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THE MECHANISM OF ACTION OF THE SULFONE DERIVATIVES IN LEPROMATOUS LEPROSY [1]

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". . . even incorrect theories are often of great help in unveiling the secrets of nature, as long as we regard them merely as concrete formulations of possibilities, which, by virtue of their concreteness, lend themselves to be proven or disproven by subsequent observation."—SELYE (12).

This paper conveys the personal opinions of the authors with regard to the apparent mechanism of action of the sulfones on human lepromatous leprosy, based on experience acquired in some thousands of histopathologic and bacteriologic examinations realized in daily routine work in accordance with classical techniques. This paper is not, therefore, the result of experiments purposely made in accordance with a planned aim or with a special technique for the purpose of proving a definite hypothesis previously arrived at.

The series of photomicrographs that is presented is meant only to be a visual support of what is said. They have been selected from material from untreated cases and from others treated with sulfones. They have nothing special about them, and they show only an aspect of the question which any pathologist working in leprosy can see easily in similar material at his disposal. This has to do with lepromatous leprosy; we do not have sufficient material from sulfone-treated cases of the

[1] This article was originally published in the *Revista brasileira de Leprologia* **18** (1950) 59-68, in the Portuguese language. In this translation, which was made by Mr. S. A. Baba to whom we are indebted, certain modifications have been made for purposes of clarification. Some bibliographic references have been added, and the numbering of the figures has been changed.

tuberculoid type or of the indeterminate group of leprosy to permit us to form opinions or draw conclusions as to the action of the sulfone derivatives in those forms of the disease.

As everybody knows, lepromatous leprosy is characterized by the presence of fundamental lesions: the lepromatous infiltration and the leproma, found mainly in the skin. Both are formed, essentially, by variable numbers of histiocytic (Virchow) cells, assembled together and sometimes forming tumor-like masses. Within these cells are found the agent of this disease, the Hansen bacillus.

Before the advent of the sulfone era it had already been observed that such lesions do not last indefinitely. Sometimes, either due to the then-used drugs or spontaneously, they underwent regression. This regression, in some cases, was complete, healing taking place to leave only scars.

The histologic and bacteriologic appearance of lesions showing this regressive evolution were studied and described in 1942 by one of us (R. de S.) and Alayon (9). That paper dealt principally with the behavior of the lipids of the lesions, and it also described in some detail the morphological alterations of the cells which contained them, i.e., the Virchow cells in regression. At that time, however, we had less experience than now, and for that reason we did not perceive, with precision, the correlation which exists between the morphological appearance of the cell and its contents of bacilli and lipids. Our present point of view is as follows:

The Virchow cell in regression is definitely swollen, with a pycnotic nucleus and a cytoplasm with a great number of rounded vacuoles of various sizes which give it a foamy appearance (Figs. 4, 10, and others). After staining with scarlet red these vacuoles are seen to be filled with lipids (Figs. 6, 8 and 14), forming masses and droplets and taking on a hue which is redder than the lipids seen in Virchow cells which are in less pronounced regression. In the latter case the lipids are less abundant, and they take a clearer color which tends towards orange.

Virchow cells which are not in regression are smaller than those in regression. They have a vesicular nucleus. Their cytoplasm, contrary to what is currently believed, is not vacuolated, and still less is it foamy. This cytoplasm has an appearance *sui generis*, accurately described by Leon Blanco and Fite

(5), with whose description we concur. The most highly active lesions:

". . . . are recognized histologically both from the large numbers of organisms present and from the character of the cells containing the organisms. These are not the vacuolated cells of the older leproma but are fairly simple macrophages of a wide variety of shapes and forms. In ordinary sections the parts of the cell occupied by the bacilli can be identified only by hollow areas in the cytoplasm which are not distinct vacuoles. Indeed, the numbers of organisms present would not be suspected, with the abundant and well stained cytoplasm."

This morphological appearance can be seen in Figs. 1, 2 and 3, to be compared with those referred to which show the foamy cells. In this phase of their evolution the Virchow cells do not contain lipids.

With regard to the content of bacilli, we can see that these cells which are in regression contain few or rare acid-fast "bacilli of granular appearance" (Figs. 9 to 11), and they may even contain no bacilli whatsoever.[2] Virchow cells which are

[2] With respect to granulations, we differentiate two types of bacilli: (*a*) the "granular bacillus," and (*b*) the "bacillus of granular appearance." This terminology is used, conventionally, for the purpose of distinguishing between forms of the bacilli which, we believe, are different. For a better understanding of this division it will be convenient to recall the following aspects of the granulations of the Hansen bacillus when stained with Ziehl-Neelsen:

1. Acid-fast granulations inside of distinct, individual bacilli. In this case the granulations are set in a line the diameter of which is sometimes greater than that of the bacillary cylinder, and they take more stain than does the body of the bacillus. This aspect characterizes the "granular bacillus" which we regard as a particular departure from the typical bacillus, which is an evenly-stained acid-fast rod.

2. Acid-fast granulations which no longer are inside distinct, individual bacilli. In this case they have the appearance of a string of beads kept in line by thin filaments which are much less deeply stained than the granulations, and are therefore hardly visible. Frequently one finds only two granulations joined together by one filament, giving to the bacillus a bipolar appearance (Fig. 10). These are the characteristics of what we call the "bacillus of granular appearance," which is different from the "granular bacillus."

3. Acid-fast granulations which are scattered and not bound together by filaments. This is the aspect of the so-called "acid-fast granulations" which, in our opinion, result from disintegration of the "bacilli of granular appearance."

4. Acid-fast granulations which are definitely smaller than the previous one, and which constitute the so-called "bacillary dust" of Marchoux.

These aspects correspond, in general, to those described by Chaussinand (1).

Since, in our opinion, the "acid-fast granulations" represent the dis-

not in regression are seen teeming with typical bacilli forming globi (Fig. 2).

It is important to emphasize that, according to our present experience, the quantity of lipids found is in general inversely proportional to the number of bacilli. Virchow cells which definitely are not in regression and are loaded with bacilli (Figs. 1 and 2) do not show any lipids when stained with scarlet red, while cells that are definitely in regression and have very few acid-fast bacilli of granular appearance are found to be loaded with lipids when similarly stained.

During the last few years, with the widespreading use of sulfones, it was noticed that lepromatous lesions in regression began to be seen more frequently than previously, and as time passed this frequency increased steadily and sharply. This fact is in agreement with the clinical observations of leprologists that, when sulfones are used, the regression of the lesions is certainly more frequent than under other treatments and comparatively rapid. In some cases the clinical improvement of the patients is spectacular. This regression of the lesions should not, however, be confused with the actual healing of the patient, which takes a longer time.

In the great majority of cases, however, the lepromatous lesions in regression of patients treated with sulfones show the already known histopathological appearance.[3] They are formed by Virchow cells that are swollen, with pycnotic nuclei, the cytoplasm riddled with vacuoles which contain lipids in the same proportion and with the same morphological and staining characteristics as we have already described for cells that are in regression.

Bacteriological findings go hand in hand with the histopathological findings, and nothing new is seen. Instead of the

integration of the "bacillus of granular appearance," both being signs of bacillary injury, it is likely that it would be very difficult to find in a given preparation one of these aspects without having the other at the same time. For this reason, and to simplify our terminology, when we speak of "bacilli of granular appearance" we mean to convey the idea that both kinds of structures are present.

[3] In comparatively rare cases of lepromatous leprosy treated with sulfones L. de Souza Lima and Rath de Souza ([13]) observed that, clinically, there had been an apparent exacerbation of the lesions. Histological examination revealed, however, that invasion by cells of epithelioid type was not answerable for this. This invasion gave to the lesions an appearance that was more or less similar to that of tuberculoid reactional leprosy ("pseudoexacerbation").

great concentration of typical bacilli, in globi, which fill the cytoplasmic cavities of the cells that form the lepromatous lesions which are not in regression, we find a great scarcity of bacilli. Those which are found show the granular appearance, while in certain cases the lesions may be completely free of bacilli.

It is clear that the histopathologic and bacteriologic picture of the lesions of sulfone-treated patients coincides with that of the lesions in regression of cases studied before the beginning of the sulfone era. Although Fite and Gemar (2) arrived at a different conclusion, they were nevertheless of the opinion that:

> "Under promin treatment, the improvement in leprosy is not accompanied by characteristic cellular changes . . . . These changes do not differ materially from similar changes occurring in spontaneous remission without treatment of any sort, ..."

This lack of distinction seems to us surprising, because with the application of new drugs which presumably should exercise a direct action against the Hansen bacilli without injury to the Virchow cells we did not expect to find these cells degenerated to the extent that they are. What we anticipated finding was, (*a*) well-preserved Virchow cells and (*b*) very rare and disintegrated bacilli or even no bacilli at all.

We have never seen, however, either before or after the sulfone era, lesions predominantly formed by cells of nonregressive appearance, containing rare and disintegrated bacilli. Such lesions, according to our experience, always contain numerous acid-fast bacilli, the majority of which are typical.

On the other hand, in lesions of regressive appearance we have already stated that the rule is to find few or rare bacilli of granular appearance and sometimes no bacilli at all. In some cases, however, it has been possible to find in lesions which histologically were regressive in appearance, with or without sulfone treatment, bacilli still numerous and partly typical. This fact, it seems to us, indicates that the process of degeneration of the cell begins *before* the bacillary disintegration. If this is proved to be the case it will be evidence of the *indirect* action of the sulfones against the Hansen bacillus.

Let us, now, consider some aspects of the biology of Hansen bacillus. It is our personal conviction that this bacillus is a parasite strictly adapted to the histiocyte of human beings in whom the conditions are favorable for the development of the lepromatous type of the disease. The Virchow cell, therefore,

actually constitutes the habitat of the Hansen bacillus, and for this reason we think that it can live and multiply, at least substantially, only inside the histiocyte which itself proliferates and is "colonized" by the bacillus. This essentially, characterizes the lepromatous type of leprosy. We have already expressed this view in a previous paper with other collaborators (14), and many other workers are of the same opinion. From among more recent authors whose statements strengthen this point of view, although not all of them mention leprosy specifically, the following are quoted:

McCutcheon (7) says, with regard to the eventual failure of phagocytosis: "In kala-azar and leprosy the parasites are able to live and multiply inside macrophages, which thus constitute their natural host: Thus phagocytosis may prove not to be a defense mechanism but a source of danger to the host."

Pinkerton (8) says: "The obligate intracellular parasites include primarily the ricketsiae and viruses, although certain protozoa, fungi and bacteria (*Mycobacterium leprae*, for example) also belong to this group. The metabolites necessary for the growth of these agents are unstable compounds which are formed by intracellular enzymes as intermediary metabolic products, and which are appropriated by the parasites for their own use."

Mauri and Hadler (6), in reporting on an experimental study of murine leprosy, which is similar to the lepromatous type of human leprosy, stated *inter alia* that phagocytosis of the causative germ has no favorable influence on murine leprosy, contrary to what is observed in other diseases, and that it is even necessary for the normal evolution of the Stefansky disease. They stated further that "blockade" of the reticuloendothelial system acts to delay the evolution of murine leprosy, and that "stimulation" of the histiocytic system favors its development.

Forbus (3) says with regard to the pathologic significance of granulomata, and using histoplasmosis as an example, which in our opinion can be applied *mutatis mutandis* to lepromatous leprosy: "Surely the activities of the macrophages, whose reactions above all else characterize granulomatous inflammation, are not in all respects defensive. Indeed, some are most unfavorable to the host. An especially good example of what I mean is to be found in the case of the granuloma of histoplasmosis. In this disease the reticuloendothelial cells actually serve as the specific host of the fungus, *without which one may doubt that the disease could exist.*" (The italics are ours.)

Rich (10) says in his book on tuberculosis: ". . . the fact that the monocytes in the lesions often do not destroy all of the bacilli that they phagocytize, led Doan and Sabin to regard these cells as 'a liability rather than an asset to the defenses of the body,' and they attempted to increase resistance to tuberculosis by destroying the body's monocytes by means of an anti-monocytic serum." Tuberculosis, however, has its peculiar aspects, and Rich refutes the measure proposed by Doan and Sabin when he says: "Imperfect as these cells undoubtedly are in ridding the body of the bacilli

in many cases, they constitute, in the light of present information, the only protection that the body possesses against the tubercle bacillus."

In murine leprosy—which, we repeat, is similar to human lepromatous leprosy—the experiment of Mauri and Hadler already referred to shows, however, that the histiocytes are clearly unfavorable and are in no way of use in the defense of the body, and that they can, therefore, be "blockaded" without causing harm and even to advantage.

With these notions on the biology of a certain group of microorganisms one can easily realize that the Hansen bacillus, in order to live and multiply successfully, would need a Virchow cell in a morphologically and functionally good form. Degenerated cells, with their altered metabolism, are evidently a bad culture medium for it. If the sulfone derivatives have a direct action against the Hansen bacillus, it seems to us strange that the numerical diminution and the disintegration of this microorganism should always be accompanied by evident alteration in the morphology of the cells containing it, in the same manner as was observed before the advent of the sulfones. This fact is much easier to understand if we admit that the sulfone derivatives act principally against the Virchow cell. The degeneration of this cell, in its turn, would reflect unfavorably on the Hansen bacilli within it.

Clinical observation shows that the present treatment of leprosy with sulfones requires the administration of extremely large quantities of the drugs during a notably long period. This, however, is not the case in the treatment of other infections in which the action of the drugs used—chemotherapeutics and antibiotics—is undoubtedly bacteriostatic. Johansen and Erickson (4) support this view when they say: "The slow disappearance of the organisms from leprous lesions, however, would indicate that sulfones have little if any direct destructive effect upon the acid-fast organisms in the tissue cells."

We do not intend to deny that the sulfone derivatives may exercise a direct action against the Hansen bacillus because we do not have sufficient evidence to support such an intention, while other investigators (11) have demonstrated that sulfones may have an action which is exclusively bacteriostatic against different organisms—but which are not the leprosy bacillus. We also do not want to deny that, besides the action against the Virchow cells which seems to us to be the most important effect, there may be a concomitant or conjugated direct action against the bacillus.

However, for the morphological, biological and clinical reasons that we have presented we are strongly inclined to believe that, although the sulfones exercise a definite beneficial effect, they do not go further than to start, accelerate and intensify some mechanism which seems to be already present in what we call the *Virchow cell-Hansen bacillus complex*. The sulfones, in our opinion, act principally on the Virchow cell component, altering in some way its metabolism and rendering its cytoplasm unsuitable for the multiplication and survival of the bacillus.

An identical mechanism is also put to work, although not so regularly or effectively, either when other methods of treatment are applied or under natural conditions without treatment. To explain what happens in cases where no treatment is applied we are of the belief that the Hansen bacillus, because of biological factors which are not yet known but which may be connected with its multiplication beyond a certain degree, may be able by itself to create the disequilibrium of the Virchow cell-Hansen bacillus complex. This disequilibrium determines the degeneration of the Virchow cell and consequently the disintegration and disappearance of the bacillus itself and the regression of the lesion.

We are of the opinion that if consideration is given to the circumstances involved a better understanding of the mechanism of action of these drugs will be possible.

SUMMARY

This present work is based on the experience gained by the authors in the histopathological and bacteriological examinations of material from some thousands of cases of lepromatous leprosy, done before and after the introduction of the sulfone derivatives in the treatment of leprosy.

The authors do not deny the existence of a direct action of these drugs against the Hansen bacillus. However, for morphological, biological and clinical reasons, they are strongly inclined to believe that, although the sulfones exercise a definite beneficial effect, they do not go further than to start, accelerate and intensify some mechanism which seems to be already present in what they call the Virchow cell-Hansen bacillus complex. The sulfones act principally on the Virchow cell component, in some way altering its metabolism and making its cytoplasm unsuitable for the life of the Hansen bacillus.

An identical mechanism is also operative, although not so regularly or effectively, either when other ways of treatment are applied or in natural conditions when regression of the lesions occurs without treatment. This hypothesis has been arrived at on the strength of the following facts:

1. Morphological: The lepromatous lesions that start regressing when the sulfone treatment is applied are like the lesions in regression observed, although not so frequently, when no treatment whatsoever is applied. The characteristics of these lesions are: (*a*) swelling of the Virchow cells, pycnosis of the nuclei, vacuolation of the cytoplasm with foamy appearance, the presence in the cytoplasmic vacuoles of lipids found in quantities inversely proportional to the number of bacilli—these constituting signs of injury of the cells; and (*b*) scarcity and granular appearance of the Hansen bacilli—signs of bacillary injury. It is believed that the degeneration of the Virchow cell begins before the bacillary disintegration.

2. Biological: The biology of Hansen bacillus indicates that it depends strictly on the Virchow cell in order to live and multiply successfully.

3. Clinical: The present treatment of leprosy with sulfones requires the administration of extremely large quantities of the drugs during notably long periods. This, however, is not the case in the treatment of other infections in which the action of the drugs used—chemotherapeutics and antibiotics—is unquestionably bacteriostatic.

RESÚMEN

Los autores presentan sus opiniones personales sobre el mecanismo aparente de la acción de las sulfonas en la lepra lepromatosa humana. Basan sus opiniones en experiencias de miles de casos lepromatosos estudiados, antes y después de la introducción de las sulfonas.

Los autores no niegan la posible acción directa de las drogas sobre el bacilo de Hansen. Sinembargo, creen ellos que tales drogas actúan por medio del comienzo, acceleración, o intensificación de mecanismos celulares ya existentes y los cuales ellos llama "complejo célula-Virchow bacilo-Hansen." Las sulfonas actúan principalmente en el componente célula-Virchow, en alguna forma alterando su metabolismo y haciendo su citoplasma no favorable al bacilo-Hansen.

Mecanismos idénticos o similares ocurren ya con otras formas de tratamiento, ya con regresiones clínicas espontáneas.

Razones clinícas, biológicas y morfológicas, respaldan, de acuerdo con los autores, la hipótesis anterior.

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DESCRIPTION OF PLATES

(The magnifications given are those of the original photographs, before reduction in reproduction.)

PLATE 18.

FIG. 1. Biopsy No. 17708-50. Leproma not in regression, from the skin of the dorsal aspect of the left hand. There is no swelling of the Virchow cells, the nuclei are vesicular, and the cytoplasm is not vacuolated. The clear spaces in this photomicrograph represent either capillaries or empty tissue spaces, and not cytoplasmic vacuoles. Sections of the same lesion stained with scarlet red were negative for lipids. Hematoxylin and eosin, 780×.

FIG. 2. Same specimen as in Fig. 1, stained by the Ziehl-Neelsen method (Faraco's modification), the nuclei stained with hematoxylin. The Virchow cells contain great numbers of typical acid-fast bacilli, forming globi. 780×.

FIG. 3. Biopsy No. 11013-47. Leproma not in regression, from the skin of the right forearm, before sulfone treatment. Sections stained for bacilli showed them in great numbers, of typical morphology. Hematoxylin and eosin, 220×. (Courtesy of Dr. R. Braga.)

FIG. 4. Biopsy No. 12923-48. Same lesion as in Fig. 3, after one year of sulfone treatment. The leproma is now in regression: swelling of the Virchow cells, pycnosis of the nuclei, and definite vacuolation of the cytoplasm. Sections stained for bacilli showed rare "bacilli of granular appearance." Hematoxylin and eosin, 220×. (Courtesy of Dr. R. Braga.)

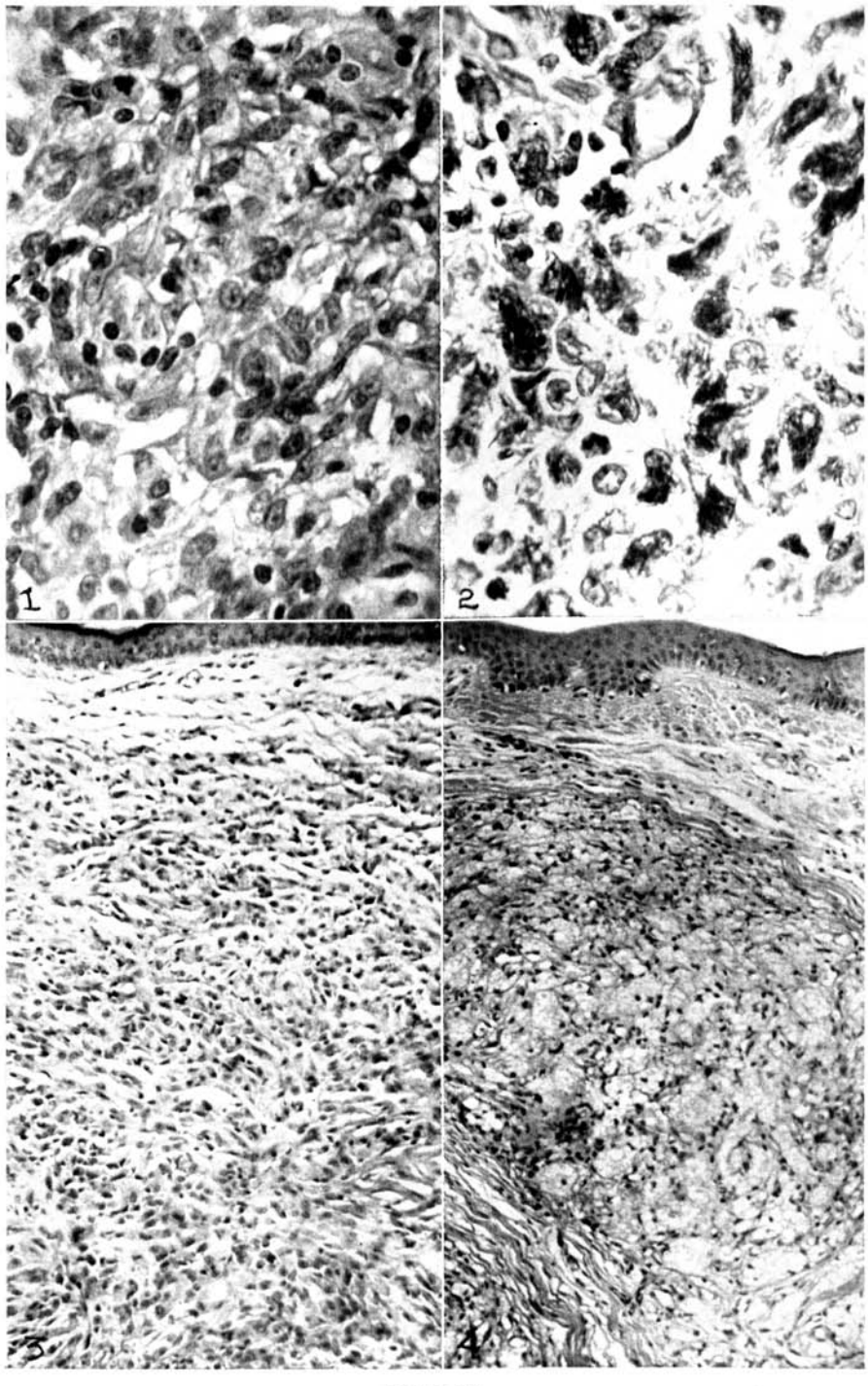

PLATE 18.

PLATE 19.

FIG. 5. Biopsy No. 6300-42. A lepromatous lesion in regression, from the skin of the left thigh. Swelling of the Virchow cells, nuclear pycnosis and definite cytoplasmic vacuolation. No sulfone treatment. Sections stained for acid-fasts showed rare bacilli of granular appearance. Hematoxylin and eosin, 130×.

FIG. 6. Same material as in Fig. 5, stained with scarlet red. Showing the great amount of lipids in cells identical to those shown in Fig. 5. 130×.

FIG. 7. Biopsy No. 6262-42. A lepromatous lesion in regression from the skin of the thigh, showing a similar appearance to that in Fig. 5. No sulfone treatment. No bacilli found in this instance. Hematoxylin and eosin, 130×.

FIG. 8. Same material as in Fig. 8, stained with scarlet red. Appearance similar to that in Fig. 6. 130×.

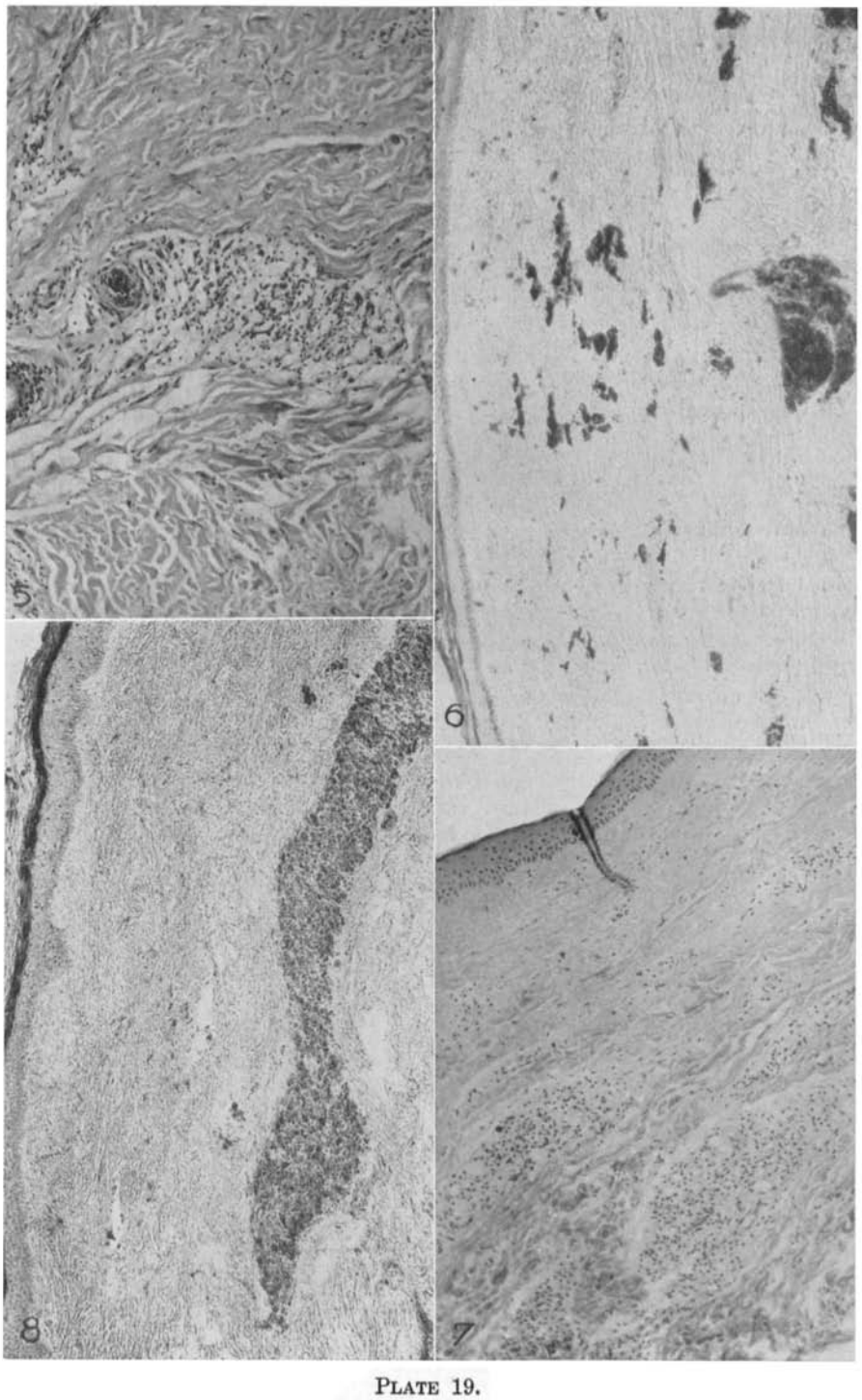

PLATE 19.

PLATE 20.

FIG. 9. Biopsy No. 17,772-50. Skin of the right leg, from a sulfone-treated case. Showing an isolated Virchow cell in regression, with cellular swelling, nuclear pycnosis and advanced cytoplasmatic vacuolation. Some bacilli of granular appearance are shown in this cell. Ziehl-Neelsen (Faraco), 770×.

FIG. 10. Biopsy No. 4263-50 (Sanatorio Padre Bento). Skin of the left thigh, from a sulfone-treated case. Showing a group of three Virchow cells in regression, bordered on one side by a capillary containing red blood cells. Cellular swelling, nuclear pycnosis and cytoplasmatic vacuolation. Rare bacilli of granular appearance, some bipolar, are seen. Ziehl-Neelsen (Faraco), 770×.

FIG. 11. Biopsy No. 17942-50. Skin of the left thigh, from a sulfone-treated case. Some Virchow cells in regression and a foreign-body giant cell with an "asteroid body." A very few bacilli of granular appearance are seen inside the Virchow cells and near the asteroid body. Ziehl-Neelsen (Faraco), 770×.

FIG. 12. Same material as Fig. 11, stained with scarlet red. Virchow cells loaded with lipids. These cells are similar to those shown in Figs. 4, 9, 10 and 11. 770×.

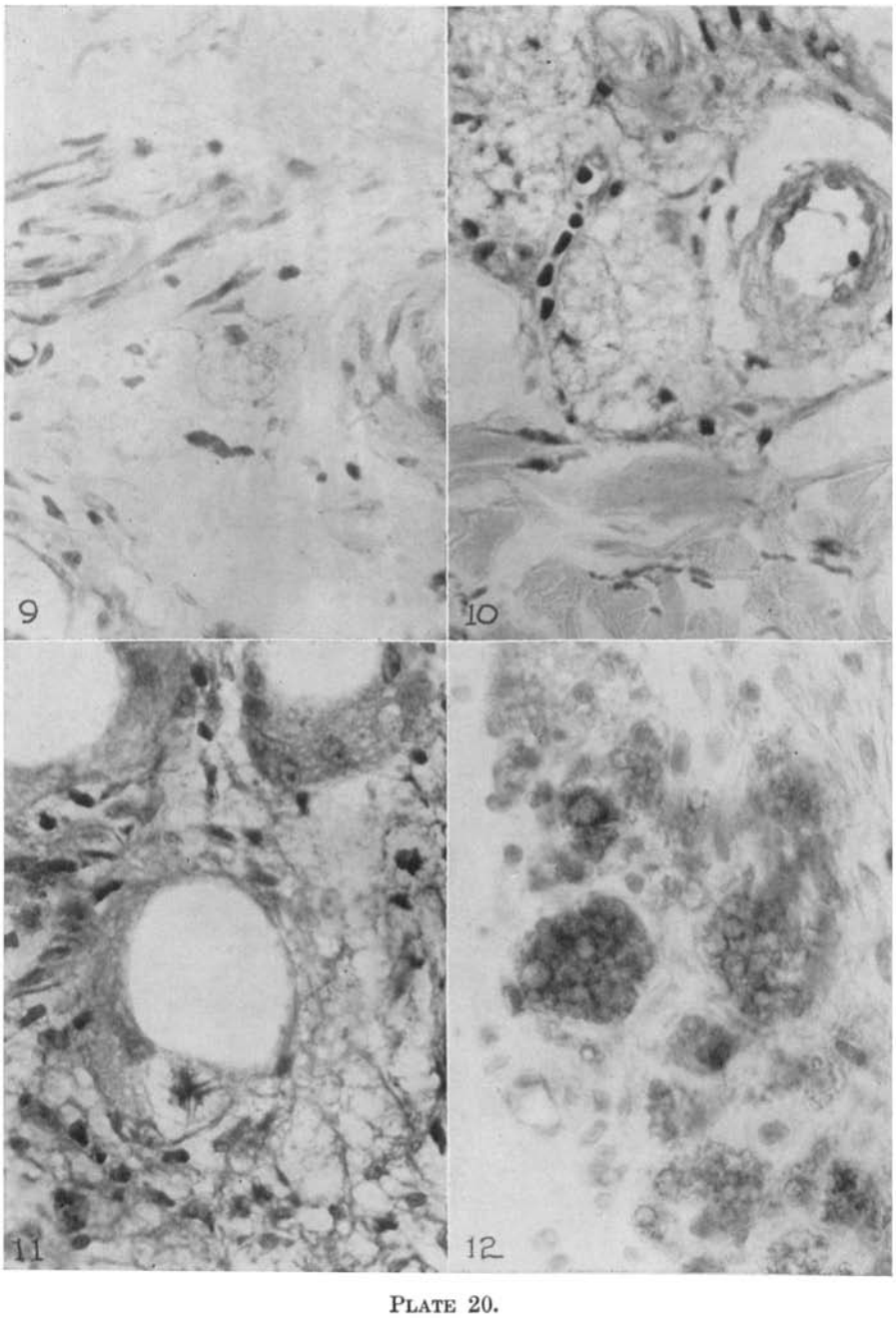

PLATE 20.