

**MYCOBACTERIUM LEPRAE IN DEEP ORGANS IN FIFTEEN  
"QUIESCENT" AND "ARRESTED" CASES OF LEPROSY  
THAT GAVE NEGATIVE SMEARS AT NECROSPY<sup>1</sup>**

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Pineda (2), in 1927, reported finding *Mycobacterium leprae* in deeper organs of 10 out of 11 cases that had come to autopsy at Culion after becoming "negative" under treatment, but he did not discuss the histological findings. The present writer has reported (1) that smear examinations made in 42 "quiescent" and "arrested" cases showed bacilli in 27 cases in nerves, lymph nodes, or testes; smears from the spleen, liver, kidney, and adrenal were always negative. In the other 15 cases no bacilli could be found, though macroscopically the lymph nodes usually showed yellowish infiltration and the nerves were quite fibrous. The present report deals with the results of histological search for the bacillus in the preserved (Zenker-fixed) tissues of these 15 negative cases.

Of the nerves, the most thickened portions were saved, usually the ulnars at the elbow, the common peroneal in the popliteal fossa and the superficial peroneal in the distal third. The median at the wrist, the radial, and the posterior tibial were examined in a few cases. Axillary and inguinal lymph nodes were examined in all cases and the submental in five. Examinations of the spleen, liver, kidney, adrenal and testis are indicated in Table 1.

Since these tissues contained few organisms, if any, the freezing method of sectioning was used as more suitable than the paraffin method. However, paraffin sections were made of duplicate pieces for a histologic control. The tissues had been in alcohol for from one to two and half years.

Organs known to contain the bacillus and preserved for over three years, used as control, showed bacilli in very large numbers. Tissues from a non-leper who died as a result of an accident also served as a control; they showed clumps of acid-fast granules in normal nerve tissue, and the acid-fast neurokeratin of nerve fibers, which have to be kept in mind in judging the nature of acid-fast structures seen in leprous tissue.

<sup>1</sup>This is a condensation, revised with approval of the author, of an article which appeared in Trans. Ninth Congress, Far Eastern Assoc. Trop. Med., Nanking, 1934. Shanghai, 1935, vol. 1, pp. 705-713.

TABLE 1.—Data on cases examined (stage of leprosy on admission and duration of the negative period) and findings in the organs examined.

CASE DATA															
Case number	4	8	9	11	13	14	16	17	19	23	31	32	34	37	41
Leprosy on admission	1326	1409	1467	1496	1506	1507	1526	1528	1543	1568	1655	1662	1677	1690	1720
Negative period	4 mo.	7 yr. 2 mo.	3 mo.	1 yr. 9 mo.	10 mo.	15 da.	6 mo. 2 mo.	2 yr. 5 mo.	8 mo.	3 yr. 3 mo.	4 yr. 7 mo.	1 yr.	4 yr. 1 mo.	1 yr. 2 mo.	2 mo.
Organs examined	C2 N1	C1 N1	C1 N1	C1 N2	C1 N2	C2 N3	C1 N3	C2 N3	C2 N2	C2 N2	N3 (x)	C2 N1	C2 N2	C1 N2	C1 N1
RESULTS OF EXAMINATION															
Nerve, ulnar	F,++	-,-	-,-	F,+?T,F,++	F,+	F,+	F,-	F,+?	F,-	F,+?T	F,-	F,++	F,++	-,+	-,-
Nerve, median															
Nerve, radial				F,+?											
Nerve, peroneal															
Nerve, posterior tibial															
Lymph Node, axillary	F,-	-,-	F,+	-,-	F,+	F,+	F,-	F,+	F,-	F,-	F,+	F,+	F,+	-,+	F,-
Lymph Node, inguinal	F,-	-,-	F,+?	-,-	F,+	F,+	F,+?	F,+	F,+	F,+	F,-	F,-	F,-	-,-	F,+?
Lymph Node, submental															
Spleen															
Liver	F,+?	-,-	-,-	-,-	F,+?										
Kidney															
Adrenal															
Testis	F,-	Female	Female	-,-	F,+	F,+	F,-	F,-	Female	Female	F,-	-,-	Female	Female	-,-

F = Foamy cells.

- = Negative for bacilli.

+? = Doubtful; one bacillus seen, or atypical granules.

+ = Two or more bacilli found with difficulty.

++ = Several, or many bacilli, easily found.

T = Tuberculoid lesions.

(x) = No record. N3 at time of death.

The frozen sections were stained for acid-fast organisms,<sup>2</sup> and also with Sudan and Nile blue sulphate to detect the foamy cell accumulations; these stain brownish-red with the former and pinkish with the latter. Nile blue differentiates these foamy cells from phagocytic cells, found especially in the lymph nodes, that contain globules of the antileprosy drug; these stain dark blue (3, 4). In the fuchsin sections these globules retain the stain and interfere with the examination. Drug globules were never found in the nerve trunks. Search for the bacilli was prolonged, and especially directed to the places where foamy cells were present, although the entire sections were always examined.

#### FINDINGS AND COMMENTS

The organs examined, the results obtained, the type of leprosy on admission, and the duration of the negative period are shown in Table 1, where the manner of recording findings is indicated.

In twelve cases bacilli were demonstrated in one or more organs, usually in very small numbers, always associated with the foamy cells (F,+ or F,++ in the table).

In all cases sections of other organs showed acid-fast structures or shadows of them in the foamy cells, but in many instances such cells showed nothing significant, their foam spaces appearing empty. In three of these cases there were tuberculoid lesions in the nerve trunks, but none was ever observed in the other organs.

#### GROUP I

To illustrate the observations, some of the protocols of the microscopic findings are quoted briefly.

CASE 4-1326.—In the liver a very few granular forms in foamy cells (+?).

CASE 9-1467.—In one lymph node foamy cells with acid-fast granules (+?); and in another node five granular bacilli seen in foamy cells (+).

CASE 13-1506.—In an axillary node acid-fast granules in foamy cells (+).

CASE 14-1507.—In an axillary node acid-fast granules in foamy cells (+).

CASE 17-1528.—In the liver a very few bacilli, mostly granular forms, in foamy cells (+).

CASE 23-1568.—In an inguinal node foamy cells with acid-fast granules (+); most of them empty. In an submental node granulated bacilli in foamy cells (+). In the liver two acid-fast rods and acid-fast granules (+).

CASE 31-1655.—In an axillary node acid-fast granules are aligned in rod formation in foamy cells (+); many foamy cells with none. In two other nodes shadows of granules in foamy cells.

CASE 32-1662.—In an axillary node acid-fast granules in foamy cells (+).

<sup>2</sup> Carbol-fuchsin overnight, decolorized in 15 per cent nitric acid, and counterstained with Loeffler's methylene blue.

The findings indicate that the infection was being overcome in these cases. It was evident that the microorganisms were undergoing intracellular digestion and that many had died out, for they were often very few and difficult to find at all, whereas ordinarily in our leprosy cases the nerves and lymph nodes are heavily loaded with them. Marked fibrous changes in the nerve trunks were in keeping with this. Whether the empty foamy cells contained a non-acid fast form or an invisible "virus" stage of the organisms (5, 6, 7, 8), if such forms really exist, remains to be determined.

#### GROUP II

Of the other three cases, the infection had apparently been overcome in one and almost overcome in the other two.

CASE 8-1409.—No foamy cells or bacilli found. Ulnar and peroneal nerves appear normal, without fibrosis, round-cell or foamy cell infiltration. Lymph nodes, spleen and liver without foamy cells.

The patient, female, 53 years old, was admitted in July, 1910, with eyebrows three-fourths gone and ears atrophied; face, hands and feet infiltrated; parchment skin. October, 1911, still bacteriologically positive (right cheek). Presented to the Negative Examining Committee in September, 1923. Records of about that time describe slight infiltration of the earlobes and cheeks, small reddish spots on the right hand and below the left elbow; slight contracture right small finger, ulnar nerves small, epitrochlear nodes not palpable, inguinals barely palpable. Bacteriological findings repeatedly negative. Paroled March, 1924; returned to the colony a year later, and died November, 1930, still negative.

CASE 11-1496.—A very few foamy cells found in sections of the ulnar and peroneal nerves, but not confirmed in paraffin sections. Marked fibrosis of nerves with round cell infiltrations and tuberculoid lesions of the ulnars. Only one isolated bacillus found free in the connective tissue of each of the ulnar and peroneal nerves, and only after repeated staining; this was graded +?.

This patient, male, 48 years old, was admitted in November, 1923, with slight diffuse infiltrations in the cheeks, moderate on feet; pinkish areas on upper extremities and back; ulcers of nose; extensive anesthesia over arms and legs with deformities of big toes and left thumb. Bacteriologically positive (++) from neck and back. The Negative Examining Committee, in October, 1929, found apparently tuberculoid lesions on various parts of the body; these were extensive, depigmented, slightly thickened, with faintly elevated borders. Extensive anesthesia of extremities with some deformities. Bacteriologically negative nine times from October, 1929 until his death in July, 1931.

CASE 37-1690.—No foamy cells found. Ulnar and peroneal nerves very fibrous, with round cell infiltrations. Four or five bacilli found isolated in the thick connective tissue of the nerves; graded +.

This patient, female, 70 years old, was admitted in November, 1929, with a few pinkish infiltrations in the cheeks, chin, earlobes, brows and glabella, and marked contracture of all fingers. Presented to the Committee in May, 1931, she was found negative in eight examinations before her death in July, 1932.

#### DISCUSSION

Of these last two cases, in one only a very few foamy cells were found in the nerves, and they only after repeated examinations, while in the other none were found. In the first case somewhat doubtful bacilli were found (+?), in the other a few definite ones (+); in both the microorganisms were free in the connective tissue, without any cellular reaction. It may be asked: (1) whether they were still alive, potentially pathogenic, capable of multiplication on favorable opportunity but unable to excite the usual foamy-cell reaction to their presence; or (2) or whether they were dead and, so to speak, buried in the connective tissue; or (3) whether the acid-fast form had reverted into, or had been replaced by, the hypothetical non-acid-fast-forms (5, 6, 7, 8). These questions have to be left unanswered, but the fact that in all cases but the three in Group 2 foamy cells, alone or in association with bacilli, were demonstrated in lymph nodes inclines one to believe it highly probable that in these three cases the infection had been or was being overcome.

In view of the scarcity of the microorganisms in all these cases, it is not surprising that they were missed by the smears at necropsy. In no instance were "globi" demonstrated. Evidently proliferation of the bacillus was being held in abeyance, but from the high incidence of persistent bacilli in the deeper organs it may be said that clinically "arrested" cases of leprosy can mean only that. Many more years of observation are required to determine the permanence of arrest in our paroled cases. A start in this direction has been made in an inquiry (9) which revealed that 195, or 46 per cent, of 420 paroled cases examined had become positive within a limited number of years.

#### SUMMARY

1. It was previously reported (1) that in 15 (36 per cent) out of 42 necropsies on "quiescent" and "arrested" cases of leprosy the bacillus was not demonstrated by direct smears from the deeper organs. Histological study of these cases is now reported.

2. In 12 of these 15 cases the bacillus was found in association with foamy cells. Of the remaining 3 cases, in one neither

foamy cells nor bacilli were found, in another a very few foamy cells and one bacillus, and in the third a few bacilli without foamy cells.

3. The three cases last mentioned are looked upon as instances in which the infection had probably been almost or quite overcome.

4. Foamy cells were found in the spleen in 3 out of these 15 cases, in the liver in 5 out of 14 cases, in the adrenal in 2 out of 9 cases, and in the testes in 5 out of 9 cases.

5. Tuberculoid lesions were found in the nerve trunks in three cases, never in the lymph nodes or visceral organs.

6. Absorbed globules of the injected drug were found in some of the lymph nodes examined, never in the nerve trunks.

7. The histological evidence indicates that the bacilli found within the foamy cells were undergoing intracellular digestion.

8. When the microorganisms are very scarce in the tissue, demonstration is probably influenced by chance when the "scraped-incision" method of obtaining smears is used.

9. The necessity of a regular, systematic and continuous follow-up of all paroled cases, in order to determine the permanence of "arrest," is emphasized.

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