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Studies on Sulfone Resistance in Leprosy

I. Detection of Cases[1]

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Almost to be expected, as the concomitant of a new chemotherapeutic agent, is the development of drug resistance by formerly sensitive organisms. Sometimes this is such a frequent occurrence that the drug ceases to be useful; with other drugs the appearance of resistant strains is a rarity. Throughout the twenty year experience with sulfones in leprosy, the incidence of resistance has appeared to be low. There are, however, a number of patients, both in and outside leprosaria, who are supposed to have been on adequate antileprosy treatment but fail to show the improvement expected. Wolcott and Ross [23] claimed that such patients had developed resistance to sulfones, but many leprologists could not accept this because of lack of supportive laboratory evidence.

In the last few years considerable advances have been made in the study of leprosy. Work [12, 13] on the morphologic appearance of the bacilli in stained smears taken from patients has now been brought to the point where it has been claimed [2, 22] that a change in the morphology of *Mycobacterium leprae* can be used to assess the response to antileprosy treatment. If the percentage of viable bacilli (solid-staining organisms), referred to as the Morphologic Index (MI), does not fall significantly, and usually to 5 or less, within six months of starting treatment, it is probable that the patient is not responding properly. Furthermore, studies initiated by Shepard [17] and confirmed by Rees [10] and others [4, 7], have shown that *M. leprae* can be grown in the foot pads of mice and that this growth is dependent upon the number of live bacilli injected [19] and can be inhibited by giving the animals dapsone (DDS) and other [11,18] antileprosy drugs. The application of these two advances provides, for the first time, more definitive methods for the investigation of patients suspected of having developed drug resistance. Suspicion would be strengthened by finding a high MI in such patients and confirmed by showing that their bacilli multiplied in mice receiving the suspected drug.

It was decided, therefore, that a search should be made in Sungei Buloh Leprosarium for patients who had been receiving treatment for a long period but had apparently failed to respond. As a secondary objective, the opportunity has been taken to make a histologic study of acute relapsing leprosy, the results of which are reported in an appendix.

SELECTION OF PATIENTS

Sungei Buloh Leprosarium has a popula-

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tion of some 2,500 patients; many stay for a relatively short time, but others have spent years in the Settlement for medical, social, or economic reasons. A search was made in this community to see if any patients could be found who gave *prima facie* evidence of sulfone resistance based on a lack of clinical improvement over at least five years and an absence of a satisfactory fall in the Bacterial Index (BI). Such patients were screened by a brief clinical examination. Smears were taken from six different sites, including both ear lobes. The BI was recorded using Ridley's logarithmic scale ([14]) and the MI was estimated. Some patients were found who still had fairly large nodules, but whose MI was low. These patients were considered to be responding to treatment, perhaps more slowly than usual, and the satisfactory bacterial picture indicated that such cases need not be further studied. We include below the details of nine patients who were suspected of having sulfone-resistant infections, this suspicion in all cases being based both on a failure of clinical improvement and a high average BI and MI. Seven of the patients have already been referred to in a preliminary communication ([8]), and for ease of reference these are the first seven cases reported here.

Case 1. (No. 5075) Indian male, age 41. This patient was admitted to the Settlement in 1937 and treated with hydnocarpus oil until 1948. From then until 1961 he had injections of sulfone, 2 ml. (equivalent to 400 mgm. DDS) twice weekly. In May 1961, because of the apparent failure of treatment, he was changed to thiambutosine (DPT), 2 tablets twice daily (2 gm. per day) for 10 months, but clinically this produced little effect and he returned to sulfone treatment early in 1962. All skin smears recorded in the hospital laboratory were at this time strongly positive and the patient was referred to the Research Unit for study. He had extensive lepromatous leprosy with large shiny nodules on all parts of the glabrous skin, and looked like an untreated patient. Smears taken in February 1963 gave an average BI of 4.8 and MI of 37, and a biopsy taken at that time was reported as showing a very heavy and active infiltration leproma (LL) ([3]). There were many densely packed macrophages stuffed with bacilli and a slight cellular infiltration of nerves which suggested that the patient had been BL and that he might possibly revert to BL or BB with successful treatment. The lepromin (Mitsuda) test was negative (0 mm.).

Case 2. (No. 9055) Chinese male, age 46. This patient was admitted to Sungei Buloh in April 1946 and treated with hydnocarpus oil until 1948. He was given injections of sulfone (usually 2 ml.) twice weekly from 1948 until 1958 and then was changed to two 100 mgm. tablets of DDS twice weekly until he was referred to the Research Unit. At this time the patient had extensive lepromatous leprosy with large nodules particularly on the chest and upper limbs. The lepromin (Mitsuda) test was negative (3 mm.), and the biopsy was reported as showing a very large and active infiltrative granuloma with many bacilli in foamy cells at the center, but some epithelioid-like cells in a focus near the periphery. The histologic classification was BL to LL. Skin smears taken from six different sites in the Research Unit showed an average BI of 4.2 and MI of 32.

Case 3. (No. 9386) Chinese male, age 58. This patient entered the Settlement in December 1946 and was treated with hydnocarpus oil until 1948, after which time he had injections of sulfone, 2 ml. (400 mgm. DDS) twice weekly, except for two years when he was given two 100 mgm. tablets of DDS twice weekly instead. On his admission the hospital smears had been heavily positive and at no time did any site become negative. When he was referred to the Research Unit, clinical examination showed multiple small nodules on most parts of the trunk and limbs and it was thought that, although he may have changed from purely lepromatous to near lepromatous in the course of 15 years of sulfone therapy, he certainly had not responded adequately and was probably a resistant case. The Research Unit smears

[3]All histologic diagnoses are based on the research classification described by Ridley and Jopling ([15]).

showed an average BI of 3.7 and MI of 38. Biopsy of a nodule was reported as showing a large "expansile" granuloma with slightly foamy histiocytes and a number of lymphocytes and plasma cells. The histologic classification was BL or LL. The lepromin (Mitsuda) test was negative (3 mm.).

Case 4. (No. 10458) Chinese male, age 50. This patient came into the Settlement in May 1949 and from then until 1952 received 2 ml. of injectable sulfone twice weekly. From 1952 to 1953 he was given one Sulphetrone tablet (500 mgm.) three times a day and from then until 1958 he received Sulphetrone injections, 4 ml. (2 gm.), twice weekly. Early in 1959 treatment was changed back to sulfone injections and from late 1959 until the end of 1961 he was given DDS tablets. For the first ten months of 1962 he again received sulfone injections and toward the end of 1962, at his own request, he received further treatment with aqueous injections of Sulphetrone. On his admission the smears had been strongly positive in all sites, and, although by 1960 they were reported as uniformly negative, from 1961 onward examinations of sites apparently not previously studied were invariably positive. The patient had severe nodular lepromatous leprosy and was convinced that he did not respond to sulfone treatment either by mouth or injection. The Research Unit smears taken in March 1963 showed an average BI of 3.9 and MI of 43. A biopsy taken at this time was reported as showing a large active infiltrative granuloma composed of large undifferentiated histiocytes. The classification was probably LL. The lepromin (Mitsuda) test was negative (0 mm.).

Case 5. (No. 10607) Chinese male, age 34. This patient came to Sungei Buloh in November 1949 and received aqueous injections of Sulphetrone (4 ml.) twice weekly until 1957. From 1958 until mid-1962 he received injectable sulfone (300 mgm. DDS) twice weekly and then started a further course of Sulphetrone. From 1949 until he was referred to the Research Unit no smear from any site had ever been negative, and when he was first seen in our Unit he was covered with large active nodules of lepromatous leprosy and the average smears were BI 4.7 and MI 53. Biopsy showed a large active infiltrative granuloma composed of undifferentiated histiocytes; probably LL. The lepromin (Mitsuda) test was negative (0 mm.).

Case 6. (No. 10735) Chinese male, age 43. This patient entered the Settlement in May 1950 and received 4 ml. (2 gm.) of aqueous Sulphetrone twice weekly until 1957. He was then put on injections of sulfone until mid-1962 when, because of persistent positivity of smears, he was changed to DPT, two tablets twice daily (2 gm.). All hospital smears had been positive throughout his stay and the Research Unit studies in March 1963 showed an average BI of 4.3 and MI of 36. When first seen at our Unit he had many large, shiny, erythematous nodules on the body, face and limbs and enormous plaques of lepromatous infiltration over the arms and hands. The lepromin (Mitsuda) test was negative (0 mm.) and the histology was reported as showing so many bacilli that it was almost impossible to count them. There was a very active tumor-like mass of macrophages in a large "expansile" nodule. Histologically as well as clinically the classification was LL.

Case 7. (No. 10757) Chinese male, age 60. He was admitted in May 1950 and treated until 1953 with Sulphetrone injections 5 ml. (2.5 gm.) twice weekly and then he was changed to sulfone injections 1 ml. (200 mgm. DDS) twice weekly until late 1958. After that time he was given DDS tablets (400 mgm.) twice weekly until January 1963. The patient was referred to us because of obvious persistent activity of his lepromatous leprosy, and the Research Unit smears showed an average BI of 4.0 and MI of 48. The patient had a rather thick skin and seemed to have a more diffuse type of lepromatous leprosy than the others in the series, although he too had a number of nodules on the skin, situated particularly over the bony prominences of the limbs. The lepromin (Mitsuda) test was negative (0 mm.). Histologically he showed a large, partly infiltrative and partly "expansile" granuloma with

many macrophages and a few lymphocytes and plasma cells. There were foci of polymorphonuclear leucocytes under the epidermis. The classification was LL.

Case 8. (No. 5285) Chinese male, age 39. This patient was admitted to Sungei Buloh in January 1938. There is some doubt as to what treatment he received for the first ten years, but it was probably hydnocarpus oil. From 1949 until 1957 he was treated with aqueous injections of Sulphetrone, and after a few injections of sulfone early in 1958 he was put on DDS tablets until March 1960. From March 1960 he was given further injections of sulfone, 1.5 ml. (300 mgm. DDS), twice weekly until early in 1963, when the dosage was increased to 2 ml. twice weekly. The smears in the hospital were reputed to have steadily diminished from 1948, but when he was referred to the Research Unit on 8 November 1963, because of clinical failure to improve, smears then taken showed an average BI of 3.7 and MI of 43. The lepromin (Mitsuda) test was positive (+5 mm.). The biopsy was reported as showing the fibrotic cytologic structure that seems to be the most important feature of histoid leprosy, but in this case the lesion was infiltrative and not "expansile." It was classified as very active LL.

Case 9. (No. 10663) Malay male, age 41. He came into the Settlement in 1950 and was said at that time to have negative smears from both ears but positive smears from the back. By the middle of 1963, however, both ears had become positive and he was covered with nodules with a flat shiny surface. He had been having injections of sulfone since admission to the hospital, usually 2 ml. (400 mgm. DDS) each week, but had not taken the treatment regularly and in 1962 and 1963 had had only about two-thirds of the injections that he should have received. When he came to our Unit in August 1964, the average BI was 5.0 and the MI 34. At that time he showed a large number of small shiny nodules and plaques on the trunk, face and limbs. The lepromin (Mitsuda) test was negative (0 mm.) and the histology was reported as that of a very active case of LL, similar to the previous case in that there was a fibrotic lesion which had some histoid features but was not "expansile."

STUDY OF SELECTED PATIENTS

The nine selected patients from the Leprosarium with *prima facie* evidence of sulfone resistance were admitted to the Research Unit for a six months trial period on DDS and on admission bacilli were obtained from a biopsy of skin for DDS sensitivity tests in mice. The "trial period" was rigorously controlled, all the criteria for a chemotherapeutic trial ([21]) being used to determine the response of these patients. To make certain that the patients were actually receiving the prescribed drug, DDS was given by injection, 1.5 ml. (300 mgm.) twice weekly, and all the injections were administered by our own staff.

INVESTIGATIONS

Clinical. The first seven patients were studied together and the other two later and separately. On admission to our Unit each patient was carefully investigated with detailed charting and photographing of his lesions. Skin smears for recording the BI and MI were taken from six sites, and two skin biopsy specimens were sent to London, one on ice for the experimental bacteriologic studies and the other in fixative for histologic study. Skin smears from the same six sites were repeated after three, four and six months of therapy. The following investigations also were performed: hemoglobin, white blood cell and differential counts, serum proteins, urine analysis, and pulmonary radiographs. The blood and urine studies were repeated monthly, but are not mentioned further in this paper, as no significant abnormality was detected. Similarly, the serum proteins were found to be within normal limits at the beginning of the trial, and it was not considered necessary to repeat them.

To ensure that there was no metabolic defect and that absorption of the injected material was satisfactory, blood samples were taken immediately prior to an injection, and 24 and 72 hours after injection, for sulfone assay by the method of Francis

and Spinks ([3]). In order to ensure that there was no change in the situation of these patients this assay was made not only at the commencement of treatment but again three months later.

Each of the patients was reexamined after six months of treatment in the Research Unit, including approximately 56 injections. An occasional patient had missed an injection when he was on leave, but none had received less than 53 injections in the previous six months. Clinical assessments were made as accurately as possible, being aided by the clinical photographs and the charts of lesions that had been prepared, and an attempt was made to determine if the leprosy had improved during the period of the study. It was also noted whether the patients had erythema nodosum leprosum (ENL) or any other type of reaction during the previous six months. The patient's own view as to his progress also was sought. At the same time biopsy specimens were taken from as near as possible to the previous site. In retrospect it is regrettable that only a single biopsy was made on each occasion. The use of single biopsies to estimate an individual patient's progress is not reliable and such an investigation is of value only when the biopsy indices of a large series were estimated. It is strongly recommended that if these studies are to be repeated two biopsies should be made on each occasion, as is the usual practice in the Research Unit's drug trials. Two biopsy specimens were taken from patient No. 9, the most recent admission to this study. There is, however, a practical difficulty in the matter of repeating two biopsies in these resistant cases. Most of the patients did not have large plaques of infiltration, such as are normally selected as the sites of serial biopises, but were studded with small nodules, frequently rather flat-topped and shiny. It is a matter of some doubt whether comparison can fairly be drawn between biopsies of such nodules at six-month intervals.

Experimental. The DDS sensitivity of the strains of *M. leprae* from the nine patients was determined by use of the newly developed mouse foot pad infection. These investigations were undertaken at the National Institute for Medical Research, London. Biopsy specimens reached London from Malaya by air on wet ice, and were processed and the bacteria inoculated into mice within 36 hours of taking the specimen. Suspensions of bacilli were prepared by homogenizing the specimens in 1 per cent albumin in saline and diluted, for inoculation into the mouse, so as to contain 10^4 acid-fast bacilli per 0.03 ml. Female mice (18-20 gm.) of the albino P strain were inoculated into the right or both hind foot pads with the nine strains of *M. leprae*. For each strain 10-12 mice were used as untreated controls and batches of 6-12 mice were given one or other level of DDS in their diet. Details of the technic used in the foot pad infection have been described by Rees ([10]). The sensitivity of the strains of *M. leprae* to DDS was assessed by counting the yield of bacilli from homogenates of the foot pads from untreated and DDS-treated mice (receiving either 0.1 or 0.025% DDS in their diet) at intervals from six to ten months from the day of inoculation.

RESULTS

Clinical. After the investigations and the treatment described above, the patient's progress was assessed by the medical staff of the Unit, on the basis of the clinical, bacteriologic (BI and MI) and histologic results. An attempt was made to decide clinically whether or not patients had improved.

Clinical progress was assessed by a detailed examination of the patient after six months' treatment with DDS, and was scored on the following scale:

2+	Marked improvement
1+	Slight improvement
0	No change
−1	Slight deterioration
−2	Marked deterioration

Table 1 shows the progress of the nine suspected patients, including the occurrence of reactions during that period.

The changes in the bacterial smears were assessed entirely on the MI, as it was not expected that changes in the BI would be

TABLE 1. *Assessment of the clinical response of the nine patients with* prima facie *evidence of sulfone resistance after a six month trial period on DDS.*[a]

Case number	Clinical assessment	Reactions during trial period	Clinical response (score[b])
1	Some improvement	Nil	1+
2	Condition resolving	Slight ENL[c] during last week	2+
3	Lesions much reduced	Nil	2+
4	No change	Nil	0
5	Some improvement	ENL during last 2 months	1+
6	Leprosy worse: sudden swelling of many patches	Nil	−2
7	Condition resolving	Nil	2+
8	No change	Nil	0
9	Flattening of all lesions	Nil	1+

[a]1.5 ml. (300 mgm.) injectable 4,4'diaminodiphenyl-sulfone twice weekly.
[b]2+ = marked improvement; 1+ = slight improvement; 0 = no change; −1 = slight deterioration; −2 = marked deterioration.
[c]Erythema nodosum leprosum.

TABLE 2. *Bacteriologic status at time of selection and assessment of response after a six month period on DDS.*[a]

Case number	Bacteriologic assessment of skin smears (average of six sites)				Assessment of improvement (score[d])
	At time of selection		After six months		
	BI[b]	MI[c]	BI[b]	MI[c]	
1	4.8	37	4.5	12	1+
2	4.2	32	3.7	4	2+
3	3.7	38	3.7	32	0
4	3.9	43	4.7	49	0
5	4.7	53	4.3	1	2+
6	4.3	36	4.8	31	0
7	4.0	48	3.7	4	2+
8	3.7	43	4.0	19	1+
9	5.0	34	4.5	1	2+

[a]1.5 ml. (300 mgm.) injectable 4,4'diaminodiphenyl-sulfone twice weekly.
[b]Bacteriologic index.
[c]Morphologic index.
[d]Based on MI: 2+ = marked fall in MI (to 5 or less); 1+ = moderate fall in MI; 0 = no significant change.

sufficiently marked for an assessment of improvement in a period of only six months. The following scores were used to indicate whether or not improvement had been found after six months of treatment:

2+ Marked fall in MI (to 5 or less)
1+ Moderate fall in MI
0 No significant change

These results, together with the MI and BI at the beginning and end of treatment, are shown in Table 2. The MI and BI were also assessed after three and four months of treatment, but are not reported here because the changes that took place in the intermediate periods are not essentially different from those at six months. Although not much weight can be given to the small change in the BI in a six month period, it is of interest that only the four patients with a moderate or no significant fall in MI had a rise in the BI in the same period.

A summary is given in Table 3 of the histologic findings at the beginning and end of six months of treatment, together with the fall in the biopsy index expressed

in terms of a percentage reduction scored on a 2+ to 0 scale as follows:

2+	A fall in the biopsy index of greater than 30 per cent
1+	A fall in the biopsy index of between 10 and 30 per cent
0	A change in biopsy index of less than 10 per cent

Table 4 correlates the clinical, bacteriologic and histologic findings. It has already been explained why there is doubt about the reliability of the histologic assessments (biopsy indices), based, as they were, on single biopsies only of nodules that were not always comparable and often too small for an accurate assessment. Therefore the

TABLE 3. *Histologic status at time of selection and assessment of response after a six month trial period on DDS.*[a]

At time of selection			After six months		Assessment of improvement	
Case number	Histologic Report	Biopsy index	Histologic Report	Biopsy index	Fall in biopsy index (per cent)	Score[b]
1	Very active leproma: many densely packed macrophages stuffed with bacilli (LL).	3.0	Slightly active; no striking change.	2.3	23	1+
2	Very large active granuloma: many bacilli in a center of foamy cells with some epithelioid cells at periphery (BL to LL).	4.2	Inactive: good progress.	0.9	78	2+
3	Slightly foamy granuloma with lymphocytes and plasma cells (BL or LL).	4.0	Inactive: remarkable improvement.	0.6	85	2+
4	Active: slightly foamy or macrophage cells with some epithelioid tendency (probably LL).	3.5	Still active; no significant change.	3.3	6	0
5	Active: similar to Case 4 (Probably LL).	5.4	Inactive: better than average progress.	2.0	61	2+
6	Very active: tumor-like mass of macrophages with many bacilli, almost too dense to count (LL).	5.4	Inactive: very small lesion, remarkable improvement.	0.15	97	2+
7	Large active granuloma with many macrophages, few lymphocytes and plasma cells, foci of polymorphs: probably in reaction (rather atypical LL).	3.3	Inactive lesion.	2.5	24	1+
8	Very active: fibroid cytotogic structure, but infiltrative and not expansile (LL).	4.4	Very active: (histology taken only aften one year)	4.3	0	0
9	Very active: histology like that in other resistant cases (LL).	2.8	Inactive:	1.65	41	2+

[a] 1.5 ml. (300 mgm.) injectable 4,4'diaminodiphenyl-sulfone twice weekly.
[b] 2+ = fall in the biopsy index of greater than 30 per cent; 1+ = a fall in the biopsy index of between 10 and 30 per cent; O = a change of biopsy index of less than 10 per cent.

TABLE 4. *Correlation of the assessments of the clinical, bacteriologic and histologic response of the nine patients with* prima facie *evidence of sulfone resistance after a six months trial period on DDS.*[a]

Case number	Clinical (score[c])	Bacteriologic (MI)[b] (score[d])	Histologic (Biopsy Index) (score[e])
1	1+	1+	1+
2	2+	2+	2+
3	2+	0	2+
4	0	0	0
5	1+	2+	2+
6	−2	0	2+
7	2+	2+	1+
8	0	1+	0
9	1+	2+	2+

[a]1.5 ml. (300 mgm.) injectable 4,4'diaminodiphenyl-sulfone twice weekly.
[b]Morphologic index.
[c, d, e]Scores as per Tables 1, 2 and 3 respectively.

histologic results were excluded from the overall assessment. On the basis of the clinical and bacteriologic (MI) findings there was good agreement in Cases 1, 2, 4, 5, 6, 7 and 9; Cases 1, 2, 5, 7 and 9 showed improvement, and Cases 4 and 6 showed no improvement or deterioration on both counts, respectively. Cases 3 and 8 gave discrepant results; Case 3 showed clinical improvement but no bacteriologic improvement, and Case 8 showed moderate bacteriologic improvement but no clinical improvement. As we believe that a fall in the MI is the most accurate measure of response to treatment, it was reasonable to suspect that Case 3 was resistant to treatment and, on the basis of the same criterion, together with the unsatisfactory clinical response, there was good evidence to suspect that Cases 4 and 6 were resistant to DDS. Because the MI usually falls to less than 5 in patients treated with DDS for six months, the changes obtained in Cases 1 and 8, respectively, being no more than moderate (viz., 12 and 19), were suggestive, but not conclusive enough by themselves to predict with certainty resistance to DDS. The associated failure to show clinical improvement somewhat strengthened the prediction of drug resistance in Case 8. All these predictions were made before the animal studies were completed at the National Institute for Medical Research in London.

There was no abnormality worthy of comment in any of the other investigations.

It was concluded from the clinical and bacteriologic findings at the end of the six months test period on DDS that Cases 1, 2, 5, 7 and 9 were not resistant and all five cases were continued on DDS. Cases 2, 7 and 9 were followed for at least another six months on DDS, during which time their bacterial indices showed no sign of deterioration, nor did the morphologic index increase, and the patients have maintained their clinical improvement. Case 5 has been discharged on DDS and is said to be well. Case 1 has caused considerable worry because of fluctuations in the MI, and further details are to be reported later. Of the four patients who showed evidence of resistance, three (Cases 3, 4 and 6) are being treated satisfactorily with the riminophenazine derivative B.663 ([1]) and the other (Case 8) is responding well to thiambutosine (DPT).

Experimental. In the foot pad infections all nine strains of *M. leprae* multiplied satisfactorily in a high proportion of the untreated mice, thus behaving similarly to strains of bacilli derived from previously untreated leprosy patients ([11]). Five strains, from Cases 1, 2, 5, 7 and 9, showed no significant multiplication of bacilli in the foot pads of mice receiving 0.1 per cent DDS in the diet. Only one (from Case 9) of the five strains was tested in mice receiving

0.025 per cent DDS in the diet, and multiplication was inhibited by this lower dose. On the other hand, three strains (from Cases 3, 4 and 6) multiplied as freely in mice receiving 0.1 per cent DDS in the diet as in the untreated animals, and one strain (from Case 8), while being inhibited in mice receiving 0.1 per cent multiplied freely in animals receiving 0.025 per cent DDS in their diet. The results of these sensitivity tests to DDS are summarized in Table 5 and indicate that the bacilli from Cases 3, 4, 6 and 8 are resistant to DDS on the basis of the now considerable evidence that the multiplication of strains of *M. leprae* from previously untreated patients is inhibited in the foot pad of animals receiving 0.025 per cent or less DDS in their diet ([11,18]). The first seven strains were tested in mice receiving only the highest dose of DDS (0.1% in their diet), because at that time it was the only dose known to inhibit strains of *M. leprae* from untreated patients. With the more recent knowledge that ordinary strains of *M. leprae* are sensitive to much lower doses of DDS, the two later strains (from Cases 8 and 9) were tested at lower levels of DDS, and it is of particular interest that the organisms from Case 8 were resistant to a level of 0.025 but not 0.1 per cent. The results of these studies also indicate that DDS-resistant strains of *M. leprae* are pathogenic in the mouse foot pad, since they multiply as freely as susceptible strains. Further studies on three of the resistant strains (from Cases 3, 4 and 6) passaged in mice and retested against DDS, have shown that resistance was maintained.

DISCUSSION

In an extensive search of one of the largest inpatient leprosaria in the world nine patients were discovered who gave *prima facie* evidence of sulfone resistance. This was a clinical decision based on the failure of patients to improve over many years of treatment and, when the skin smears were taken from several sites, it was invariably found that positivity was associated with a high MI as well as a high BI. These were the cases that were taken for study and from whom biopsy specimens of fresh tissues were sent for DDS-sensitivity tests using the mouse foot pad technic. All of them showed extensive nodular leprosy clinically and, although one of them had a tendency to diffuse lepromatous leprosy, he too had multiple small shiny nodules scattered over the limbs and also on the face. It is interesting to note that, although we see many patients with diffuse lepromatous leprosy in Malaya, all those who were clinically suspected of resistance were covered with yellowish-red shiny nodules. It has not been possible to ascertain whether any of these patients were originally of a diffuse type or whether all the cases that we collected had always been of

TABLE 5. *DDS sensitivity as shown by the mouse foot pad injection of* M. leprae *from nine patients with* prima facie *evidence of resistance.*

Case number	Multiplication of *M. leprae* in foot pads			Results of sensitivity tests
	Untreated mice	DDS-treated mice (per cent DDS in diet)		
		0.1	0.025	
1	+	0	--	Sensitive
2	+	0	−	Sensitive
3	+	+	−	Resistant
4	+	+	−	Resistant
5	+	0	−	Sensitive
6	+	+	−	Resistant
7	+	0	−	Sensitive
8	+	0	+	Resistant
9	+	0	0	Sensitive

+ = Multiplication (10-600 fold).
0 = No significant multiplication (less than 10 fold).

the nodular form. It remains to be shown whether or not there is a greater tendency to sulfone resistance in cases with primary nodular lepromatous disease, but the nodularity at the time of relapse is clearly due to the somewhat unusual histologic features, which are described in the appendix.

In retrospect there was no clinical or histologic difference between those patients who were proved to have sulfone resistance and those who improved when they received sulfone treatment under supervision in our Research Unit. It is interesting that all the proven cases of resistance had received sulfones for at least 14 years. At present it is impossible to forecast clinically whether a patient is truly resistant or simply one of those who has somehow or other relapsed. The latter type of patient improved during the trial period on DDS. It is extremely difficult at the moment to understand how these relapses have occurred. Despite persistant cross questioning most of the patients who improved deny that there was any irregularity in their treatment and the only patient who was demonstrably erratic (Case 9) responded as well as Cases 1, 2, 5 and 7. It is presumed that these patients were afraid of rejection by the outside world and, finding life in the Settlement to be pleasant and undemanding, decided to stay in this protective atmosphere as long as possible. Such patients would probably have avoided therapy if they believed that discharge automatically followed smear-negativity.

These studies on sulfone resistance in leprosy were undertaken because there are now available, for the first time, new bacteriologic technics that could be applied successfully to this problem. In particular it is now possible to determine the viability of leprosy bacilli from their morphologic appearance (MI) in routine smears, and it is possible to grow the bacilli isolated from patients in experimental animals by use of the mouse foot pad technic. Therefore, in planning these studies, great weight was placed on the value of the MI as a simple means of determining the viability of the bacilli in the patients to be selected as showing *prima facie* evidence of sulfone resistance, and in confirming or otherwise their inability to respond to a six months trial period on DDS in our own research wards. Studies ([21]) consistently demonstrate that previously untreated patients with lepromatous type leprosy show a reproducible and significant diminution in their MI, usually to less than 5, on standard doses of 300 mgm. of DDS injected intramuscularly twice weekly for six months. Patients who show an abnormal response probably have a sulfone-resistant infection. Therefore, although in the present studies the selected patients were assessed clinically, bacteriologically (BI and MI), and histologically for their response to DDS in the six months trial period, it was anticipated that the MI would be the most sensitive assessment. In a short period of six months little weight can be placed on either the clinical or BI assessments. Normally the biopsy index ([14]) would be expected to have considerable significance, but the present studies have shown that, because the majority of these potentially resistant patients had small nodular lesions, rather than diffuse lesions, the biopsy index was unreliable.

Our previous experience in untreated patients and retrospect from the current resistance studies have fully endorsed the value of the MI, since this index alone has correlated with the response of the patients to treatment and the DDS sensitivity tests carried out in experimental animals. Thus the three patients (Cases 3, 4 and 6) whose MI failed to fall during the six months trial period, proved to be fully resistant in the mouse foot pad test. Furthermore, of the two patients (Cases 1 and 8) whose MI fell to only 12 and 19 respectively, the latter proved to be resistant and the former sensitive in the mouse foot pad test. The rate of fall in the MI of the remaining four patients (Cases 2, 5, 7 and 9) was satisfactory, indicating a good response to treatment and no evidence of drug resistance. The subsequent response of these four patients had been maintained and there has been no increase in their MI's during at least another six months on DDS. Of the group of patients who failed to respond satisfactorily during the trial period on

DDS, four (Cases 3, 4, 6 and 8) have responded satisfactorily, with a fall in their MI to less than 5, since being put on alternative antileprosy treatment, B.663 for Cases 3, 4 and 6 ([9]) and DPT for Case 8. The remaining patient (Case 1) was continued on DDS because, although his MI had fallen to only 12, rather than the more usual 5 or less, during the six months trial period, it was felt that this small difference was too rigid a criterion to justify immediate change of treatment. The subsequent progress of this patient while maintained on DDS has been unsatisfactory, with fluctuations in the MI, and further details will be published later. Nevertheless, in retrospect, it is of interest that at the completion of the six month trial period on DDS the MI of this patient had fallen somewhat less than that of the previously untreated lepromatous patients on standard DDS therapy.

The multiplication of *M. leprae* in the mouse foot pad, with between ten- and 100-fold yields of bacilli, in a period of six to eight months, has provided the first real opportunity for experimental studies on human leprosy bacilli in the laboratory. Furthermore, it has been shown that strains of *M. leprae* derived from previously untreated patients fail to multiply in the foot pads of animals treated with DDS. With this knowledge we assumed that the mouse foot pad infection could provide a means for determining whether or not the failure of patients to respond to treatment with DDS was due to the emergence of drug-resistant bacilli. Bacilli from all nine patients presenting *prima facie* evidence of sulfone resistance were tested in mice, but only those from Cases 3, 4, 6 and 8 multiplied in the animals treated with DDS and therefore could be definitely classed as drug-resistant. The same four patients also were the ones who failed to respond to the initial test period on DDS, and thus, for the first time, it has been possible to correlate clinical and laboratory findings and establish experimentally the existence of DDS-resistant strains of *M. leprae* in man. At the time these studies were initiated on the first seven patients, our knowledge of the inhibitory activity of DDS in the foot pad infection was limited and we were aware only that 0.1 per cent DDS in the diet was active. Later studies have revealed that doses of only 0.01 per cent are equally active against strains of *M. leprae* from previously untreated patients, and therefore Cases 8 and 9 were screened against high and low doses. It is of interest that the bacilli from Case 8 were shown to be resistant, but only to an intermediate dose (0.025%) and not the high dose (0.1%) used for the first seven cases. Therefore, in the future, studies on drug resistance should always include sensitivity tests in the mouse with high and low doses of DDS in order to detect different levels of resistance.

From our experience gained in these studies we have demonstrated that the mouse foot pad infection now provides a sensitive method for detecting DDS-resistant strains of *M. leprae* from patients, and that the method could be successfully adapted for other drugs also. The method has practical limitations because the test takes at least eight months to complete, but no more rapid test can be anticipated until *M. leprae* can be cultured *in vitro*.

Although DDS has been the main drug used in the treatment of leprosy for some twenty years, fortunately there are still very few patients who relapse under treatment. While the present studies for the first time have demonstrated beyond doubt that a proportion of such relapses are due to the development of drug resistance, we believe it is a rare occurrence. Thus the leprosarium from which these nine patients were selected has at the moment 2,500 inpatients, and many thousands of others have been admitted and discharged or treated as outpatients, without any suspicion that their leprosy did not respond. From a rough estimate we believe that these four cases of DDS resistance have probably arisen from not less than 5,000 patients with lepromatous leprosy. Now that it has been demonstrated that, albeit rarely, drug resistance to DDS does occur, it can be anticipated that sooner or later primary cases of drug resistance will arise in patients infected with DDS-resistant strains of leprosy bacilli.

APPENDIX

Note on the histology of acute relapsing leprosy. The histologic and bacteriologic features of the lesions biopsied have been described briefly in the case reports. The histologic interest of resistant patients lies in the fact that their lesions illustrate the stages of the host response to an acute increase in numbers of bacilli better than do most untreated patients, whose lesions are often more chronic or more mature. The histologic features are associated with relapse due to drug resistance, not to resistance itself.

That these lesions were more acute than in the average untreated patient was confirmed by bacteriologic and histologic examination of the sections. From the morphology of the bacilli and the character of the infiltration ([16]) five of the initial lesions were classed as very active and the other four as active (Table 3). Whereas the average routine biopsy from untreated leprosy patients in Malaya shows a relatively granular bacterial morphology, with perhaps 30 to 40 per cent of solid forms, many of the initial lesions in this series showed a much higher proportion. The five lesions described as very active had many more solid bacilli than is indicated by the MI, which is based on the average of several smears. But not all the lesions of a relapsing patient are active. Thus in Cases 3 and 6 the follow-up lesions were undoubtedly inactive, although these patients are now known to have had sulfone-resistant organisms.

From this point of view the picture was partly obscured in some of the initial biopsied lesions because the relapse appeared to have taken place at the site of a substantial preexisting lesion; other lesions were almost wholly new (Case 9). Considering as far as possible only the new features, we noted that in Cases 2, 8 and 9 a number of the host cells were somewhat elongated or spindle shaped and showed no foamy change. Indeed, in Cases 8 and 9 spindle cells of this type predominated (Fig. 1). In the earliest and most active lesions spread of the granuloma was by infiltration; in the largest and most mature it was expansile (Case 3), while Cases 5 and 7 showed some expansile tendency. In Case 7 there were accumulations of polymorphonuclear leucocytes in the granuloma beneath the epidermis, indicative of a localized "reaction." Two cases showed some borderline features; in Case 1 there was increased cellularity of nerves due to infiltration or Schwann cell proliferation, which is never normally seen in LL leprosy; in Case 2 there were one or more small tubercles of epithelioid-like cells in each section (Fig. 2), in which there were far fewer bacilli than in other parts of the granuloma. These cells, of course, are indicative of tuberculoid or borderline leprosy and are never seen in lepromata; yet in other respects these patients were lepromatous (LL). It is interesting, therefore, that all the features described as characteristic of "histoid" leprosy ([20]) were present individually in these nine patients; a predominance of spindle shaped host cells, an absence of globi, the presence of localized areas of reaction in the granuloma, or of epithelioid tubercles, an "expansile" type of spread and, finally, the size of the bacilli, which were longer than average. None of the lesions studied combined all these features, and none possessed the dimensions of the specimens studied by Wade ([20]). However, there is more than one type of histoid lesion: Wade recognizes subcutaneous nodules, cutaneous nodules, and plaques. All our cases had cutaneous nodules, although, perhaps surprisingly, they did not all show an expansile type of development; at least there was no evidence of this at the stage at which they were biopsied.

The significance of a histoid histology has not been elucidated. It would seem that the essential feature of both relapsing and histoid lesions is that they are, or were at some stage of their development, acutely progressive to an unusual degree. Wade states that his cases developed as a result of reactivation or relapse after a period of successful treatment. What is difficult to explain is why the histoid lesions continued in the acute phase until they

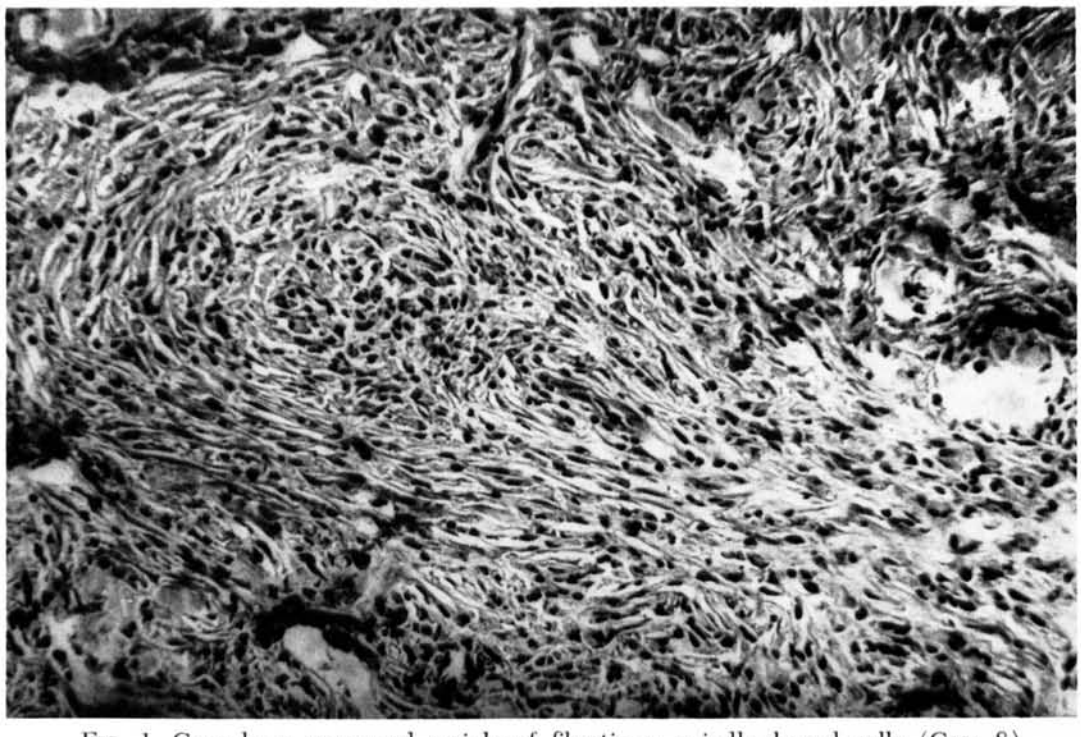

FIG. 1. Granuloma composed mainly of fibrotic or spindle-shaped cells (Case 8). Bacilli in this part 6+. X240.

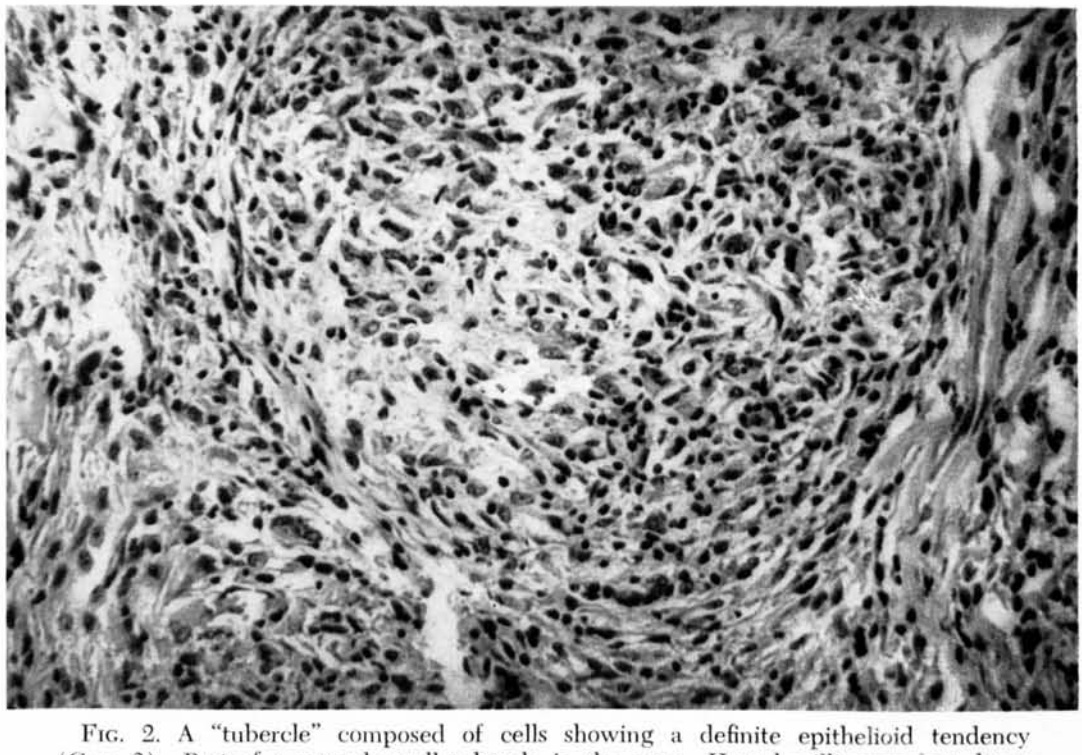

FIG. 2. A "tubercle" composed of cells showing a definite epithelioid tendency (Case 2). Part of a second small tubercle is also seen. Here bacilli were less dense (4+) than in the other parts of the granuloma. X300.

became so large and why even then they did not mature in the usual way with fatty change and globus formation. In contrast, our cases reverted to the usual histiocytic cytology with fatty change on maturation.

As early as 1936 Mitsuda ([6]) noted that in fresh nodules the host cell is spindle shaped and devoid of vacuoles, and that the bacilli are in rod form. It is impossible to avoid questioning whether the spindle cells are fibroblasts or cells derived from them. The initial response of the dermis to a relapsing lesion is fibroblastic proliferation ([16]); many of the spindle cells in the progressing lesion resemble fibroblasts even though they are phagocytic; and in the case of histoid lesions a fibrous stroma develops in the late stage. It is disputed whether fibroblasts may evolve into histiocytes, and the literature on this question is too vast to review here. Lever ([5]) believes that in skin adventitial cells around the blood vessels, which normally develop into fibroblasts, may under pathologic conditions produce histiocytes. It seems possible that in leprosy an acute increase of bacilli causes these adventitial cells to act as host cells for bacilli, retaining some fibroblastic properties though not that of producing large amounts of collagen. Such cells might differ somewhat from the normal reticuloendothelial host cells and so modify the attributes of the granuloma.

The classification of these patients proved difficult and sometimes uncertain, histologically as well as clinically. This was due partly to a relative lack of experience with this type of case or to the undifferentiated cytology of the granuloma, but more particularly it was because borderline features were sometimes superimposed on a pattern that seemed otherwise to be purely lepromatous. Thus in Case 1 the mild neural infiltration was out of keeping with the cytology of the granuloma, which consisted of macrophages heavily laden with bacilli, and, likewise, the epithelioid-like tubercles in Case 2 were quite out of context with the other aspects of the lesions in which they occurred. The same applies to the finding of tubercles in histoid lesions that are definitely lepromatous ([20]). This, taken in conjunction with the occasional occurrence of localized reactions in these lesions, prompts the question if an acute increase of bacilli at one site may not occasionally goad even a pure leproma (LL) into a show of tissue reactivity. The hypothesis is that this unusual response is elicited because the bacterial challenge at one site becomes very much greater than that prevailing in other lesions. It has already been mentioned that the states of activity of different lesions in a relapsing patient are unequal, and the same applies to histoid patients.

SUMMARY

From an extensive search of one of the largest inpatient leprosaria in the world, at Sungei Buloh, Malaysia, nine patients with lepromatous leprosy were discovered who gave *prima facie* evidence of sulfone resistance. The evidence was based on a failure to show clinical improvement over at least five years despite treatment with sulfones and an absence of a satisfactory fall in the bacteriologic (BI) or the morphologic (MI) index.

The selected patients were admitted to our Research Unit for (a) a further six month, rigorously controlled, trial period on DDS (as injectable sulfone, 300 mgm. twice weekly) and (b) DDS sensitivity tests, based on use of the foot pad infection in mice with bacilli obtained from skin biopsies.

The response of the nine patients to the six month trial period on DDS was assessed clinically, bacteriologically and histologically, and revealed that only four of the patients failed to respond satisfactorily. Furthermore, the sensitivity tests in the mouse foot pad infection showed that only the strains of *M. leprae* from the four patients who failed to improve were insensitive to DDS. Thus there was a good correlation between the results of the clinical and experimental studies and for the first time direct proof for the existence of DDS-resistant strains of *M. leprae*. The MI proved to be the most sensitive of the assessments used to determine the response of the selected patients to a trial period on DDS. The histology of patients with drug resistance is essentially that of relapsing

or very acute leprosy. Its features have much in common with those of "histoid" lesions, the latter being distinguished mainly by the absence of cytologic maturation. Classification is complicated by the presence of borderline features in otherwise lepromatous lesions.

RESUMEN

De una búsqueda extensiva de uno de los leprosarios mas grandes en el mundo, en Sungei Buloh, Malaysia, se descubrieron nueve pacientes con lepra lepromatosa que dieron a primera vista, evidencia de tener resistencia a la sulfona. La evidencia se basó en el fracaso a mostrar mejoría clínica en el transcurso de cinco años, a pesar del tratamiento con sulfonas y en ausencia de una disminución satisfactoria del indice bacteriológico (BI) o morfológico (MI).

Los casos seleccionados fueron admitidos en nuestra Unidad de Investigación para (a) un nuevo período de ensayo de seis meses con DDS (en forma de sulfona inyectable, 300 mgm. dos veces por semana) rigurosamente controlados y (b) pruebas de sensibilidad a DDS basado en la infectión del colchón plantar de ratón, con bacilos obtenidos de biopsias de la piel.

Las respuestas de los nueve pacientes en los seis meses en que se ensayó DDS fué avaluado clínica, bacteriológica e histologicamente y demostró que solo cuatro de los pacientes no respondieron satisfactoriamente. Mas todavía las pruebas de sensibilidad mediante la infección del colchón plantar de ratones demostró que solamente las cepas de *M. leprae* de cuatro enfermos que no mostraron mejoría eran insensibles a DDS. Hubo una buena correlación entre los resultados de los estudios clínicos y experimentales y por primera vez una prueba directa de la existencia de cepa de *M. leprae* resistente a DDS. El índice morfológico (MI) demostró ser la prueba mas sensible de evaluación empleada para determinar la respuesta de pacientes en un período de ensayo con la droga DDS. La histología de los pacientes con resistencia a la droga, es esencialmente aquella de recaída o de lepra muy aguda. Sus características tienen mucho en común con las lesiones "histoides" distinguiéndose esta última principalmente por la ausencia de maduración citológica. La clasificación es dificil por la presencia de características difíciles de clasificar, en lesiones que de otra manera serían lepromatosas.

RÉSUMÉ

Une investigation approfondie menée parmi les malades hospitalisés dans l'une des plus grandes léproseries du monde, Sungei Buloh, en Malaisie, a permis de découvrir neuf malades lépromateux avec évidence à première vue de résistance aux sulfones. L'évidence était basée sur l'absence d'amélioration clinique au cours de cinq années, malgré le traitement sulfoné, et sur le fait que l'index bactériologique (BI) et l'index morphologique (MI) n'avaient pas montré de diminution satisfaisante.

Les malades ainsi choisis ont été admis dans notre Unité de Recherche, afin (a) d'être soumis pendant une période supplémentaire de six mois à un traitement d'essai par la DDS rigoureusement contrôlé (sulfone injectable à raison de 300 mgm. deux fois par semaine), (b) de subir des épreuves de sensibilité à la DDS, basée sur l'infection de la sole plantaire de la souris par des bacilles obtenus de biopsies cutanées.

La réponse des neuf malades à cet essai de DDS s'étendant sur six mois a été évaluée cliniquement, bactériologiquement et histologiquement. Il a été ainsi révélé que quatre seulement des malades ne répondaient pas de façon satisfaisante. De plus, les épreuves de sensibilité par infection de la sole plantaire de la souris ont montré que les seules souches de *M. leprae* insensibles à la DDS étaient celles qui avaient été obtenues chez les quatre malades qui n'avaient pas témoigné d'amélioration. Il y avait donc une bonne corrélation entre les résultats des études cliniques et expérimentales, et pour la première fois a été fournie la preuve directe de l'existence de souches de *M. leprae* résistantes à la DDS. Le MI (index morphologique) s'est révélé l'épreuve d'évaluation la plus sensible pour déterminer la réponse de malades sélectionnés à un traitement d'essai prolongé par la DDS. L'histologie des malades avec résistance médicamenteuse est essentiellement celle que l'on trouve dans des récidives de lèpre ou dans des cas fort aigüs. Ses caractéristiques ont beaucoup de points communs avec les lésions "histoïdes." C'est surtout l'absence de maturation des cellules qui permet de distinguer ces dernières. La classification est rendue difficile par la présence de caractéristiques de lèpre "borderline" dans des lésions qui, autrement, sont lépromateuses.

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