Electron Microscope Study of the Dental Pulp of Lepromatous Patients[1]

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Studies of histopathologic changes in dental lesions of leprosy patients were reported by Kitami ([6]), Tochihara and Murakami ([9]), Hirashita ([2, 3]), Tajiri and Kamio ([8]), Itakura ([4]), Kamio ([5]), and Asano ([1]). Most of the papers of these authors were published during the period before sulfones were used in Japan. Only the paper of Asano belongs to the later period, when sulfones were employed commonly in the treatment of leprosy.

Almost all of the authors named examined the teeth of autopsied cases of leprosy; only a few used teeth extracted from patients.

This paper reports electron microscope observations of the dental pulp of lepromatous patients. In the study we could see the details of leprosy bacilli and lepra cells in dental pulp more clearly than was possible in the histopathologic studies of the authors named above.

MATERIALS AND METHODS

Seven teeth, taken from six lepromatous patients, were examined by light and electron microscopy. Among these teeth, only one had lepromatous changes of the dental pulp; the others showed no changes due to leprosy in the pulp.

Report of a lepromatous case in which lepromatous lesions were found in the dental pulp. A male Japanese patient (J.S.) with lepromatous leprosy was born in May 1940. A reddish infiltration alpeared on his face in 1964, which expanded, with papules and nodules in the infiltrated area. In January 1965 the case was diagnosed as lepromatous leprosy and in February the patient was admitted to National Leprosarium Oku-Komyo-en. At the time of his admission to the hospital a diffuse red infiltration was observed on his face, and pea-sized or finger-tip-sized nodules were seen on his forehead and cheek. The hair was normal. The eyebrows and eyelashes had fallen out. Both hands were anesthetic. There were lepromatous macules on the trunk. The bacterial index was 3.2, and the lepromin reaction was negative. The tooth examined was extracted on 8 April 1965. The left upper central incisor was chosen for extraction because of its strong rotation. The outer appearance of the tooth was normal.

The patient has been treated with DPT (Ciba 1906), 1-2 gm. daily, and at the time of writing nearly all the lepromatous skin lesions have been well absorbed.

All of the electron microscope descriptions of lepromatous lesions of the dental pulp in this paper are based on the findings of the extracted tooth of this patient.

Processing of the specimens for histopathologic examination and electron microscopy. After extraction of the tooth and removal of attached gum, tooth debris, and calculus dentalis in physiologic saline, two grooves were cut with a Vulcarbo disk to make cleavage lines on the mesial and distal surface of the tooth. During the grinding the tooth was dipped in physiologic saline. It was then broken with a crown-cutting forceps. Dental pulp was taken out and fixed in 6 per cent glutaraldehyde in phosphate buffer at pH 7.2-7.5.

For the histopathologic examination the fixed pulp was dehydrated and embedded in paraffin. For electron microscopy the glutaraldehyde-fixed tissue was fixed again in osmium tetroxide for three hours. After the reosmication, the tissue was dehydrated and embedded in 6:4 mixture of N-butyl and methyl methacrylate.

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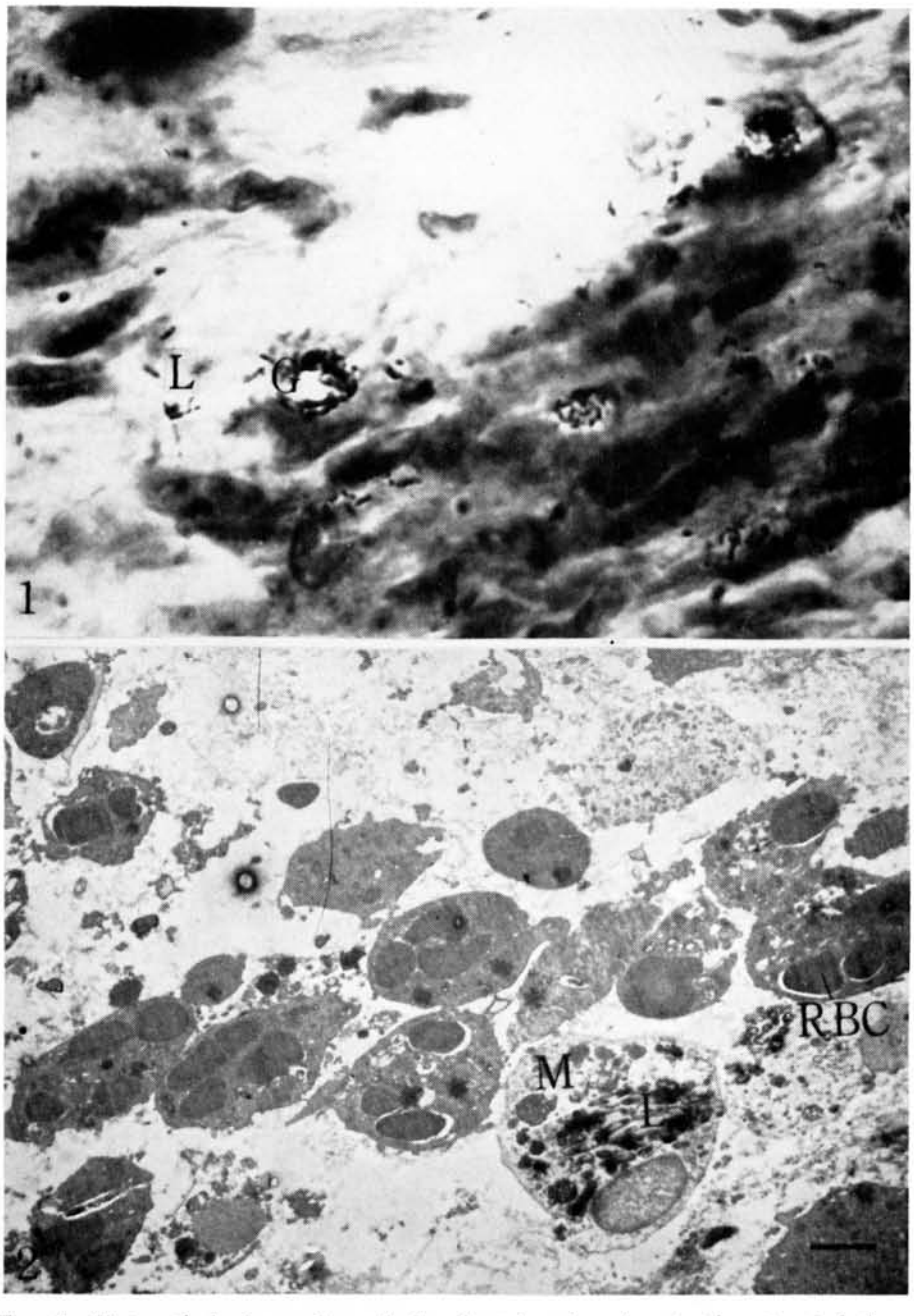

FIG. 1. Histopathologic section of the dental pulp of a tooth extracted from a lepromatous patient. **G** = globus; **L** = leprosy bacillus. Magnification: 2,800X.

FIG. 2. Macrophages in the dental pulp. Many leprosy bacilli and red blood cells can be seen inside the cytoplasm of the macrophages. **L** = leprosy bacillus; **M** = macrophage; **RBC** = red blood cell. Magnification: 9,000X. Scale: 1μ.

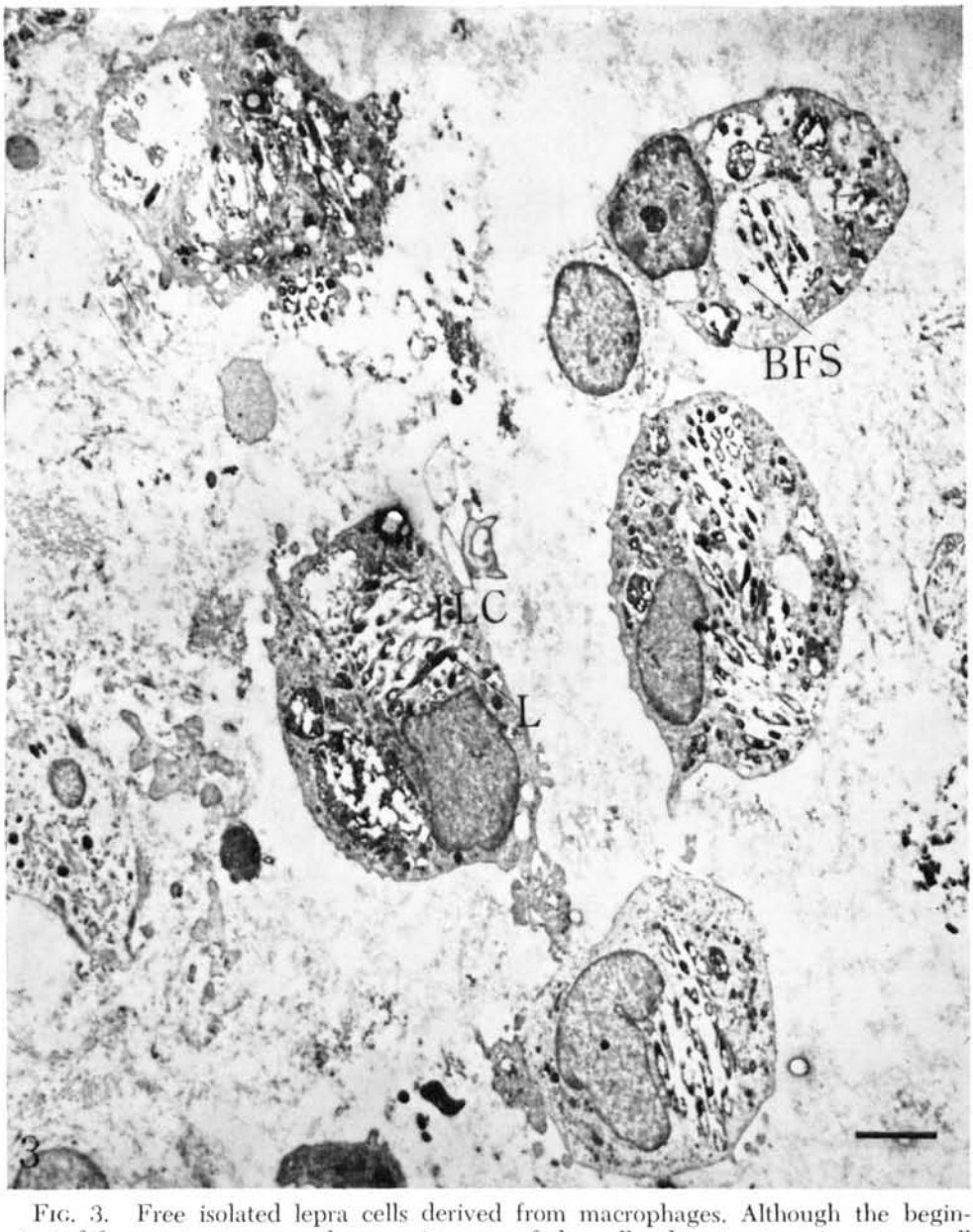

FIG. 3. Free isolated lepra cells derived from macrophages. Although the beginning of foamy structure can be seen in some of the cells, this structure is not so well developed as in lepra cells derived from pulp cells. **BFS** = beginning of foamy structure; **ILC** = isolated free lepra cells; **L** = leprosy bacillus. Magnification: 12,000X. Scale: 1μ.

Ultra-thin sections were made with a Reichert ultra-microtome, and electron microscope pictures were taken with the Akashi Tronscope 50 electron microscope at the accelerating voltage of 50,000 volts.

ELECTRON MICROSCOPE OBSERVATIONS

Fundamental structure of the dental pulp. The basic structure of the connective tissue of the dental pulp consists of pulp

cells and small collagen bundles arranged alternatively in parallel layers. The pulp cells show electron-dense cytoplasm with oval or elongated mitochondria, a slightly more electron-dense nucleus, and membranous stretching of the cytoplasm.

Blood vessels and myelinated nerve fibers can be seen embedded in the connective tissue. Nonmyelinated nerve fibers could not be seen clearly in these specimens, although they have been reported by Miyoshi ([7]) in normal dental pulp.

Lepra cells in dental pulp. Two kinds of lepra cells were found in lepromatous lesions of the dental pulp.

1. *Free isolated lepra cells* (derived from macrophages) (Figs. 2, 3). It appeared that free isolated lepra cells are more liable to break down and when broken discharge leprosy bacilli from the cell body (Fig. 4). Because of this, extra-cellular bacilli are observed more frequently in the infiltrated edematous area of the dental pulp, where most of the lepra cells are isolated and floating freely in the tissue fluid. Leprosy bacilli have only a small amount of electron-transparent zones, and no distinct foamy structures can be seen in free isolated lepra cells.

2. *Fixed lepra cells* (derived from pulp cells). In contrast, fixed lepra cells are usually seen among the connective tissue elements composed of pulp cells (a kind of fibrocyte, Fig. 5), arranged in parallel fashion. These pulp cells engulf leprosy bacilli, and usually develop into more mature foamy cells with a distinct electron-transparent intracytoplasmic foamy structure. From these observations it appears that the fixed lepra cells derived from pulp cells have a longer life than free isolated lepra cells. Because of this longer life of pulp cells, leprosy bacilli that have become degenerated in the cytoplasm are retained longer within them. The pulp cells loaded with bacilli are usually located near the blood vessels.

Leprosy bacilli in the nerve tissue of the dental pulp. In histopathologic sections of the same dental pulp, many bacilli were observed along the nerve fibers of the root pulp. Some bacilli that appeared to be located along the nerve fibers are presumably to be considered as in Schwann cells.

In a picture of the nerves of the dental pulp, leprosy bacilli were seen in a tubular structure that appeared to be a nerve fiber (Fig. 7). We considered this tubular structure to be a nerve fiber because it lay end-to-end close to neighboring nerve fibers, and it had also a basement membrane similar to that of the Schwann cell. But, for final conclusion as to the location of bacilli in the nerve tissue, we must wait for the decisive electron micrographs of leprosy bacilli in the nerves of the dental pulp.

DISCUSSION

Histopathologic features of the evolutive stages of lepromatous lesions of the dental pulp. Lepromatous lesions of the dental pulp are usually divided into three stages of pathologic evolution (Hirashita ([2]) and Kamio ([5])). The findings of these named authors can be summarized as follows:

1. *Initial stage.* Leprosy bacilli and round cell infiltration can be seen along the dental nerves. When the lesion advances, a few lepra cells appear around the nerve, and also a few bacilli can be seen in the endothelial cells of the blood capillaries. In this stage, lepra cells are distributed evenly in the crown pulp, but in the root pulp bacilli are located around the nerve fibers. In this stage, too, the basic structure of the dental pulp is still retained fairly well.

2. *Proliferating stage.* Capillaries are stacked with leprosy bacilli, and lepra cell infiltration can be seen in perivascular spaces. A few bacilli can be seen in odontoblast layers. Bacilli are seen also between the nerve fibers of the dental pulp. When the lesions advance, the lepra cells are formed in the odontoblast layer.

3. *Final stage.* In the final stage, the basic structure of the dental pulp is no longer visible, and the tissues are replaced by lepra cells. Finally, the lepra cells change to foamy cells, and necrosis of the dental pulp can be seen. All the nerve and blood vessels are destroyed.

The pathologic changes in the dental pulp of the case here described appear to belong to the later phase of the initial stage, or to the beginning of the prolifer-

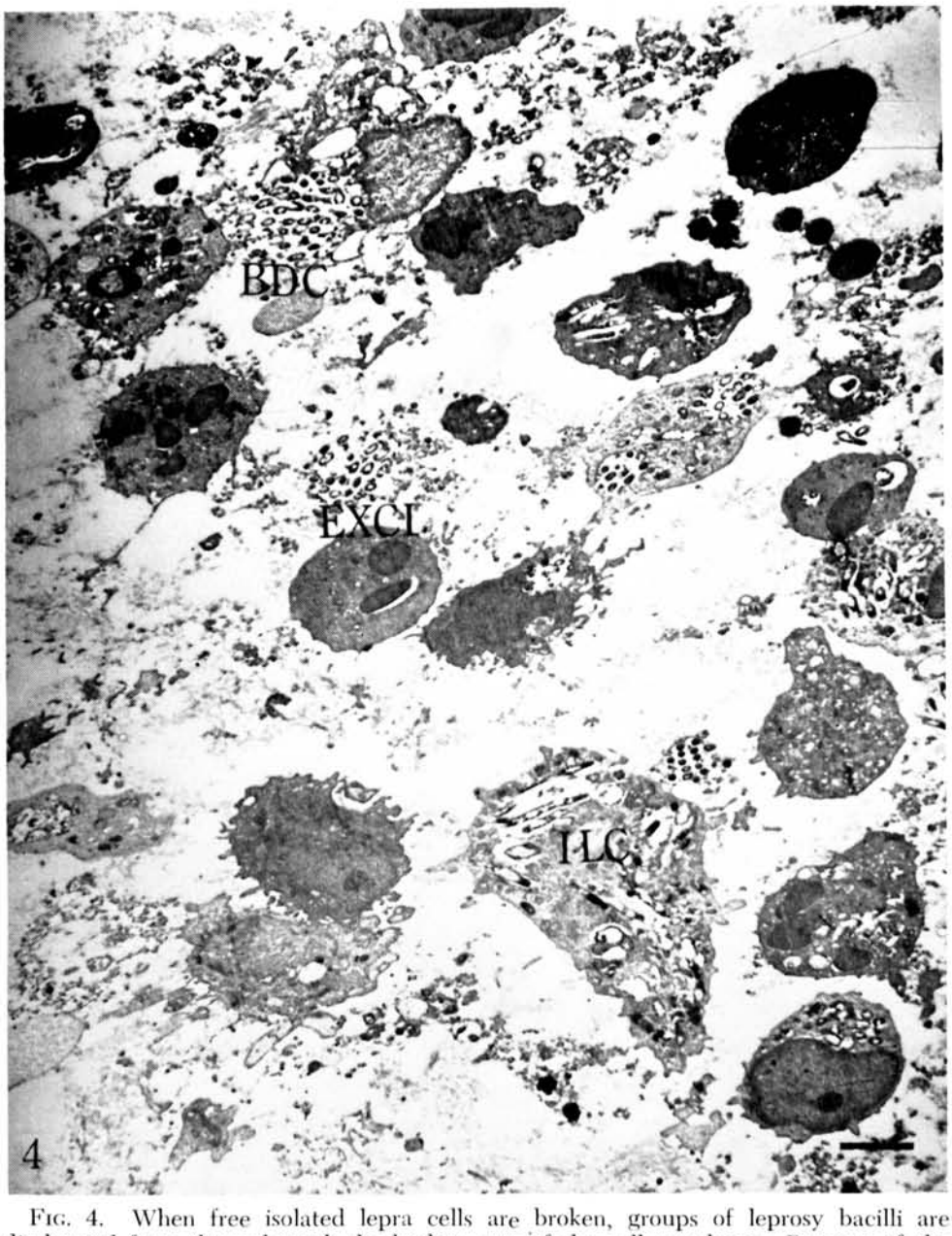

FIG. 4. When free isolated lepra cells are broken, groups of leprosy bacilli are discharged from them through the broken part of the cell membrane. Because of the destruction of lepra cells, many leprosy bacilli can be seen extra-cellularly. **BDC** = leprosy bacilli discharged from a broken lepra cell. **EXCL** = extracellular leprosy bacilli; **ILC** = isolated free lepra cells. Magnification: 10,600X. Scale: μ1.

FIG. 5. Fixed lepra cells derived from pulp cells. They have well developed foamy structures in the cytoplasm. The cytoplasm of such cells has the same electron-density as that of the pulp cells. Also they show membrane-like stretching of the cytoplasm going out of the cell body just as in the case of pulp cells. The background structure of the dental pulp is rather well retained in this part of the specimen. **CE** = capillary endothelium; **CF** = collagen fibrils; **LCP** = lepra cells derived from pulp cells; **MS** = membrane-like stretching of the cytoplasm; **PC** = pulp cell. Magnification: 10,500X. Scale: 1μ.

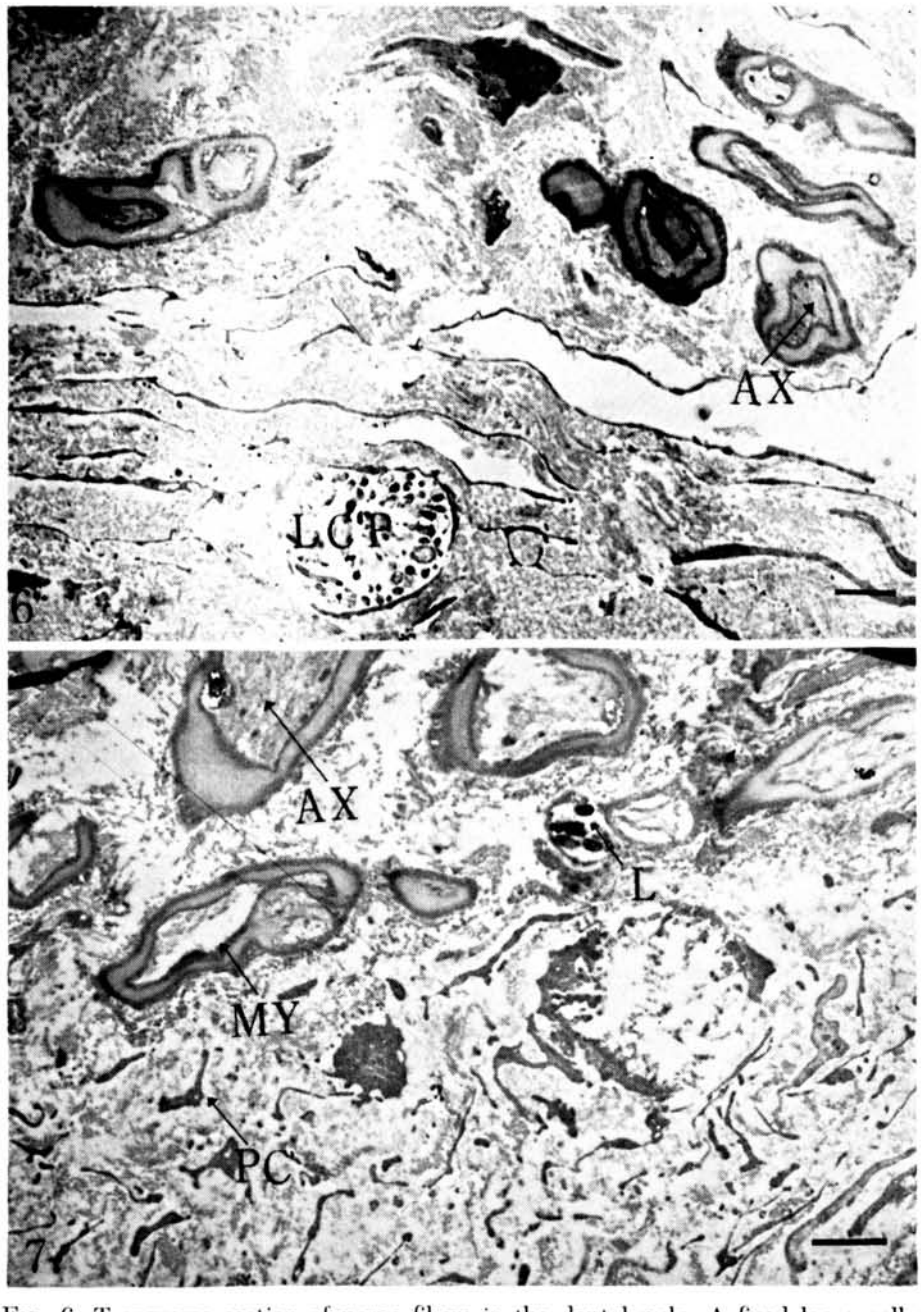

FIG. 6. Transverse section of nerve fibers in the dental pulp. A fixed lepra cell derived from the pulp cell can also be seen. **AX** = axon; **LCP** = lepra cell derived from the pulp cell. Magnification: 8,700X. Scale: 1μ.

FIG. 7. A group of leprosy bacilli are seen in a tubular structure that appears to be a nerve. **AX** = axon; **L** = leprosy bacilli, which seem to be located in the nerve; **MY** = myelin sheath; **PC** = pulp cell. Magnification: 10,600X. Scale: μ1.

ating stage, according to the definition given above of the succeeding stages. Electron microscopy in this case has shown isolated free lepra cells derived from macrophages, and also distinct foamy structure formation in the cytoplasm of the pulp cells near the blood vessels. However, the basic structure of the connective tissue of the dental pulp, which is composed of parallel layers of collagen bundles and pulp cells, is not yet destroyed.

Route of invasion of leprosy bacilli in the dental pulp. It seems probable that the bacilli were carried by way of the alveolar anterior maxillary arteries to the dental pulp of the upper central incisor of the patient studied, and that macrophages loaded with leprosy bacilli migrated from the blood vessels into the dental pulp tissue. When these macrophages had been broken, the bacilli in them were discharged from the cells (Fig. 4). These extra-cellular leprosy bacilli appear to have been phagocytized again by new macrophage and pulp cells. Some of them might be phagocytized also by Schwann cells of the dental nerves, since the Schwann cells have some phagocytic property. As there are no endoneurial and perineurial cells in the nerve tissue inside the dental pulp (Fig. 6), the bacilli that could be seen in the histopathologic sections of the root pulp of this patient may be in Schwann cells or macrophages or pulp cells near the nerve fibers (Fig. 7).

SUMMARY

Lepromatous changes of the dental pulp were found by both light and electron microscopy, in one of seven teeth extracted from six patients with lepromatous leprosy.

Two kinds of lepra cells are found in the dental pulp, viz., free isolated lepra cells derived from macrophages, and fixed lepra cells derived from pulp cells.

Free isolated lepra cells are often broken and bacilli are discharged from the broken cell body; in such cases extracellular bacilli are frequently encountered.

Intracystoplasmic foamy structures are most distinct in fixed lepra cells derived from pulp cells. This fact may be due to the longer life of pulp cells than that of macrophages. Usually pulp cells near the blood vessels show distinct foamy structure formation attributed to degeneration of leprosy bacilli in them.

Although leprosy bacilli were found in the nerve tissue of the dental pulp, they seem to have been carried there by the blood vessels and later taken into the Schwann cells of the pulp.

RESUMEN

Se encontraron cambios de tipo lepromatoso en la pulpa dentaria en uno de siete dientes extraídos en seis pacientes con lepra lepromatosa y en cuyo estudio se empleó el microscopio de iluminación corriente como también el microscopio electrónico.

Dos tipos de células leprosas se encuentran en la pulpa dentaria, v.gr. células de lepra libres, aisladas, que proceden de los macrófagos, y células fijas, de lepra, que derivan de las células de la pulpa.

Las células de lepra libres, aisladas, están corrientemente rotas y los bacilos son descargados del cuerpo celular roto; en tales casos se encuentran con frecuencia bacilos extracelulares.

La estructura vacuolar intracitoplasmàtica está más diferenciada en las células leprosas fijas que derivan de las células de la pulpa. Este hecho puede deberse a la vida más larga de las células de la pulpa que la de los macrófagos. Por lo general, las células de la pulpa próximas a los vasos sanguíneos muestran una distinta estructura vacuolar, lo que podría atribuirse a la degeneración del bacilo de la lepra en ellos.

Aunque se encontraron bacilos de la lepra en el tejido nervioso de la pulpa dentaria, parecería que fueron llevados allí por los vasos sanguíneos y luego trasladados a las células de Schwann de la pulpa.

RÉSUMÉ

Parmi sept dents extraites chez six malades atteints de lèpre lépromateuse, on en a observé une qui présentait des altérations lépromateuses de la pulpe dentaire, pouvant être mises en évidence à la fois par le microscope optique et par le microscope électronique.

Deux espèces de cellules lépreuses ont été trouvées dans la pulpe dentaire, à savoir des cellules lépreuses libres isolées, dérivées des macrophages, et des celules lépreuses fixes dérivées des cellules de la pulpe.

Les cellules lépreuses isolées et libres étaient souvent brisées et des bacilles étaient déversés du corps cellulaire brisé; des baciles extracellulaires ont souvent été rencontrés dans ces cas.

La structure spumeuse intracytoplasmique était mieux discernable dans les cellules lépreuses fixes, dérivées des cellules de la pulpe. Ceci peut être du au fait que la vie des cellules de la pulpe est plus longue que celle des macrophages. Habituellement, les cellules pulpaires proches des vaisseaux sanguins montraient une formation en structure spumeuse distincte, que a été attribuée à la dégénérescence des bacilles de la lèpre qu'elles contiennent.

Quoique des bacilles de la lèpre aient été trouvés dans le tissu nerveux de la pulpe dentaire, il semble que ces bacilles y aient été amenés par les vaisseaux sanguins, et englobés ultérieurement dans les cellules de Schwann de la pulpe.

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