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Pathology of the Kidney and Liver in the Experimental Leptospirosis of the Guinea-Pig*

A Light and Electron Microscopy Study

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A. Introduction

Liver (DOTTI and SABBIONI, OSTERTAG) and kidney biopsies (PENNA et al.) can be performed in human leptospirosis, thus opening new possibilities in the study of the basic lesions of the disease.

Electron microscopy of the biopsied kidney in human leptospirosis (BRITO et al.) disclosed a slight, but definite glomerular lesion which provides an anatomical basis to explain proteinuria. However, tubular pathology was more marked, beginning at the cell membrane, thus adding further support to the idea that the pathogenesis of leptospirosis is linked to a toxin production by the parasite (AREAN, STAVITSKY).

Before further studying by electron microscopy the human liver in leptospirosis, a reevaluation of the experimental disease is in order.

B. Material and Methods

Forty-three guinea-pigs were used, five experiments being performed. Sex did not differ in the animals of each experiment and no differences regarding course of illness and pathologic lesions were seen when using female or male guinea pigs. In four experiments animals weighing an average of 421 g were used. In one experiment, in order to try to obtain a less severe disease, animals weighing an average of 798 g were used. However, in this experiment this aim was achieved only in two animals. The course of illness and pathological lesions in the others were similar to those seen in the other four experiments.

The strains of *L. icterohaemorrhagiae* used were originally isolated from rats and cultivated from fragments of liver and kidney in Fletcher's medium for 8 to 10 days at 28° C. Virulence was enhanced through an initial inoculum of 0.5 ml of culture into the peritoneum of healthy guinea pigs weighing an average of 250 g. At the terminal phase of the disease these animals were killed with a blow in the head and 1:5 liver-kidney homogenates in saline were prepared. About 1 ml of the suspension was administered intraperitoneally in the animals used in the experiment.

The guinea pigs, after being bled through a heart puncture, were killed with a blow in the head in the initial phase of the disease, usually around 3—4 days after the inoculum, and in the terminal phase of the disease, usually around the 5—7th day of illness. Two healthy guinea pigs were also bled and killed after each experiment in a similar manner and used as controls.

Necropsy was performed immediately and representative fragments of liver and kidney were fixed in 10% formalin, Bouin's, Helly's fluid and, after paraffin embedding, were cut 5 μ thick and stained routinely by H.E., PAS (periodic acid-Schiff reagent with and without salive digestion) and a modification of Mallory's phosphotungstic hematoxylin for mitochondria. In part of the animals a modification of Perl's technique for iron and the silver technique of Warthin-Starry to show leptospires were used.

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In all experiments fragments of liver and kidney were cut 7 μ thick in a cryostat microtome either immediately after the necropsy or after 24 hours fixation in 4% formalin, pH 7.2, plus 7.5% of sucrose (HOLT and HICKS), at 5°C. The fixed fragments before cutting were transferred to a mixture of sucrose and gum acacia for 24—48 hours at 5° C. In the non fixed fragments succinodehydrogenase activity was studied using as substrates the Nitro B.T. (ditetrazolium chloride) and the M.T.T. [3-(4,5-dimethylthiazolyl-2)-2,5 diphenyl tetrazolium bromide] (PEARSE). In only two experiments cytochrome oxidase activity was also studied using as substrate the para-aminodiphenylamine, according to technique proposed by BURSTONE. In fixed fragments of 33 animals alkaline (using as substrates either Gomori's medium or sodium α-naphthyl phosphate), acid phosphatase (Holt's technique) and unspecific esterase (using as substrate the sodium α-naphthyl acetate) were studied.

In the last experiment, comprising 10 animals, 0.3—0.5 mm thick slices of liver and kidney were cut with a razor blade and fixed during 2 hours at 5° C in 1 per cent osmium tetroxide buffered to pH 7.2—7.4 with veronal acetate buffer (PALADE) plus 0.045 g of sucrose for each cm^3 of the solution (CAULFIELD). The tissues were rapidly dehydrated in a graded series of ascending alcohols and embedded in a calatysed epoxy resin mixture, Epon 812, by a method similar to LUFT. Thin sections were cut in a Porter-Blum microtome equipped with glass knives. The sections were doubly stained first in uranyl acetate (WATSON) and then in lead citrate (REYNOLDS). The preparations were examined either in a Siemens Elmiskop I or in a Philips EM 200 electron microscope.

C. Results

1. Gross and light microscopy study. Aminals examined at an early phase of the disease showed grossly kidneys either between the normal limits or slightly enlarged. Occasionally, small hemorrhagic striae were seen at the cortex going down to the medulla.

Histopathology of the kidney in the animals of this group disclosed occasional proteic deposits at the Bowman space and focal glomerular endothelial cells tumefaction.

The interstitium was usually edematous and focal inflammatory infiltrate was seen, made up of an admixture of histiocytes, lymphocytes and few eosinophils, occasionally surrounding the Bowman's capsule. Tubular lesion was seen throughout the nephron, chiefly distal tubules, being also focal and mainly degenerative. It was characterized by cloudy swelling and, occasionally, by hyalin droplets in cells of proximal tubules. Hyalin and hematic casts were seldom seen in distal tubules lumina. No marked changes were observed in the blood vessels and mitochondria at this phase of the disease.

At the late phase of the disease the kidneys were enlarged and the cut surface disclosed hemorrhagic striae chiefly at the cortex going down to the medulla. Between the hemorrhagic areas parenchyma was pale and edematous. Histopathology showed glomeruli markedly congested with proteic deposits inside the Bowman's space. The interstitium was strongly edematous, chiefly at the cortico-medullary limit, and an inflammatory infiltrate, still focal and made up of the cells previously described was observed, producing sometimes a disorganization of the tubules. Tubular lesions were now marked, affecting the entire nephron and characterized by a degenerative process and, occasionally, by areas of cellular necrosis with rupture of the tubular basal membrane. Hyalin, hematic and cellular casts were seen inside the lumina of the distal tubular cells. Side by side with degeneration and necrosis evidence of tubular regeneration was occasionally seen characterized by flattened cells with basophilic cytoplasm and typical mitosis. Brush border

disappears from most the proximal tubular cells. Mitochondria lacks their normal basal disposition inside the proximal tubular cells. Individual cells showed mitochondrial numerical depletion. The distinction between proximal and distal tubules sometimes was quite difficult.

The liver at the early phase of the disease was slightly enlarged and usually congested. Otherwise, it appeared normal.

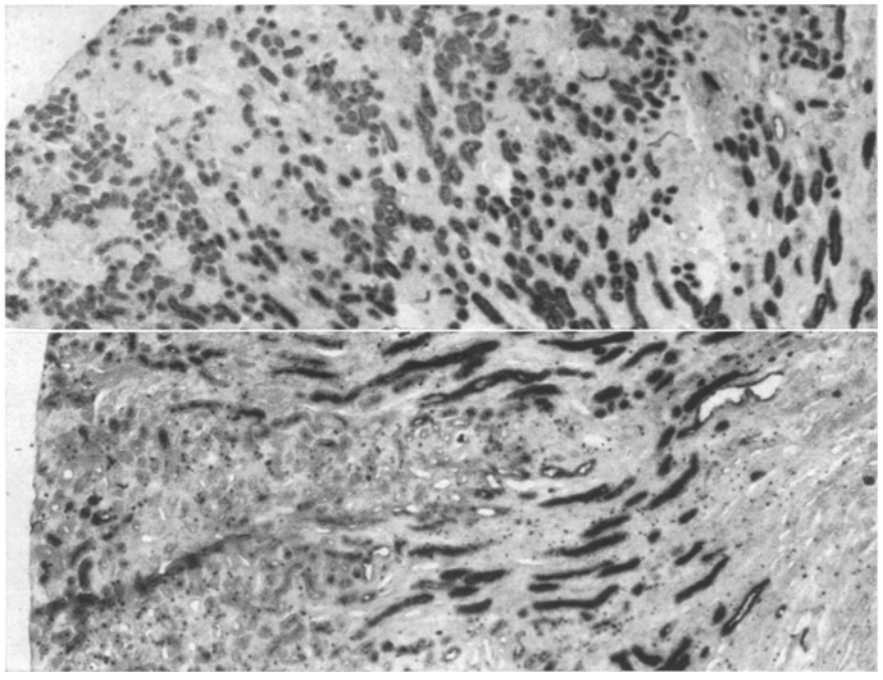


Fig. 1. a Kidney of guinea pig at an early phase of the disease showing an almost normal disposition of alkaline phosphatase activity at the border of tubular cells. Compare with b, showing a marked reduction of alkaline phosphatase activity in areas of kidney cortex in guinea pig at the late phase of the disease. Gomori's technique, cold buffered formalin fixation 40×

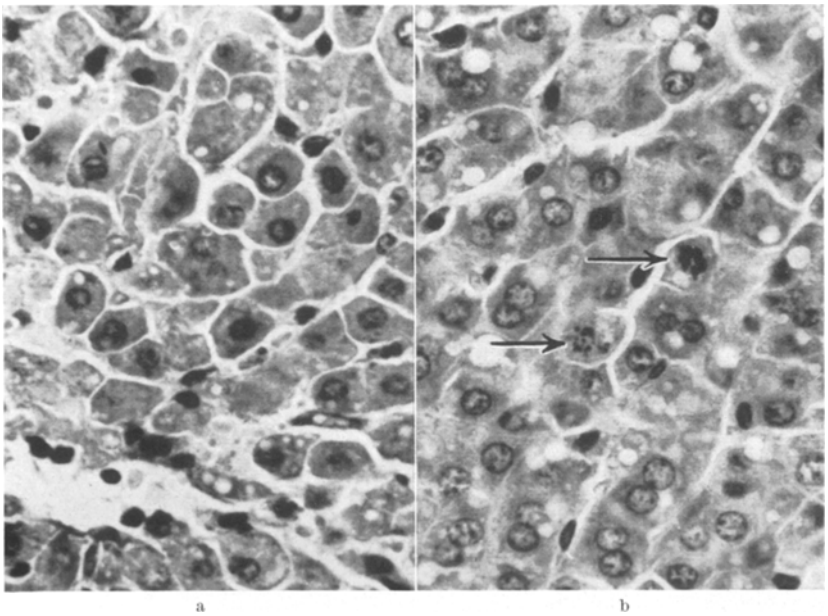
Histopathology disclosed mild hepatic lesions characterized by Kupffer cells hyperplasia and portal inflammatory infiltrate made up of histiocytes, lymphocytes and few eosinophils. Groups of hepatic cells showed fatty change.

At the late phase of the disease liver was enlarged, edematous, stained by bile. Lobular markings were blurred in areas. Some cases showed small irregular yellowish areas of necrosis, chiefly beneath the liver capsule.

Histopathology disclosed diffuse Kupffer cells hyperplasia plus chronic cellular infiltrate and edema of the portal spaces. Occasionally, focal necrosis of hepatic parenchymal cells were well seen, chiefly beneath the capsule. Hepatic cells usually showed degenerative process such as fatty change and, seldom, intracellular hyalin deposits. Few cases exhibited lack of the trabecular arrangement of the liver, the cells appearing small, with an angular contour, basophilic cytoplasm and, sometimes, pyknotic nuclei (Fig. 2a). Between these cells a faintly eosinophilic PAS negative deposit could occasionally be seen. Mitotic figures were seen in Kupffer's and hepatic cells as well as many multinucleate hepatic

cells (Fig. 2b). The mitochondrial lesion was also marked and characterized by numerical depletion, lack of an uniform location inside the cell and, sometimes, by an altered volume and contour of the organelle.

2. Histochemistry. Only the alkaline phosphatase activity correlates well with the degree of kidney lesion. At the early phase of the disease its activity was seen to disappear from groups of nephrons and, at the late phase large areas of depletion of the enzyme activity was observed at the cortex (Fig. 1a, b).



a b

Fig. 2a and b. Liver of guinea pigs at the late phase of the disease. a Cells appear with a well delimited and angular contour and with an irregular trabecular disposition. A faintly stained material is seen between the cells of the centrolobular area. 480×. H.E. stain. b Fatty change and mitotic figures (arrows) are seen in the hepatic parenchyma. H.E. stain. 480×

Acid phosphatase, inspecific esterase, succino dehydrogenase and cytochromo-oxidase did not show alteration when compared to controls.

Few animals only showed evidence of hemosiderosis. However, an increase of granules of PAS positive material, which resists salive digestion was seen chiefly at the late phase of the disease located at the biliar pole of the hepatic cell and inside the cytoplasm of Kupffer cells.

3. Electron microscopy findings. At the early phase of the disease kidney lesions were mild and mainly tubular. They were characterized by a focal partial disappearence of the brush border of the proximal tubules. Seldom observed, a widening of the intercellular spaces, the lumina exhibiting a granular electron dense deposit. However, no abnormalities were seen regarding the epithelial tight junction. Distal tubules failed to show microvilli lesion.

In general tubular cells failed to show marked abnormalities of their organelles, mitochondria, endoplasmic reticulum and Golgi complex appearing normal. However, at this phase of the disease was seen definite increase of round or ovalar dense bodies surrounded by a single membrane, usually located nearby

the luminal part of the cells. They were about the size of a mitochondrion and few of them have not a homogeneous matrix, a suggestion of altered cristae being observed.

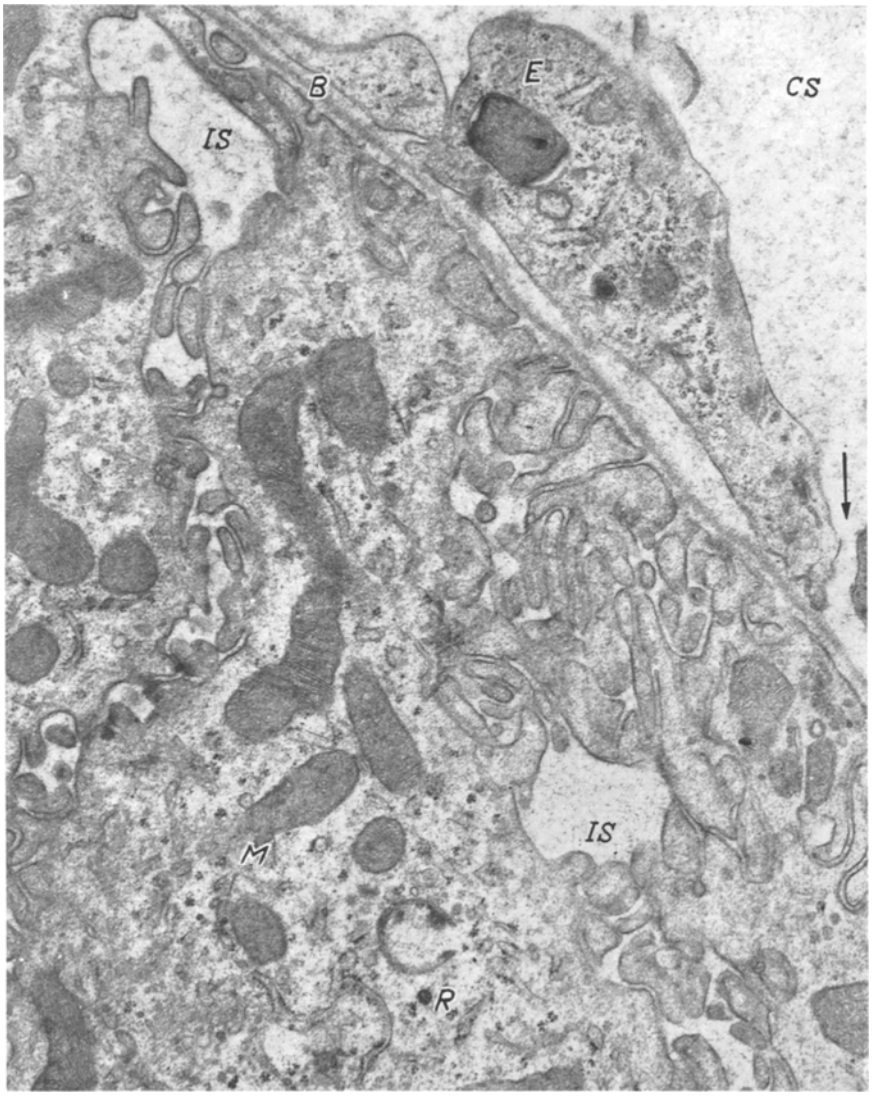


Fig. 3. Tubular cells and interstitial capillary of the kidney of a guinea pig at the late phase of the disease. Endothelial cells (*E*) appear swollen, with areas of detachment and disjunction (arrow). Tubular basal membrane (*B*) is normal. There is also a definite enlargement of the intercellular space (*IS*) between the tubular cells. Mitochondria (*M*) without abnormalities. Original magnification 5,000×, enlarged 3.5×

Besides focal mononuclear cells infiltration and a small capillary dilation no abnormalities were seen regarding the kidney interstitium.

At the late phase of the disease, tubular pathology becomes more marked, with frequent areas of brush border disappearence as well as edema of the remainder villosities (Fig. 5a). Distal tubuli exhibited also partial disappearence

of the microvilli. The widening of the intercellular space was also marked and sometimes mononuclear cells and even granulocytes could be seen in this space, although the tight junction and the tubular basal membrane did not appear abnormal (Fig. 3).

Regarding the tubular cell, in spite of the fact that there was still an increase of dense bodies with the previously described morphology and that occasional cells appeared light, with less mitochondria, these organelles usually had preserved double membrane and cristae. Seldom, in areas, they were enlarged, round, appearing edematous.

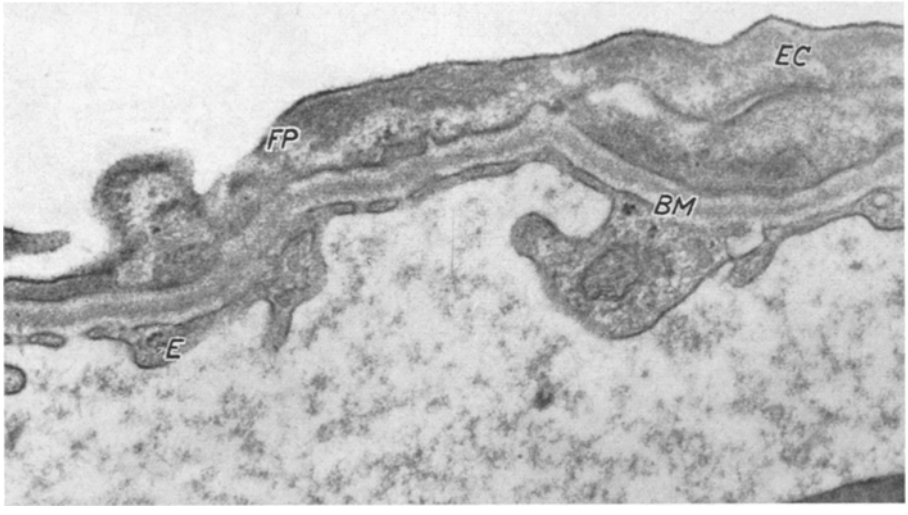


Fig. 4. Glomerular loops of a guinea-pig at the late phase of the disease showing focal areas of epithelial foot process fusion (*FP*). Endothelial cell (*E*) cytoplasm and basal membrane (*BM*) appears unaltered. Original magnification 9,000×, enlarged 3.5×

Besides the dense bodies described, ovular or round structures with a single membrane having in their lumen a small amount of slightly electron dense material could be made out throughout the nephron at this phase of the disease. They were usually smaller than a mitochondrion and were located nearby the tubular cell lumen.

Endoplasmic reticulum and Golgi complex appeared preserved in most of the cells. Nuclei appeared in areas light, with less chromatin, but with nucleoli and membrane preserved.

Interstitium is edematous with capillaries markedly enlarged showing prominent endothelial cells. Some of the capillaries exhibited areas of disjunction between the endothelial cells (Fig. 3). In areas there was detachment of the endothelial lining, the basal membrane also not been well visualized.

Usually at the late phase of the disease a mild glomerular pathology was seen. It was characterized by areas of fusion of the foot process of the epithelial cells (Fig. 4). Very rarely, areas of focal glomerular basal membrane thickening were seen. Endothelial cells appeared more prominent than usual. Mesangial cells disclosed no abnormalities.

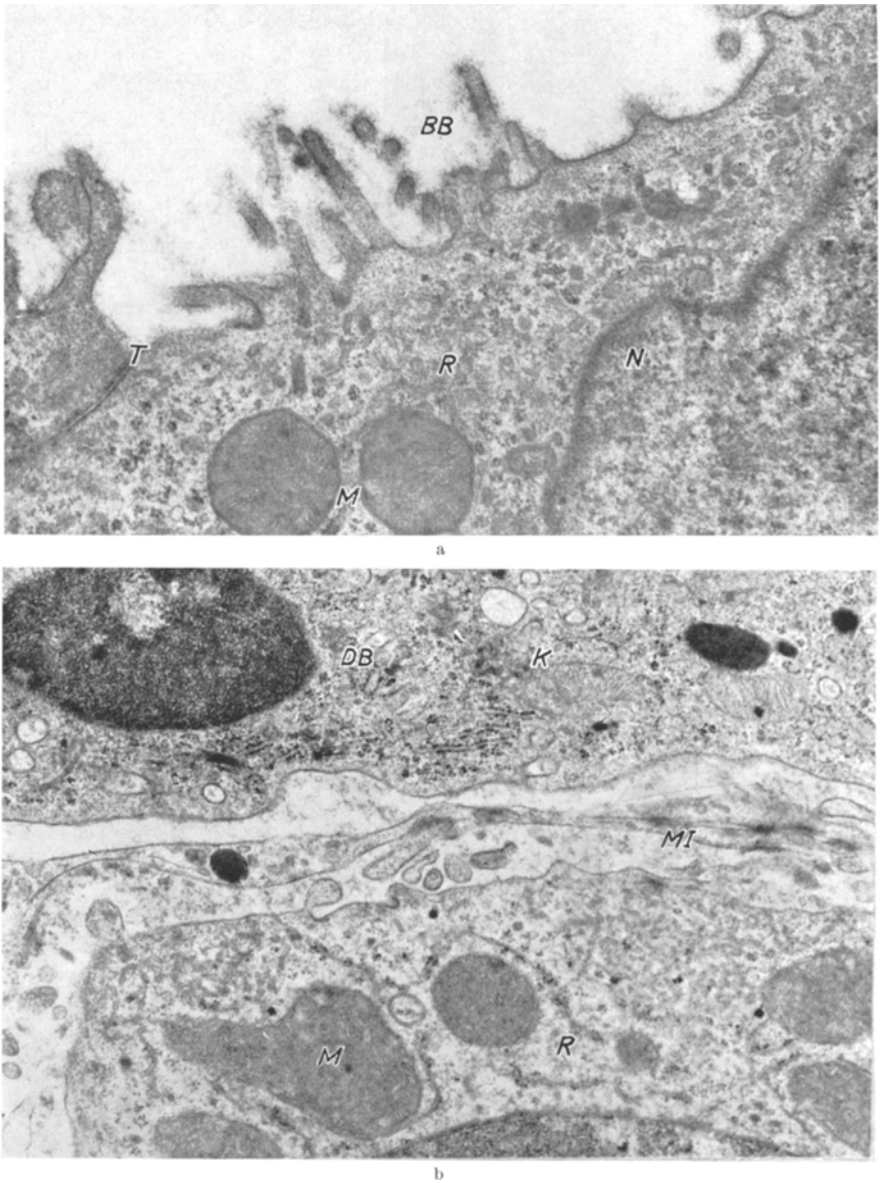


Fig. 5. a Partial disappearance of the proximal tubular cell brush border (*BB*) at the late phase of the disease. Nucleus (*N*), mitochondria (*M*) and part of the junctional complex (*T*) appear intact. Original magnification 7,000×, enlarged 3.5×. b Sinusoidal capillary of a guinea pig at the late phase of the disease. Microvilli (*MI*) are almost totally disappeared, the remainder appearing edematous. Part of the cytoplasm of a hypertrophic Kupffer's cell is also seen with many and irregular dense bodies (*DB*) in it. Mitochondria (*M*) appear intact. There is a predominance of the smooth type of endoplasmic reticulum in the cell

In the liver, at the initial phase of the disease, Kupffer cells appeared enlarged, with abundant cytoplasm plus an increased number of dense bodies. The Disse's space was either normal or slightly widened with a plasmatic material inside. Hepatic cells and bile ducts microvilli were generally unaltered. However, in areas, they appeared slightly irregular and swollen. The limit between hepatic

cells was definite and the junctional complexes were preserved. Hepatic cells showed an increased number of dense bodies, similar to the ones seen in the tubular and Kupffer cells, located chiefly around the biliary ductules. Otherwise, they showed no marked deviation from the normal.

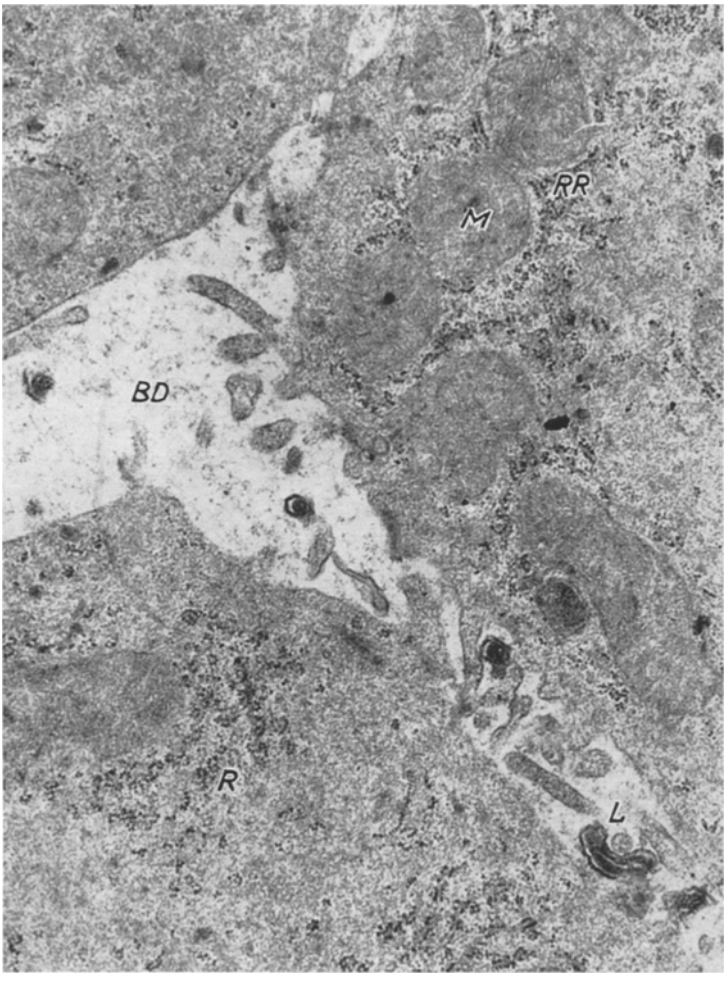


Fig. 6. Biliary ductule (*BD*) of a guinea pig at the late phase of the disease showing almost total microvilli disappearance. Hepatic cells failed to show elements of the junctional complex and in the enlarged intercellular space fragments of leptospirae (*L*) are seen. Mitochondria (*M*) appear normal. Ribonuclein particles (*R*) are also seen, together with rough endoplasmic reticulum (*RR*) around mitochondria. Original magnification 5,000 ×, enlarged 3.5 ×

At the late phase of the disease there was marked Kupffer cells hyperplasia, their cytoplasm being disposed in two partially overlapped layers, cell membrane sometimes partially fused through definite desmosomes. There was also accentuated widening of the Disse's space and the microvilli of the hepatic cells were irregular, swollen, and, in areas, completely absent (Fig. 5b). Reticular fibers were less frequently observed in the Disse's space. Microvilli of the biliary ductules were either also swollen or almost totally absent (Fig. 6).

A definite widening of the intercellular space was seen at this phase of the disease, sometimes with fragments of leptospires inside, connecting partially Disse's space and biliary ductules (Figs. 6, 7). In few animals in which the lack of trabecular arrangement of the liver cells was seen in the light microscopy study, electron micrographs showed a marked widening of the intercellular space, no precise localization of the biliary ductules being seen (Fig. 8). However, in the majority of the cases, in spite of a moderate enlargement of the inter-

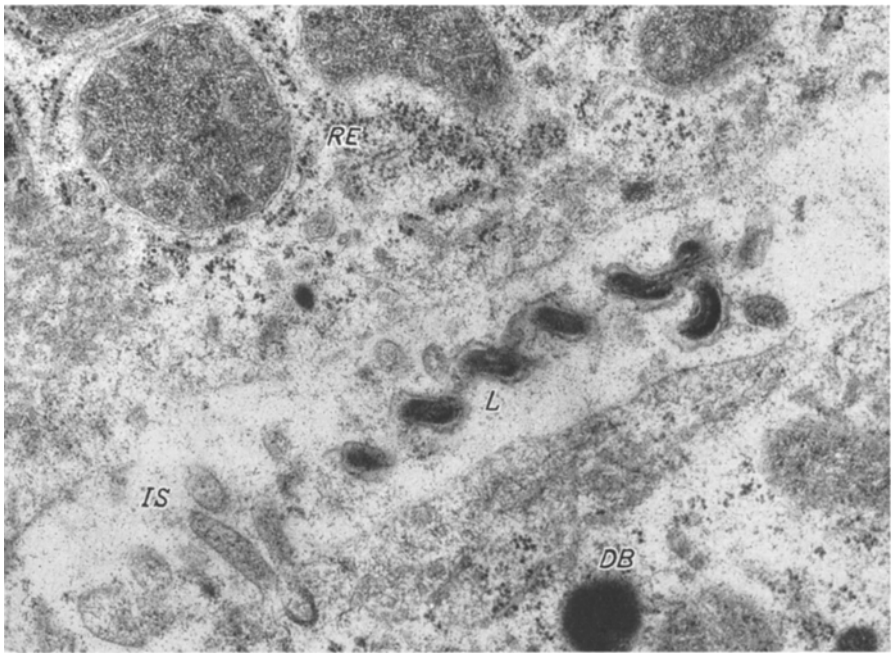


Fig. 7. In the enlarged intercellular space (*IS*) between two hepatic cells of a guinea pig at the late phase of the disease a fragment of a leptospirae (*L*) is seen. Original magnification 9,100×, enlarged 3.5×

cellular space, junctional complexes could be made out at least in some of the angles of the biliary ductules.

The hepatic cells showed, besides the already described increase in dense bodies, total or partial glycogen disappearance and a predominance of the smooth type of endoplasmic reticulum.

Mitochondria showed usually preserved membranes and cristae. However, in areas they appeared swollen and with few cristae. Some of the dense bodies, also had a size and shape similar to mitochondria and something like altered cristae could be made out. Also seen, the vacuoles previously described in tubular cells containing granules of a faintly electron dense material, delimited from the cytoplasm by a single membrane. Nuclei were in some cells light, with less chromatin than usual.

Two types of hepatic cells, one "light" and the other "dark" were more frequently seen than in the controls and in the animals killed at the early phase of the disease. The latter type of the cell differs from the first chiefly because

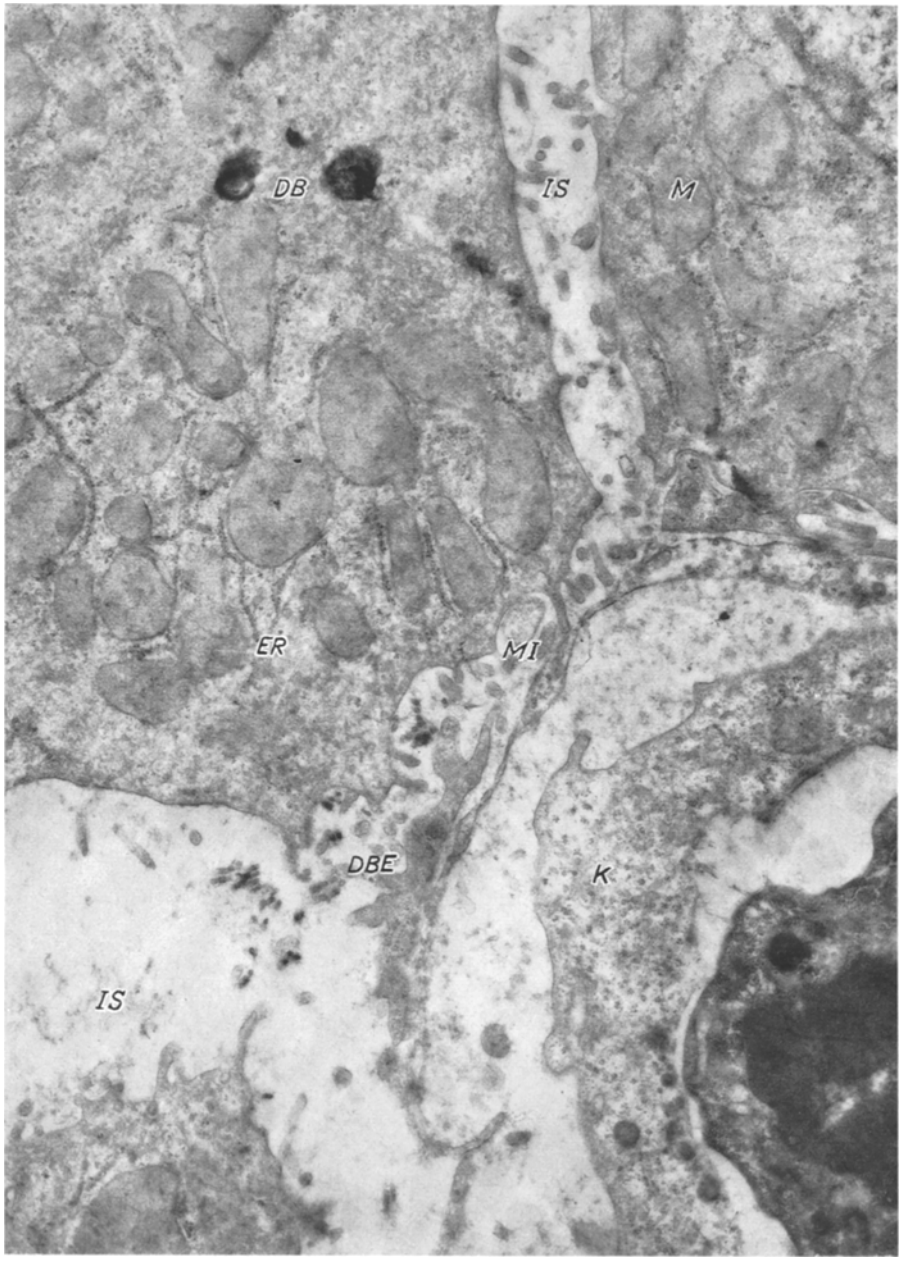


Fig. 8. Electron micrograph of the liver at the late phase of the disease in a guinea pig showing a lack of the normal trabecular arrangement of liver cells. Intercellular space (*IS*) is markedly enlarged. Microvilli are scanty and irregular. Part of an enlarged Kupffer's cell (*K*) is seen. Disse's space is enlarged (*DBE*) with a slightly electron dense material in it. Original magnification 4,000×, enlarged 3.5×

of the predominance of the smooth type of endoplasmic reticulum and in a closer disposition of the endoplasmic rete. Glycogen was usually absent in both types of cell.

As was previously referred, fragments of leptospirae were occasionally observed both in the kidney and liver. They showed a typical ultra-microscopic pattern with a moderately electron dense central axis around which the spirae were located [SIMPSON and WHITE (1, 2)] (Fig. 7).

D. Comments

Light microscopy study showed findings which are in agreement with previous descriptions (AREAN, BASILE, BUSSINELLO et al., SQUADRINI et al., STAVITSKY, WYLIE). However, some points deserve comments. In relation to the kidney it is to be noted that the lesions are focal, being more severe in groups of nephrons and mild in others. Necrosis can be seen, but it is not a common finding. The interstitial pathology is prominent chiefly at the late phase of the disease.

Tubular cells showed an irregular disposition of their mitochondria, an absence of the alkaline phosphatase activity and, in proximal tubules, partial or total disappearance of the PAS positive zone. The first finding does not correlate well with the electron microscopy findings because the mitochondrial pathology is not severe, except in animals killed at the agonic period. Histochemical findings regarding succinodehydrogenase and cytochrome-oxidase activities are in agreement with a poor mitochondrial pathology. However, this evidence must be taken carefully, because the methods employed are qualitative and not quantitative. Moreover, recent work (AREAN) showed decrease of activity of oxidative enzymes chiefly in the kidney of guinea pigs at the late phase of the disease, when there is mitochondrial pathology. On the other hand, disappearence of the alkaline phosphatase activity, which was also previously seen by SQUADRINI et al. and by AREAN and of the PAS positive zone of the proximal tubules correlates well with the electron microscopy findings which showed a definite lesion of the cell membrane, chiefly the brush border. Alkaline activity is located at the cell border and this observation was confirmed with electron microscopy studies (REALE). The PAS positive zone is also located at the brush border of the tubular cell. If we now look at the liver findings we see that there is also a lesion of the cell border, preceded by a sinusoidal pathology, and that this lesion is similar to that seen in the kidney. Moreover, an enlargement of the intercellular space is seen both in the liver and kidney, an observation which fits well with light microscopy studies showing sometimes, a lack of the trabecular arrangement of the liver in humans (ALSTON, AREAN, BEITZKE, KOPPISCH and BOND) and experimental animals (AREAN) at the late phase of the disease. Also, this is in accordance with a basic pathology of the disease, located primarily at the cell membrane.

It is tempting to admit that such enlarged intercellular space, at least in the kidney could act as a shortcut for the glomerular filtrate to the interstitium, thus contributing for the acute renal failure seen in the disease. However, the tight junction of the junctional complex [FARQUHAR and PALADE (4)] appeared preserved. It seems that, if such mechanism exists, the increased permeability of this anatomical barrier is mostly functional or it appears only at a very late period of the disease. The increased intercellular space in tubular cells of the kidney could be instead the result of a cell membrane lesion plus edema fluid of the interstitium filtered through the tubular basal membrane which appeared focally altered in the light microscopy studies.

The interstitial capillaries of the kidney showed mainly endothelial cell tumefaction and, in some cases, areas of disjunction, thus pointing out to a vascular lesion in leptospirosis which could contribute to the acute kidney failure (AUSTONI and CORÀ) and explain, at least partially, the interstitial edema. Probably, also casts, regarded by PATEL et al. as the main factor to explain oliguria and anuria in tubular necrosis and which are seen in leptospirosis, can also contribute to the pathogenesis of the kidney failure. Liver sinusoidal lining, which is a peculiar type of vessel with large pores (BENNETT et al.) showed besides Kupffer cells hypertrophy and hyperplasia, an enlarged Disse's space with the deposit of something like protein in it. This finding was interpreted as the ultrastructural manifestation of a "serous inflammation" (EPPINGER, HAENNI).

Worth while to comment, the mild but definite glomerular lesion in experimental leptospirosis, a finding previously described by us (BRITO et al.) in diseased kidneys of humans. It was characterized by focal areas of fusion of epithelial cells foot process, which, according to FARQUHAR et al. (1, 2, 3, 4) is the morphological basis of proteinuria, which is seen in leptospirosis (EDWARDS and DOMM, WOODWARD). Noteworthy that light microscopy studies by KOPPISCH and BOND had also pointed out to a glomerular lesion in human leptospirosis.

Regarding both the tubular and hepatic cells the main finding was the appearance of an increased number of "dense" bodies whose origin could be ascribed, at least in some cases, to altered mitochondria [RHODIN (1, 2)].

Another explanation is that at least some of the dense bodies, as well as the ovular structures delimited by a single-membrane are lysosomes, which, according to DE DUVE'S concept, would be "suicide bags" containing acid phosphatase and other hydrolytic enzymes which become active during cell degeneration and autolysis. In the liver some of these dense bodies were regarded as lipofuscin pigment (BIAVA) and they are related to the PAS positive non glycogenic granules seen in liver and Kupffer cells in regressive status of the organ (POPPER et al.).

Liver cells showed disappearance of the glycogen granules at the late phase of the disease. Light microscopy and biochemical studies of DRÄGERT had also pointed out to this glycogen depletion in human leptospirosis.

The predominance of a smooth type of endoplasmic reticulum at the late phase of the disease is indirect evidence of an altered protein metabolism.

No conclusive explanation for the mechanism of the icterus in leptospirosis was found. The lesions in the biliary ductules are unspecific and found both in intra- and extra-hepatic (SCHAFFNER and POPPER) forms of cholestasis. On the other hand, hepatic cell disjunction might provide a short cut between biliary ductules and liver sinusoidal lining. However, studies of the biopsied liver in human leptospirosis, now in progress, are showing marked icterus with similar lesions regarding the biliary ductules microvilli but without an accentuated disjunction of the liver cells. It is possible, then, that the failure might be located in one of the steps of bile excretion.

E. Conclusions

Although *L. icterohaemorrhagiae* has not been conclusively demonstrated to possess a toxin, the clinical and histological evidences suggest a toxin as the mechanism of leptospiral pathogenicity (AREAN, STAVITSKY). Recent work

by AREAN adds further evidence in favor of a leptospiral toxin since extracts of kidney and liver of experimentally infected guinea pigs, killed at the beginning of the fever and injected into the peritoneum of normal guinea pigs produced 12 to 24 hours later weight loss, diarrhea, hypothermia and a shock like state leading to death. Histologically, there was diffuse congestion of viscera and focal necrosis of the intestinal mucosa. The nature of the toxin or toxins responsible for the lethal effects was not determined.

Our work shows that the earliest lesion in the liver and kidney in leptospirosis is at the cell membrane, thus in accordance with a circulating toxin which, in the liver, would produce "serous inflammation", microvilli distortion or disappearence, interfering with the normal cell exchanges with the blood. In a more complex manner, it would act in bile formation and bile ductules thus providing basis for the icterus.

In the kidney toxin action would be mild in the glomerulus and more definite in the tubuli, chiefly proximal tubules, where it produces the brush border pathology through an enhanced action due to the concentration power of the proximal tubules.

The lesions seen in the capillaries and the ones regarding the intercellular spaces could also be explained by a similar toxin action.

Only at the late phase of the disease that other organelles would deteriorate in such a way that in the more severe cases cellular necrosis supervenes.

The above findings are in accordance with a low level of serum transaminase [ELKIS et al. (2)] seen in the disease. More difficult to explain is the high level of mucoproteins [ELKIS et al. (1)] unless we could admit that they were mainly located at the cell membrane, being than liberated through the toxin action.

Summary

A light and electron microscopy study of the experimental leptospirosis of the guinea pig was done. The earliest lesion found was located at the cell membrane, with partial or total disappearance of the brush border of the cells of the proximal tubuli as well as partial disappearance and distortion of the microvilli of the hepatic cells. Intercellular spaces were found to be enlarged both in the liver and kidney. Capillaries showed endothelial cell tumefaction and, sometimes, disjunction of the endothelial lining, a finding also in accordance with the basic pathology of the disease. Only at the late phase of the disease, mainly at the agonic period, that pathology of the organelles such mitochondria was found. However, a definite increase of "dense bodies" whose origin was discussed, was found since the early phase of the disease.

Also described, a mild but definite focal glomerular lesion, which provides anatomical basis for the proteinuria seen in the disease.

The above described basic pathology of the disease is in accordance with the possibility of a toxin as the main mechanism acting for leptospiral pathogenicity.

Die Pathologie der Niere und der Leber bei der experimentellen Leptospirose des Meerschweinchens

Licht- und elektronenmikroskopische Untersuchungen

Zusammenfassung

Die erste Veränderung wurde an der Zellmembran beobachtet, zusammen mit teilweiser oder vollständiger Zerstörung des Bürstensaumes der proximalen

Nierenkanälchen sowie der Mikrovilli der Leberzellen. Die intercellulären Räume der Leber und Niere waren erweitert. An den Blutcapillaren fanden sich endotheliale Schwellungen und manchmal auch Loslösung des Endothels. Eine pathologische Veränderung der Mitochondrien wurde nur während der Endphase der Krankheit gesehen, doch waren „dense bodies" schon von Anfang an vorhanden. Außerdem wurde auch eine leichte glomeruläre Läsion gefunden, welche die anatomische Grundlage der Proteinurie darstellen mag. Diese Befunde lassen die Annahme einer toxischen Wirkung als Grundlage der Pathogenese der Leptospirose zu.

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