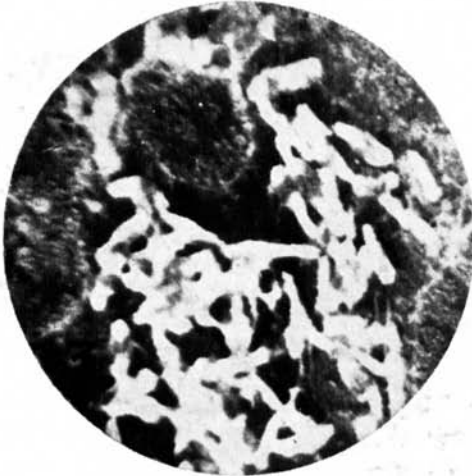


PLATE 6.

PLATE (7)

FIG. 7. Showing injured bacilli on the way to disintegration. Case 5, after 9 months of thiosemicarbazone treatment. Direct technique.

FIG. 8. Showing a small globus composed of solid bacillary units, an indication of virulence. Case 6, after 17 days of treatment with isonicotinic acid hydrazide. Direct technique.

FIG. 9. Showing various granular forms which seem to reveal injury as a result of treatment. Same patient as Fig. 8. Direct technique.

FIG. 10. Showing solid, virulent bacilli provided with peripheral envelope which as yet indicate no evident effect of chemotherapy. Case 7, after 17 days of treatment with isonicotinic acid hydrazide. Direct technique.

FIG. 11. Showing bacilli in granular disintegration and remains of the membrane. Case 6, after 47 days of treatment with isonicotinic acid hydrazide. Shadow-casting technique.

FIG. 12. Showing bacillary remnants which are almost unrecognizable. Same case as Fig. 11. Shadow-casting technique.

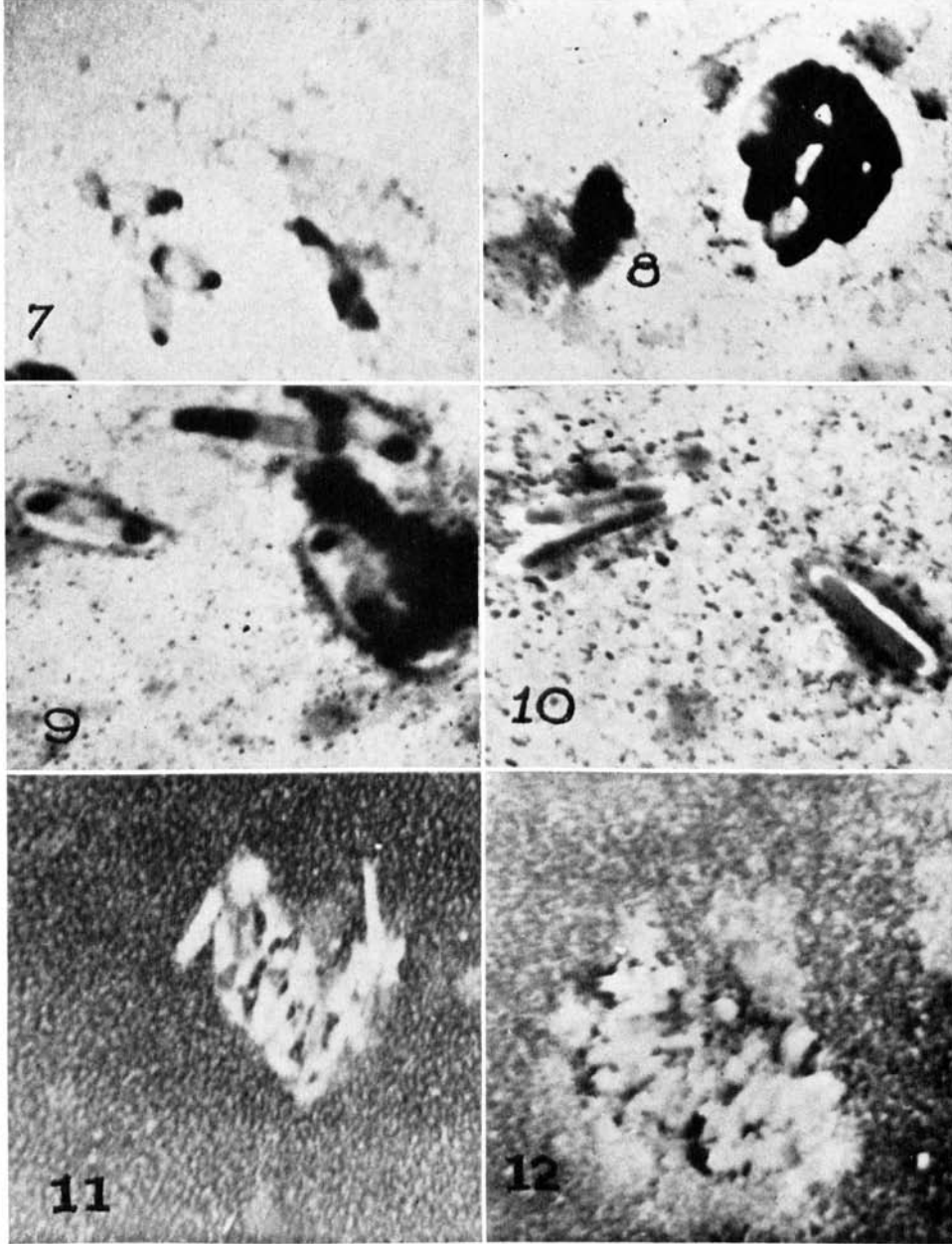


PLATE 7.