INTERNATIONAL JOURNAL OF LEPROSY

Volume 37, Number 2 Printed in U.S.A.

# The Liver in Lepromatous Leprosy I. A Biochemical, Functional and Ultrastructural Study<sup>1,2</sup>

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The liver in leprosy has been studied in necropsies (1, 6, 9, 15) and more recently, by biopsies (5, 7, 8, 12, 13, 20).

There is available only one prior report <sup>(11)</sup> on the ultrastructural aspects of the liver in two cases of leprosy. The purpose of this paper is to report on the correlation of the ultrastructural, functional and enzymatic patterns of the liver in lepromatous leprosy, as seen in the study of 29 patients.

### MATERIALS AND METHODS

Twenty-nine patients with active moderate or advanced lepromatous leprosy were selected for this study. The mean age of the group was 38 years, the oldest being 69 and the youngest 17 years. Nineteen had had leprosy for five to ten years, eight for one to five years, and two for more than ten years. Two patients with an advanced form of leprosy also presented with lepra reaction.

Acid-fast bacilli were demonstrated in the cutaneous lesions of all study subjects. Nasal mucus was positive for acid-fast bacilli in 21 patients. None of the patients had received specific leprosy treatment.

Liver biopsy was performed in 11 of these patients and part of each fragment was fixed in Helly's fluid and stained with hematoxylin-eosin, Ziehl-Neelsen stain and for iron. The electron microscopic study was done in the other part of the fragment,

as previously described (3). Three liver fragments obtained from patients with duodenal or gastric ulcers during gastrectomy were similarly treated and used as controls.

In nine other patients the whole liver biopsies were frozen. Biochemical determination of succinic dehydrogenase and acid and alkaline phosphatase activities in the specimens were carried out as previously described (3). Six liver fragments, five obtained during laparotomy in patients with mega-esophagus who underwent Heller's operation, and one from a patient with duodenal ulcer during gastrectomy, were similarly treated and served as controls.

Statistical significance of the results was evaluated by "student's test" as described by Snedecor (18).

In all patients an evaluation of the functional status of the liver was attempted using radioactive rose bengal and colloidal gold combined with liver scanning. The modified technic used will be the subject of another paper.

#### RESULTS

The biochemical Biochemical study. data regarding the enzymatic activity of the liver in patients with lepromatous leprosy as compared with the controls are presented in Table 1. There was no significant difference between the two groups.

Light microscopy. The findings were not essentially different from those previously reported in the literature (5, 7, 8, 12, 13, 20). It is noteworthy that even in young patients lipofuscin pigment was frequently found in the centrilobular hepatic cells. In three cases iron granules were detected among the lipofuscin pigment.

Electron microscopy. Changes were prominent in seven of the eleven cases while in four cases the livers were morpho-

<sup>&</sup>lt;sup>1</sup>Received for publication 1 November 1968. <sup>2</sup>This work was supported by grants of the "Fundo de Pesquisa do D.D.S." and "I.B.E.P.E.G.E." <sup>3</sup> T. de Brito. M.D., Pathologist, Instituto de Medicina Tropical, São Paulo, Brazil; N. Carvalho, M.D., Centro de Medicina Nuclear, São Paulo, Brazil; A. C. R. Marques, M.D., Dermatologist, Divisão Tecnica Auxiliar, Depto. Dermatologia Sanitaria. São Paulo, Brazil; D. O. Penna, M.D., Derete Internel, Medicine, Hermitel des Clicitan Depto. Internal Medicine, Hospital das Clinicas, São Paulo, Brazil; M. P. Azevedo, M.D., Director, Divisão Tecnica Auxiliar, Depto. Dermatologia Sanitaria São Paulo, Brazil.

Enzyme	Normal (No. pts. 6)	Lepromatous leprosy (No. pts. 8)	t test Normal vs pathologie (P > 0.05)
Succinic dehydrogenase	$12.72 \pm 6.72$	$9.96 \pm 1.23$	t = 1.150
Alkaline phosphatase	$11.08 \pm 4.17$	$16.14 \pm 8.31$	t = 1.360
Acid phosphatase	$23.26 \pm 6.61$	$20.07 \pm 5.43$	t = 0.9937

TABLE 1. Biochemical determinations of enzymatic activity in normal and pathologic liver (mean values  $\pm$  standard deviation).

logically similar to those of the controls. Minor mitochondrial abnormalities were found in two of the control cases.

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Kupffer cells were hypertrophic and hyperplasic, containing cytoplasmic dense bodies and, occasionally, degenerated bacilli (Fig. 1), morphologically similar to those described by Kramarsky *et al.* (<sup>11</sup>) in livers and by Imaeda and Convit (<sup>10</sup>) in vesicular leprous lesions. Their cytoplasms frequently overlapped and basement membrane-like material was deposited between and at the bases of the cells. The sinusoidal side of the hepatocytes appeared, in focal areas, either to be devoid of or to have distorted and swollen microvilli. Such



FIG. 1. Hypertrophic Kupffer cell containing degenerated lepra bacilli (B). Part of the nucleus (N) with nucleolus (Nu) and a few mitochondria (M) are also seen.



FIG. 2. Hepatocyte presenting with its sinusoidal pole (SP) partially devoid of microvilli. One enlarged Kupffer cell (KC) is seen showing degenerated lepra bacilli (B) and the products of their disintegration. In Disse's space, in close contact with the Kupffer cell and swollen endothelial cell cytoplasms, there is a deposit of a faintly electron dense material, interpreted as basement membrane-like material (arrow). Reticular fibers (RF), and, inside the endothelial cell cytoplasm a globule of fat (F). The hepatocyte has normal glycogen content (GL) and mitochondria (M).

findings were particularly well seen beneath distorted and swollen Kupffer cells (Fig. 2).

Hepatocyte mitochondrial alterations were frequent and of three types, as follows: (1) Marked variation in form and size. (2) Giant mitochondria with dense matrix and abnormal number and disposition of cristae (Fig. 3). The cristae were sometimes layered (Fig. 4), an appearance which is referred to as "crystalline" or "para-crystalline formation" or as "myelin or fibrillary degeneration" ( $^{2, 16, 21}$ ). (3) More rarely there were enlarged mitochondria having a light matrix and few cristae. Occasionally large irregular electron dense<sup>®</sup> masses were observed in the mitochondrial matrix.

A combination of the first two types of abnormality was frequent.

Large electron dense masses, morphologically identified as lipofuscin, were more commonly observed in lepromatous livers than in the controls and was found in its usual location near bile canaliculi (Fig. 5). This finding is in accordance with previously reported light microscopy studies (7). More granular and electron dense masses were less frequently seen in similar locations, usually surrounded by a single mem-

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FIG. 3. Hepatocyte showing an enlarged mitochondria (AM) with a dense matrix and abnormal amount and disposition of cristae. One vacuolated mitochondria (VM)is located near others that are normal (M). Granules of pigment (P), probably lipofuscin. Cell nucleus (N).

brane. They were probably iron granules.

Single membrane, round or oval, structures containing electron dense material were more often observed in hepatic cells of the lepromatous livers, chiefly, but not exclusively, near the biliary pole. These were regarded as cytosomes. Autophagic vacuoles containing altered mitochondria or degenerated portions of hepatic cell cytoplasm were less frequently seen (Fig. 6). Golgi complexes appeared with enlarged vesicles, often containing a homogeneous electron dense material. Microbodies were also more prominent and frequent.

Normal bile capillaries alternated with others showing partial loss or distortion of

Moderate alteration (5 cases)	Marked alteraton (12 cases)	Normals (12 cases)
$\begin{array}{ll} \text{RB 131 I} = 36 \text{ to } 41\% \\ \text{K}_{\text{Au}} &= 17 \text{ to } 21\% \\ \text{Dysfunction} \end{array}$	$\begin{array}{l} {\rm RB} \ 131 \ {\rm I} \ = \ 24 \ {\rm to} \ 30\% \\ {\rm K}_{\rm Au} \ \ = \ 12 \ {\rm to} \ 15\% \\ {\rm Dysfunction} \end{array}$	$\begin{array}{rl} {\rm RB} \ 131 \ {\rm I} \ = \ 44 \ {\rm to} \ 39\% \\ {\rm K}_{\rm Au} \ &= \ 24 \ {\rm to} \ 29\% \end{array}$
Predominant reticulo- endothelial = 3	Predominant reticulo- endothelial = 11	
Predominant parenchymal $= 2$	Predominant parenchyma $= 1$	

TABLE 2. Summary of the functional status of the liver in lepromatous leprosy as seen in radioactive rose bengal (RB) and colloidal gold ( $K_{Au}$ ) clearance.

Normal values of RB 131 I and KAu clearance were based on previous work of Tatit et al. (19).



FIG. 4. Giant mitochondrion (GM) with crystalline structures in its matrix. The same phenomenon is present in mitochondria of normal size (AM). One cytosome (C) is present. The hepatic cell has a normal amount of glycogen (GL). RER designates the the rough endoplasmic reticulum.

microvilli. Small, elongated, electron dense structures were sometimes observed inside the lumina of these altered bile capillaries (Fig. 7).

In one case the cells of a periportal ductule showed irregular cytoplasmic dense bodies and enlarged intercellular spaces (Fig. 8).

The glycogen content of the hepatic cells was preserved. In a few instances the smooth endoplasmic reticulum predominated over the rough reticulum and its cisternae were dilated.

These varied findings were seen in scattered groups of hepatocytes without a definite pattern of distribution.

Hepatic functional studies. Radioactive rose bengal and colloidal gold studies indicated that among the 29 patients studied, 12 were normal and 17 showed a mixed type of lesion, affecting both the reticuloendothelial (RE) and parenchymal cells, but in different proportions. These patients were further divided into two groups as shown in Table 2.

Concomitant liver scan studies of 16 patients indicated normal size, position and morphology of the liver in nine. The remaining seven presented mild enlargement of the liver and in four of them such enlargement was mainly of the left lobe.

#### DISCUSSION

Except for dilated cisternae of endoplasmic reticulum no parenchymal cell injury was seen by Kramarsky *et al.*  $(^{11})$  in two cases. In our cases it was found in seven of the 11 studied, but not in all the hepatic cells.

The mitochondrial alteration known as



FIG. 5. Biliary pole showing a bile capillary (**BD**) with few swollen microvilli, intact junctional complexes (**JC**) and Golgi apparatus (**G**). Around the bile capillary, in the cytoplasm of the three hepatocytes there are many lipofuscin granules (**LP**). Hepatocytes present normal glycogen content (**GL**) and mitochondria (**M**).

"fibrillary degeneration"  $\binom{2,14}{2}$  or crystalline structures  $\binom{16,21}{2}$ , as seen in lepromatous patients are regarded by Ruffolo and Covington  $\binom{16}{2}$  as a definite pathologic finding. We, in common with Wills  $\binom{21}{2}$ , noted it in some of our controls, but in a smaller proportion of organelles than in lepromatous liver. However, we recognize that our controls were from patients with diseases which could cause slight liver alterations.

The pathogenesis of this mitochondrial injury is not well understood and no theory has satisfactorily explained this finding. Because of its presence in so many diseases it has been regarded as a nonspecific degenerative phenomenon (<sup>21</sup>).

Enlarged mitochondria with few cristae

having a light matrix with or without large electron dense masses, infrequently observed by us, were also regarded as damaged organelles.

On the other hand, the enlarged mitochondria with many cristae and dense matrix, can be interpreted as compensatory hypertrophy, at the ultrastructural level, of undamaged organelles.

Increased deposition of lipofuscin, also observed by light microscopy, was another frequent finding even in young patients. Lipofuscin is regarded by Schaffner (<sup>17</sup>) as a residue accruing from autophagic vacuolar digestion of degenerated parts of cell cytoplasm. Pigment deposition in the lepromatous liver thus indicates preceding slight, long term injury or aging of cells.



FIG. 6. Cytosegresome (autophagic vacuole) (CS) containing part of degenerated cytoplasm in a hepatocyte. Reticulum endoplasmic profiles (RE) are slightly dilated. Mitochondria (M) are normal.

Such nonspecific injury also affects the biliary secretory apparatus, as reflected in the alterations noted in the Golgi apparatus and bile capillary microvilli. However, these changes were neither severe enough nor sufficiently widely distributed to impair bile excretion and produce cholestasis.

In the group of 17 patients showing a mixed parenchymal and RE type of lesion, as evaluated by functional study, RE predominated over parenchymal injury. Thus it appears that RE injury, chiefly characterized by Kupffer cell hypertrophy and hyperplasia (4.9.) together with intralobular circulatory disturbances due mainly to leproma formation, are the major causes of the functional deficiency seen in the hepatic parenchyma in lepromatous leprosy. This, together with excess basement membrane-like deposition and sinusoidal pole microvilli alterations are probably responsible for some deficiency in material exchange between hepatocytes and sinusoidal fluid.

The hepatocellular damage which affects the cell as a whole is nonspecific and probably secondary to either the RE or the intralobular circulatory disturbance, or both. Superimposed injuries, such as deficient nutrition and alcoholism, may contribute to the hepatocellular damage.

There are nonspecific alterations of the hepatic cell as a whole in the lepromatous liver. The lack of detectable alteration in enzymatic activity seems to indicate that the lesions in the hepatic cells are not severe enough to make themselves evident over the physiologic reserve of the liver. Compensatory mechanisms may be present within the cell itself. Thus, hypertrophic mitochondria may compensate for faulty activity of those that are damaged. Moreover, liver scanning disclosed that in a few instances, lesions were more intense in areas which are usually not readily accessible to biopsy.

Functionally there is in the liver of lepromatous leprosy a predominance of RE over



FIG. 7. Biliary pole of hepatocyte showing bile capillary (BC) with few swollen microvilli and intact junctional complexes (JC). Golgi apparatus have enlarged vesicles (G2) sometimes with a faintly electron dense material inside (G1). Microbodies (MB). Normal (M) and altered mitochondria (AM). The hepatocytes have a normal glycogen content (GL).

parenchymal injury. Probably the former appears first and, interfering with the absorptive mechanisms of the hepatic cell, is responsible for the latter.

#### SUMMARY

A biochemical and ultrastructural study of the liver in lepromatous leprosy is presented. Definite reticulo-endothelial hyperplasia was noted with hypertrophic Kupffer cells occasionally containing degenerated bacilli in their cytoplasm and appearing as lepra cells. Nonspecific, irregularly focal hepatocellular damage was demonstrated, affecting the hepatocyte sinusoidal pole, mitochondria and bile secretory apparatus. Lipofuscin pigment, peribiliary cytosomes and microbodies were more often seen in the injured cells.

Succinodehydrogenase, as well as alkaline and acid phosphatase enzymatic activity was preserved. These findings are interpreted as probably due to cell and organelle compensatory activity.

Colloidal gold and radioactive rose bengal studies pointed to a predominance of RE over hepatic cell injury. Liver scanning demonstrated in a few instances that predominant lesions were to be found mainly in the left lobe.

### RESUMEN

Se presenta un estudio bioquímico y ultraestructural del hígado en lepra lepromatosa. Definitiva hiperplasia retículo-endotelial se notó en las células hipertróficas de Kupffer conteniendo ocasionalmente bacilos degenerados en su citoplasma y apareciendo como células de lepra. Se demostro un daño hepatocelular, irregular, focal, no específico, afectando el polo sinusoidal de los hepatocitos, los mitocondrios y aparatos secretorios de la bilis. Pigmento de lipofuscina, citosomas y microcuer-



FIG. 8. Ductule showing swollen microvilli (MI), intact junctional complexes (JC) and dilated intercellular space (IS). Epithelial cells show many dense bodies (DB) in the cytoplasm and few intact mitochondria (M) and Golgi complexes (G). Basal membrane (BM).

pos peribiliales fueron frecuentemente observados en las células dañadas.

La succinodehidrogenasa, como tambien la actividad enzimática, alcalina y acido fosfatasa se mantuvieron. Estos hallazgos se interpretan como probablemente debidos a las células y a la actividad orgánica compensatoria.

Estudios con oro coloidal y rosa de bengala radioactivos señalan una predominancia de RE sobre la célula hepática dañada. La observación "scanning" del hígado demostró en unos pocos casos que lesiones notorias se encontraban principalmente en el lóbulo izquierdo.

#### RÉSUMÉ

On présente ici une étude biochimique, ainsi qu'une étude de l'ultrastructure du foie dans la lèpre lépromateuse. On a noté une nette hyperplasie réticulo-endothéliale, avec des cellules de Kupffer hypertrophiques, contenant à l'occasion dans le cytoplasme des bacilles dégénérés, et apparaissant sous l'aspect de cellules lépreuses. On a mis en évidence des lésions hépato-cellulaires non spécifiques, réparties en foyers irréguliers, qui affectent le pôle sinusoïde des hépatocytes, les mitochondries, ainsi que l'appareil secrétoire biliaire. La lipofuchsine, un pigment, des cytosomes péri-biliaires, de même que des microstructures, sont notés davantage dans les cellules endommagées.

La succino-déhydrogénase, de même que l'activité enzymatique des phosphatases alcaline et acide, étaient conservées. Ces observations sont considérées comme témoignant probablement d'une activité compensatoire de la cellule et de ses organelles.

Des études à l'or colloïdal et au rose bengale radioactif tendent à montrer une prédominance des lésions au niveau du système réticuloendothélial plutôt que de la cellule hépatique. Dans quelques cas, le "scanning" du foie a montré que les lésions principales devaient être situées dans le lobe gauche.

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