ELECTRON MICROSCOPE STUDY OF XANTHOMA CELLS IN A LEPROMATOUS LEPROSY LESION

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A diagnosis of exanthoma tuberosum was made in the case of lepromatous leprosy with yellow nodular lesions covering both of the elbows. Two possibilities were considered before this diagnosis was made, namely, the presence of a true xanthoma, and the xanthomatization of a lepromatous nodule.

Both possibilities were naturally interesting in connection with an investigation of the behavior of *Mycobacterium leprae* within the xanthoma cells, as well as for a comparative study of the metabolic aberrations of the lipid processes in the lesions.

The Kyoto group, including one of the authors (T.I.), have investigated the process of development of the components of the submicroscopic structures of both lepra and xanthoma cells, and their physicochemical properties ([11, 12, 21]). In order to clarify the nature of the present case, electron microscopic observation has been of great value. Furthermore, the present case has been very useful for the further study of the components of lepra cells. Therefore, the results of the electron microscopic investigation of this lesion are compared with the histochemical findings, and also with the results already obtained in the studies of simple xanthoma cells and lepra cells.

MATERIALS AND METHODS

The patient, A.J.C., was a 49-year-old white man with disseminated lepromatous lesions on the trunk, face and limbs. He had been aware of the disease since 1945, when paresthesia began in his limbs. About a year ago yellowish nodules began to appear on the elbows, growing in size until they measured about 4 cm. in diameter.

Laboratory tests were normal as regards the blood picture, urine and feces. Blood cholesterol was 208 mgm. per cent. Smears from the lesions, including the xanthoma nodules, were 3 positive for leprosy bacilli. The patient was treated with DPT.

Biopsy specimens were taken from the xanthomatous lesions of the elbows and prepared as follows:

1. After preliminary fixation in 10 per cent formol, a part was embedded in paraffin for histologic study.

2. Frozen sections were made for polariscopic examination, and for lipid staining with Sudan III, Sudan IV and Sudan black B.

3. Specimens from the same material were fixed for 6 hours in 1 per cent osmium tetroxide solution buffered with acetate-veronal (1/30 M) at pH 7.2 and made isotonic with sucrose, dehydrated with acetone, and immersed in a mixture of n-butyl (80%) and methyl methacrylate (20%). The samples were then polymerized at 55°C for about eight hours, sectioned on a Fernández-Morán-type ultramicrotome ([7]) equipped with a diamond knife, and examined with a Siemens Elmiskop I at 80 kv.

RESULTS

HISTOPATHOLOGIC FINDINGS

The rete cones are flattened and show a number of infiltrating foam cells, among which lymphocytes and histiocytes are found in small numbers. Neither giant cells nor eosinophils are observed in this lesion. The infiltrates usually form large islands around sweat appendages and small capillaries. The endothelial cells of the latter do not become foamy (Fig. 1).

After application of the Fite-Faraco stain, innumerable acid-fast bacilli are revealed in the foam cells, where they are either scattered or in globi (Fig. 2). With lipid stains the same cells show within their cytoplasm what seems to be a great many lipid droplets (Fig. 3). No bacilli are found in the papillary layer of the dermis.

The various staining methods employed give evidence of a typical lepromatous granuloma. However, a number of the lipid droplets present are shown to be anisotropic on polariscopic examination (Fig. 4). This finding indicates the presence of cholesterol or its esters in the foam cells of the lesion.

INTERPRETATION OF ELECTRON MICROGRAPHS

In the many vacuolated cells, which are found surrounded by collagen fibrils, various components are classified according to their structures as follows: (1) moderately dense droplets, (2) foamy structures, (3) convoluting or onion-like structures, and (4) electron-transparent vacuoles.

The droplets.—Very small, moderately dense, supposedly pinocytotic droplets, which measure less than 100 mμ in diameter, are found throughout the cytoplasm (Fig. 6). They show a tendency to fuse and form round or oval, moderately dense aggregates which measure from 400 to 900 mμ in diameter. They seem to be similar to the "coalescent droplets" in lepra cells ([11]). However, the "dense body" with dense spots, thought to be produced by the fusion of pinocytotic droplets in xanthoma cells ([12]), seems to be absent.

Besides coalescent droplets, typical opaque droplets (which are moderately dense, surrounding leprosy bacilli) can also be found (Figs. 6 and 8).

DESCRIPTION OF FIGS. 1-4

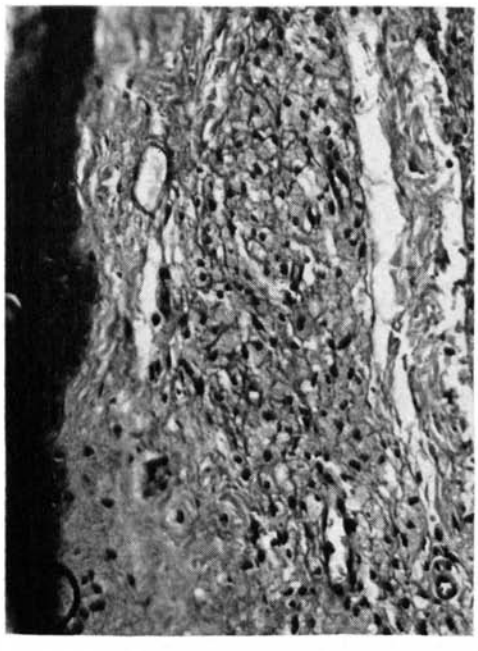
FIG. 1. This lesion shows a cellular infiltrate composed of numerous foam cells, closely resembling an ordinary leproma. In this picture, however, it is difficult to distinguish the xanthoma cells from the lepra cells. Hematoxylin-eosin stain.

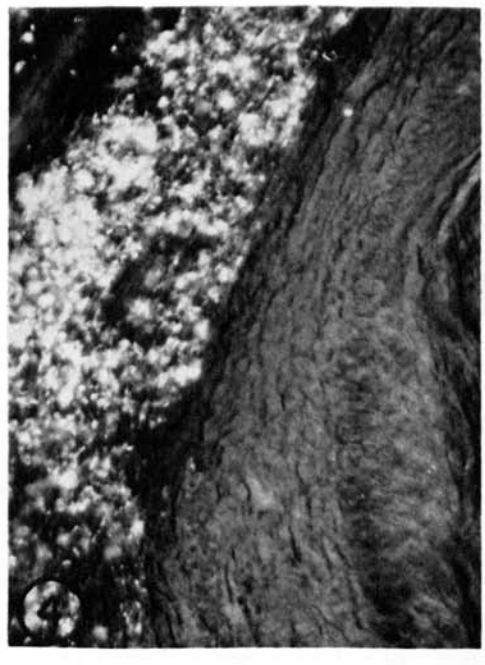
FIG. 2. In the cells of this lesion, numerous leprosy bacilli lie in clumps (globi?) and scattered. The appearance is what would be seen in a simple leproma. Fite-Faraco stain.

FIG. 3. The cellular infiltrate exhibits cells containing a number of lipid drops in their cytoplasm. Sudan black B stain.

FIG. 4. The lipid drops in the infiltrate show birefringency under polariscopic observation. Unstained section.

The foamy structures.—The foamy structures are made up, in a manner similar to those of lepra cells ([11, 21]), of moderately dense, amorphous masses, which are the remnants of opaque droplets, small vesicles and leprosy bacilli (Figs. 5, 6 and 8). These structures are clearly delimited from the cytoplasm by a single membrane.





Convoluting or onion-like structures.—These structures appear in a complex, lamellar arrangement within the coalescent droplets (Figs. 5 and 7). The cells, the object of this study, apparently produce electron-transparent vacuoles through these structures, similar to those of xanthoma cells ([12]). Leprosy bacilli are infrequently displayed in these structures, as shown in Fig. 7.

Electron transparent vacuoles.—The cells have a great many such vacuoles. These vacuoles appear to be surrounded by a membranous layer, which however does not delimit them specifically from the cytoplasm (Figs. 5, 6 and 8). It will be seen that these vacuoles begin with the vesiculation of the moderately dense droplets and pass through the stage of onion-like structure, and that at the end of that process they are electron-transparent. Those vacuoles may consist of a colloidal solution of lipid material which does not show any affinity to osmium. If these vacuoles were filled with osmiophilic material which is extracted during dehydration by the use of lipid solvents such as alcohol and acetone, the osmiophilic remnants should be found, as shown in the study of fat cells ([10]). They contain neither bacilli nor any small vesicles. As seen in Fig. 8, they show a tendency to merge with the foamy structures.

Other cytoplasmic organelles.—The mitochondria either retain their distinctive structures, or, as is sometimes the case, they become attached to transparent vacuoles or to the foamy structures (Fig. 8). It is very difficult to tell, however, whether this apparent attachment of the mitochondria to these structures, which are thought to be of a lipid nature, is a functional process or simply the result of pressure caused by the distribution of the vacuoles. As regards the Golgi apparatus, there is no evidence that it has any intimate connection with the vacuoles.

Small capillaries.—The various structures described above do not seem to be present in the endothelial cells of the small capillaries. Around the basement membrane of the capillaries, certain small adventitial cells with a complex cell membrane and with a denser cytoplasm can be observed (Fig. 5). The cells, characterized by the presence of the structures described in the foregoing, show the dense cytoplasm as well as the adventitial cells. On the other hand, clear cytoplasm can be noted in the majority of the cells located away from the capillaries.

It has been proved by histochemical and electron microscopic studies that the adventitial cells possess pinocytotic activity and a capacity for storage ([4, 10, 15]). The natural conclusion is that they take pinocytotic droplets and bacilli into their cytoplasm, which becomes vacuolated as a functional effect.

Pinocytotic droplets fuse with each other to form a dense body, which then transforms into a convoluted, onion-like structure and ends up as a transparent vocuole by losing its osmiophilic affinity.

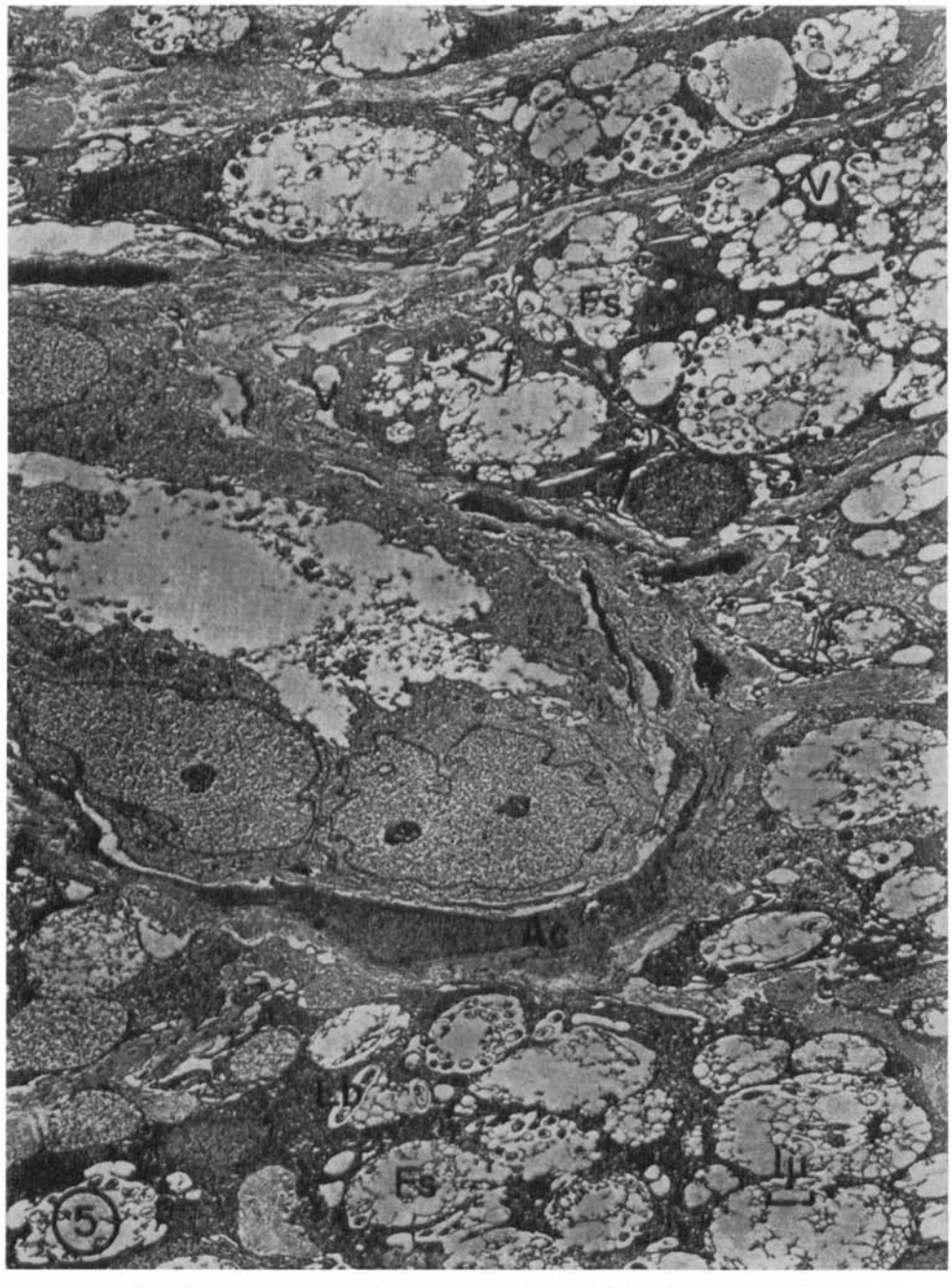


FIG. 5. An ultra-thin section of the lesion. Cells containing leprosy bacilli (**Lb**), foamy structures (**Fs**), and electron-transparent vacuoles (**V**) are observed around a small capillary. The opaque droplet and onion-like structures (arrow) are also shown in the cytoplasm. Most of the cells show dense cytoplasm, that is, these cells comprise abundant cytoplasmic organelles in the consolidated state. Adventitial cells (**Ac**) also display dense cytoplasm and complex cell membrane. Magnification × 6,500.

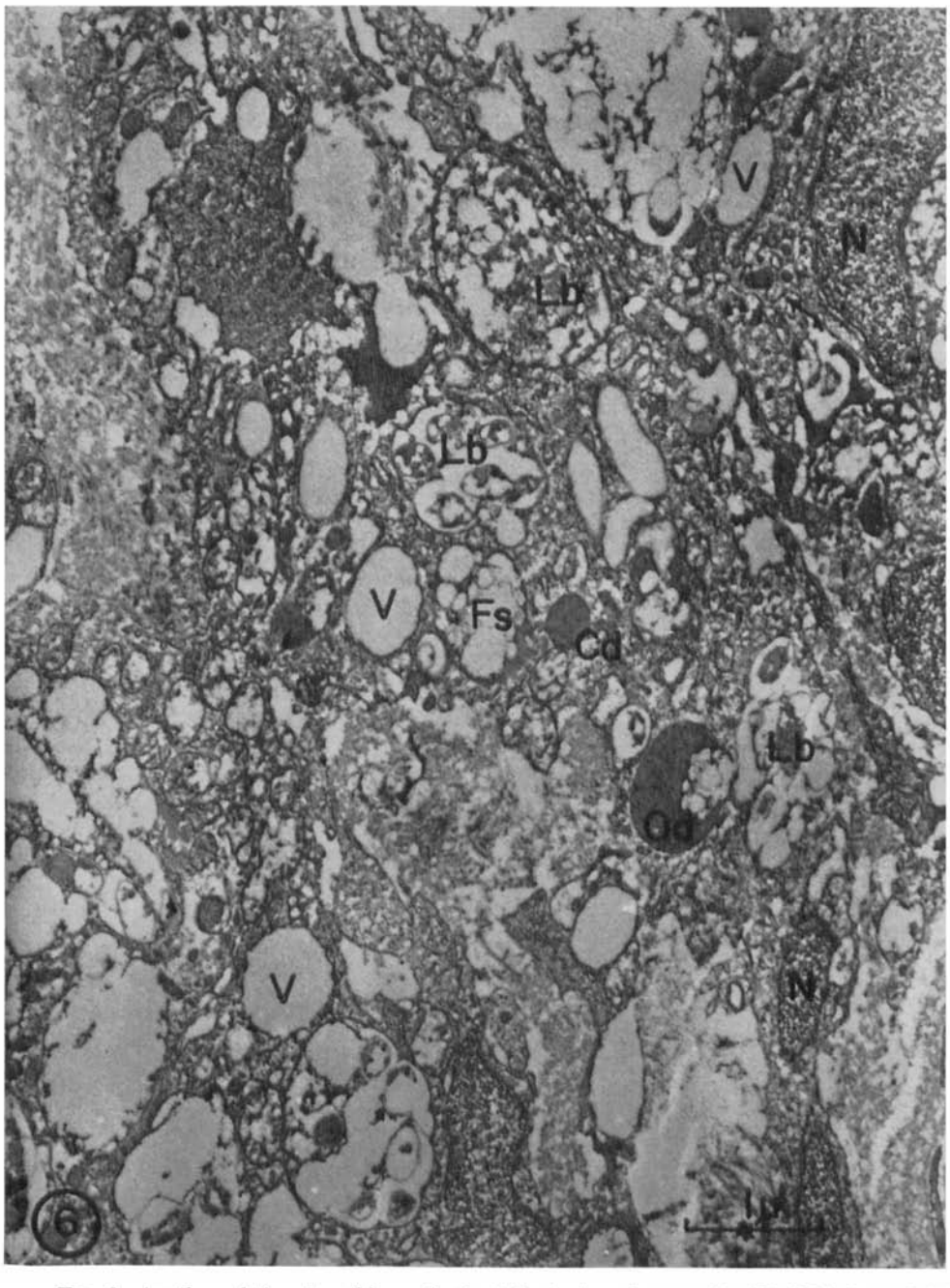


FIG. 6. Another of the ultra-thin sections of the lesion. Leprosy bacilli (**Lb**) located in foamy structures (**Fs**), opaque droplets (**Od**), and transparent vacuoles (**V**) are visible in the cytoplasm. Pinocytotic droplets (arrow) and their coalescent feature (**Cd**) are also noted. Nucleus, **N**. The transparent vacuoles do not show any relationship to the bacilli. Magnification × 20,000.

DISCUSSION

The process of xanthomatization in a leproma.—It has been shown, by histochemical study and by electron microscopy, that lepra cells contain lipids which supposedly are composed of lipoproteins, phosphatides and fatty acids ([8, 9, 11, 16, 19]). Anisotropic substances, such as cholesterol and its esters, are usually not found in a leproma ([8]). In this case, however, a number of anisotropic substances were present in the infiltrating cells and this fact is a rather strong indication of their xanthomatous nature.

In the study of the submicroscopic structures of lepra cells, it has been shown that the coalescent pinocytotic droplets accumulate around the bacilli (formation of opaque droplet), and subsequently change into foamy structures due to bacterial activity ([11, 21]). As regards the xanthoma cells, electron micrographs have revealed clearly the transformation of pinocytotic droplets into the transparent vacuoles characteristic of xanthoma cells ([12]).

In the electron micrographs of this lesion, various structures were shown in the cytoplasm, namely, moderately dense droplets, onion-like structures and the transparent vacuoles which were very similar to those of xanthoma cells ([12]). On the other hand, the bacilli, the foamy structures and also some of the moderately dense droplets, did not differ from those of lepra cells ([11]). It is evident, therefore, that the characteristic structures of both xanthoma cells and lepra cells were present in the same cells of this lesion. This being so, there is good reason to believe that the pinocytotic droplets give rise to two processes, namely, the opaque droplets characteristic of the lepra cell, and the convoluting or onion-like structures characteristic of the xanthoma cell.

Thannhauser ([17, 18]) has divided xanthomas into three groups: (1) hypercholesteremic xanthoma, (2) hyperlipemia with secondary eruptive xanthoma and (3) normocholesteremic xanthomatosis. It is to the third form, normocholesteremic xanthoma, that this case belongs since the blood cholesterol was within normal limits.

Two different postulates have been made about the causes underlying the formation of a foam cell in normocholesteremic xanthomatous tissue. It is attributed: (1) to a disturbance in the intracellular enzymatic systems that have to do with the synthesis of cholesterol ([17]); and (2) to cholesterol infiltration into the macrophages from the detritus of focal necrosis ([6]).

Regarding the first postulate, it has been asserted that the coalescent pinocytotic droplets in lepra cells are due to an aberrant lipid metabolism in the lesion ([11]). Inasmuch as the characteristic structures of lepra cells are found in this lesion, its lipid metabolism may be abnormal. Moreover, the transformation of pinocytotic droplets into transparent vacuoles, a phenomenon characteristic of xanthoma cells, is an indica-

tion that there may be an additional enzymatic disorder, presumably in the systems that have to do with the metabolism of cholesterol itself. It is doubtful if the bacilli have anything to do directly with the formation of transparent vacuoles, since bacilli are not often found in onion-like structure. It is difficult to tell, however, whether or not the formation of the xanthoma cells is due to an enzymatic disorder caused indirectly by the bacillary invasion.

The second postulate, that of absorption of cholesterol from necrotic tissue by macrophages, can now be discussed. In lepromatous lesions there is a break-down, not only of cytoplasmic organelles but also of bacillary bodies. The products of disintegration contain various lipids, which may include cholesterol or its components. However, the presence of an anisotropic substance is a very rare occurrence in a leproma. Furthermore, lepra cells show neither onion-like structures nor transparent vacuoles. Consequently, there is a minor possibility that phagocytized cell debris and bacilli contribute to the formation of the onion-like structures which characterize the xanthoma cell of this lesion.

In considering the occurrence of transparent vacuoles in xanthoma cells, it seemed most likely that these vacuoles are a final feature of the onion-like structures ([12]). In the cells observed in the present study, however, small transparent vacuoles are also distributed among large ones, and these show no correlation with onion-like structures. It is possible, therefore, that they may be formed by uptake of some electron transparent substance, thought to be free cholesterol or its esters with saturated fatty acids. However, it is not easy to determine whether this cholesterol is supplied from the blood or whether it originates from the cellular debris.

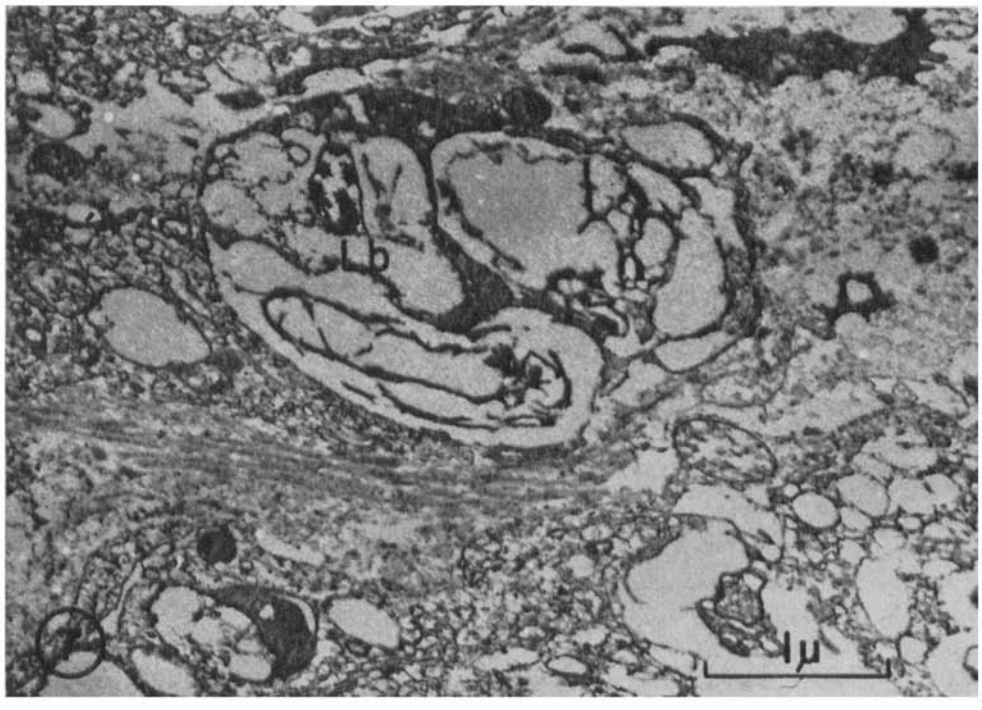
Electron microscopic analysis of lipids in lepra cells.—The opaque droplets of submicroscopic structures in lepra cells have been reported as being composed of lipoproteins. At the same time, the minute vesicles that are found within the foamy structures have been postulated as phosphatides released through lipoprotein disintegration ([11]). However, the transparent areas in the same structures leave many problems to be solved before their chemical nature can be made clear.

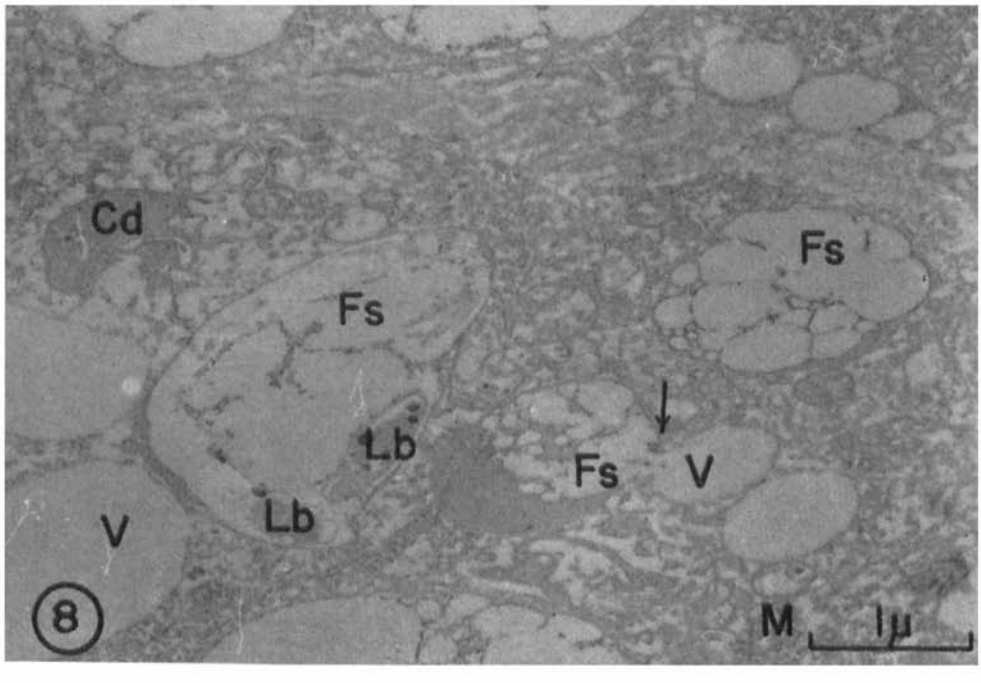
The present study, dealing as it does with a xanthoma of high

DESCRIPTION OF FIG. 7 AND 8

FIG. 7. Onion-like structures appear in the moderately dense matrix, thought to be coalescent droplets. Leprosy bacilli (**Lb**) are found in some of these structures, a finding which is very unusual. Magnification × 26,000.

FIG. 8. This picture exhibits foamy structures (**Fs**) delimited from the cytoplasm by a distinct membrane, and transparent vacuoles (**V**). The latter are surrounded by a membranous layer, but in comparison with the foamy structures are not clearly separated from the cytoplasm. The latter stage of a foamy structure containing leprosy bacilli (**Lb**) shows an appearance similar to the transparent vacuoles. However, remnants of opaque droplets and minute vesicles still remain. One of the vacuoles shows a tendency to fuse with the foamy structure (arrow). A mitochondrion (**M**) attached to the vacuole is to be noted. Coalescent droplet, **Cd**. Magnification × 23,000.


Lb
1μ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cholesterol content and complicated by a lepromatous infiltration, may contribute to a better understanding of the chemical nature of foamy structures.

The transparent vacuoles of xanthoma cells contain cholesterol and cholesterol esters, together with saturated fatty acids—transparent after osmium fixation. The vacuoles in which they are contained are not delimited by any distinct membrane. The same has been observed with the vacuoles in the cytoplasm of other cells that are known to contain such lipids, for instance, in those of the adrenal cortex ([1, 13, 14]). This is likewise observed in the cytoplasmic vacuoles of this lesion. This absence of a definite vacuolar membrane points towards cholesterol and its esters as being, most probably, the most abundant lipids in the vacuolar content.

The foamy structures form a marked contrast to the transparent vacuoles in that they are always surrounded by a single membrane measuring 50 A in width. This membrane is composed of phosphatide micelles, according to the conclusion reached in the study of lepra cells ([11]). The logical inference is that the contents of foamy structures and transparent vacuoles are physicochemically different.

The cells of xanthomatous tissue have been proven by chemical analysis to contain phosphatides, cholesterol and its esters, as well as neutral fats ([5, 18]). Among these lipids, phosphatides and neutral fats show moderate to marked density in the electron micrograph ([2, 3, 10, 11]), and, although there is as yet little clarity concerning the chemical composition of onion-like structures, those lipids are very probably contained in them. On the other hand, it must be pointed out that foamy structures never show any onion-like structures in their interior, although they probably contain phosphatides and fatty acids. When the data available on the foamy structures are composed with those pertaining to the transparent vacuoles, there is little possibility that the former contain cholesterol, while the latter almost certainly do. This was discussed in a preceding paper about lepra cells ([11]).

One of the authors (T.I.) has suggested that the transparent areas in the foamy structures may be due to a colloidal aqueous phase of certain saturated fatty acids and other molecules of comparatively low molecular weight. If there is a bacillary wax, as Wade supposed ([20]), there is—in view of the present study—little probability that it is a sterol, but it may be an aliphatic alcohol.

SUMMARY

1. A xanthoma tuberosum lesion which appeared in a lepromatous patient has been studied, using electron microscopic and histopathologic procedures.

2. The same cells in this lesion exhibit two structures characteristic of lepra cells and xanthoma cells. The coalescent pinocytotic droplets, opaque droplets, foamy structures and leprosy bacilli belong to those of lepra cells, while onion-like structures and transparent vacuoles are characteristic of xanthoma cells.

3. The interpretation of the electron micrographs indicates that the cells in this lesion deviate from the normal lipid metabolism, especially that of cholesterol.

4. Comparative observations between the transparent vacuole and the foamy structure indicate that the latter does not contain sterol derivatives.

RESUMEN

1. Se ha estudiado una lesión de xantoma tuberoso que apareció en un enfermo lepromatoso, usando para ello procedimientos electronomicroscópicos e histopatológicos.

2. Las mismas células de esta lesión muestran dos estructuras típicas de células leprosas y células xantomatosas. Las gotillas pinocitóticas coalescentes, las gotillas opacas, las estructuras espumosas y los bacilos leprosos son características pertenecientes a las células leprosas, mientras que las estructuras acebolladas y las vaculas transparentes son típicas de las células de xantoma.

3. La interpretación de las electronomicrografías indica que las células de esta lesión se desvían del metabolismo lípido normal, y sobre todo, del colesterol.

4. Observaciones comparativas entre le vacuola transparente y la estructura espumosa indican que la última no contiene derivados esterólicos.

RESUMÉ

1. Une lésion de xanthoma tuberosum apparue chez un lépromateux a été étudiée au microscope électronique et par des techniques histopathologiques.

2. Dans cette lésion, on trouve, au niveau les mêmes cellules, les deux structures cellulaires caractéristiques de la lèpre et du xanthome. Les gouttelettes pynocytotiques, les gouttelettes opaques, les structures écumeuses et les bacilles de la lèpre relèvent de la structure des cellules de lèpre. Par contre, les structures en peau d'oignon et les vacuoles transparentes sont caractéristiques des cellules xanthomateuses.

3. L'interprétation des électro-micrographies permet de dire que les cellules de cette lésion témoignent d'une anomalie du métabolisme des graisses, et spécialement de celui du cholestérol.

4. La comparaison de la vacuole transparente et de la structure écumeuse montre que cette dernière ne contient pas de dérivés stérolés.

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