

## LABREA HEPATITIS — HEPATITIS B AND DELTA ANTIGEN EXPRESSION IN LIVER TISSUE: REPORT OF THREE AUTOPSY CASES

### (PRELIMINARY REPORT)

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Labrea Hepatitis (Labrea Black Fever) is a severe liver disease found in several hamlets of the Amazon Region, where microepidemics are reported<sup>2</sup>. This morbid condition is known to occur specifically in this region, where children and young adults are mainly affected<sup>1,2,3</sup>. The clinical picture is similar to that seen in fulminant forms of viral and toxic hepatitis, and courses with a high degree of lethality<sup>1,2,3</sup>. Pathological studies have shown diffuse liver damage, with an outstanding degree of hepatocellular necrosis and degenerative phenomena, among which the most striking one is fatty change mainly of the microvacuolar type<sup>1,2</sup>. This pattern, in which small fat droplets surround a centrally placed nucleus, has been called "morula-like" cells by Brazilian authors<sup>2</sup> and is thought to be a distinctive feature of Labrea Hepatitis, as compared with other forms of fulminant hepatitis<sup>2,3</sup>. Recent studies suggest that the Hepatitis B Virus (HBV) might be related, at least partially, to the etiopathogenesis of this disease<sup>1,3</sup>.

The Delta Agent, described by RIZZETO et al. in 1977<sup>11</sup> is now considered to be a defective virus (HDV), which needs the HBV and, especially, its surface antigen (HBsAg) for in-

fection, replication and expression<sup>10</sup>. It has been shown that the HDV can cause, by coinfection with HBV or by superinfection in HBsAg carriers, nearly always, severe acute or chronic liver disease, usually more aggressive than liver damage caused by the HBV alone<sup>10,12,14</sup>.

Seroepidemiologic studies have recently characterized the Amazon Region as a high endemicity area for both viruses HBV and HDV<sup>4,5</sup>.

The present communication intends to assess the expression of HBV and HDV antigens in liver tissue and their possible role in the etiopathogenesis of Labrea Hepatitis.

Autopsy samples of liver tissue were obtained from three patients who died with a clinical picture of fulminant hepatitis, and in whom a histological diagnosis of Labrea Hepatitis was performed. All of them died in far away small villages of the State of Amazonas, Brasil, and tissue samples were referred to the Instituto de Medicina Tropical de Manaus. For this reason, clinical data were scarce and no serological markers could be tested. The available information is displayed in Table I.

T A B L E I  
Clinical and Epidemiological Aspects

Case No.	City	River	Age (years)	Sex	Onset of symptoms to death (days)
1	Tapauá	Purus	14	M	4
2	Lábrea	Purus	3	F	3
3	Itacoatiara	Amazonas	11	F	3

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Tissue samples were fixed in formalin, routinely processed in Auto-Technicon (Technicon Co., U.S.A.) and embedded in paraffin blocks; four micra thick sections were submitted to impregnation of the reticulin framework by silver and stained by Hematoxylin-Eosin.

For the detection of HBsAg and HBeAg, the highly sensitive Peroxidase Antiperoxidase (PAP) method<sup>10</sup> was used. The indirect method was used for the detection of Delta Antigen (HDAg), since Anti-Delta Antibody is a Human IgG. For each reaction, positive and negative controls were tested.

In all three cases, HBsAg was detected in small or moderate amounts of hepatocytes, always with a segmental cytoplasmic pattern. HBeAg was detected in very few nuclei, in only one of the cases. On the other hand, HDAg was strongly positive in a great amount of liver cell nuclei, especially in those of "morula-like" cells. Some of them corresponded to irregularly enlarged nuclei found in sections stained by H.E. In Table II, semi-quantitative data concerning antigen expression in liver tissue are presented whereas histopathological aspects and localization of antigens in hepatocytes are depicted in Fig. 1.

T A B L E II  
Viral Antigens Expression in Liver Cells

Cases No.	HBsAg	HBeAg	HDAg
1	+	-	+++
2	+	-	+++
3	++	+	+++

It is a well known fact that co-infection of HBV and HDV<sup>6,10</sup> and, specially, super-infection of HBV carriers by HDV<sup>7,10</sup> can lead to fulminant hepatitis or to more severe forms of HBs positive chronic liver disease<sup>10,12,14</sup>. These findings were recently reported in the Venezuelan part of the Amazon Basin, where a 3-year survey was done in 149 Yucpa Indians who developed hepatitis<sup>7</sup>.

In the present study, histopathological findings from patients who died of fulminant hepatitis are different from those of massive or

submassive hepatic necrosis found in ordinary fulminant viral hepatitis: instead of extensive areas of collapse of the reticulin framework, these regions are scarce; this finding as well as the great amount of "morula-like cells", characterized by the presence of small fat droplets, suggest a very strong and rapid block of liver cell function such as that found in Reye's Syndrome, Fatty Change of Pregnancy or due to the toxic effects of large doses of Tetracycline<sup>8</sup>. On the other hand, several cells presented enlarged nuclei with irregular borders, which could be related to a cytopathic viral effect. The fatty change and the scarcity of the mononuclear infiltrate as seen in our cases, would be other arguments favoring this hypothesis.

Furthermore, for the first time, simultaneous presence of HBV and HDV antigens is reported in Labrea Hepatitis, and emphasis should be put on the fact that HDAg was detected in large numbers of liver-cell nuclei, many of them belonging to "morula-like" cells.

The behaviour of Delta Antigen expression in liver cell nuclei in all of the three cases is similar to that described by RIZZETTO et al. in the early phases of experimental infections in chimpanzees<sup>13</sup> and, upon this fact, one could speculate that Labrea Hepatitis could be caused either by co-infection of HBV and HDV or by super-infection of HDV upon chronic carriers of HBV.

Finally, our findings on the histopathologic features of Labrea Hepatitis, and especially on the expression of HDAg in liver-cell nuclei, preferentially in "morula-like" cells, are very close to those described by POPPER et al.<sup>9</sup> in autopsy specimens obtained in Venezuela from Yucpa Indians. These similarities could suggest an identity between these two entities, suggesting also an etiologic role of HDV in both of them.

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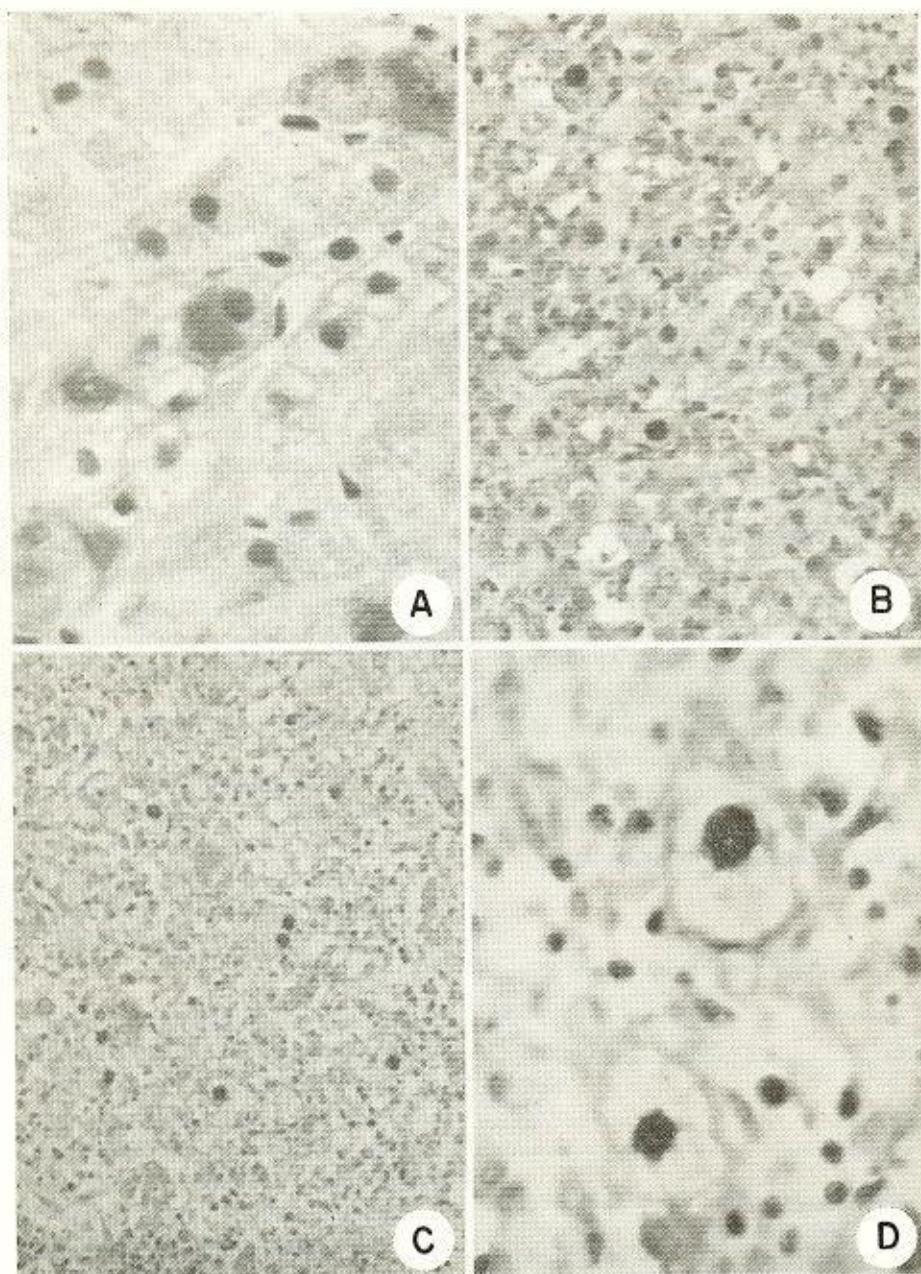


Fig. 1 — A) Case number 3 — HBsAg is found into the cytoplasm of some liver cells (PAP-Hematoxylin, 100 $\times$ ); B) Case number 3 — HBcAg expression in scarce nuclei of hepatocytes (PAP-Hematoxylin, 200 $\times$ ); C) Case number 1 — A great amount of liver-cell nuclei contains the HDAg (PAP-Hematoxylin, 100 $\times$ ); D) Case number 2 — Two "morula-like" cells, with abundant micro-goticular fatty change, present HDAg into enlarged nuclei (PAP-Hematoxylin, 400 $\times$ )

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