HBsAg and HBCAg in the livers of asymptomatic hepatitis B antigen carriers

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SUMMARY Asymptomatic blood donors with persistent HBs antigenaemia have a variety of histological lesions in the liver, and serial biopsies indicate that, in some, these lesions may progress. The immunoperoxidase technique was found to be a sensitive method for the histological demonstration of HBsAg and HBCAg. Livers showing minor histological changes contained more HBsAg than those with active lesions and there appears to be an inverse relationship between the amount of HBsAg in the liver and the severity of the histological damage. In the carriers who had more than one biopsy, the presence of stainable HBsAg, irrespective of the initial histological diagnosis, was associated with a greater likelihood of progression of the histological lesion. HBCAg was found only in the hepatocyte nuclei of carriers with chronic aggressive and chronic persistent hepatitis.

Hepatitis B antigen (HB Ag) was first identified in the liver by immunofluorescence (Millman et al., 1969; Nowoslawski et al., 1970). More recently, similar techniques have been used to demonstrate the surface antigen (HBsAg) in the cytoplasm of hepatocytes and core antigen (HBCAg) in the nuclei of hepatocytes (Gudat et al., 1975; Ray et al., 1976; Trepo et al., 1976). A disadvantage of immunofluorescence is that the level of sensitivity is such that the most intense fluorescence is produced in sections of fresh frozen tissue (Portmann et al., 1976). Moreover, the fluorescent stains are themselves only transitory, and these sections must be examined soon after staining. Consequently, the use of orcein-stained paraffin sections of liver (Shikata et al., 1974; Deodhar et al., 1975) has some potential advantages over immunofluorescence. A further advance in the identification and localisation of the hepatitis virus antigens was made when it was shown by Burns (1975) and by Nayak and Sachdeva (1975) that they could be stained in paraffin sections using the immunoperoxidase technique. This technique combines the specificity of immunological methods with all the advantages inherent in the use of paraffin sections for the study of the antigen.

In this study of the liver biopsies from asymptomatic blood donors with persistent HBsAg antigenaemia, the immunoperoxidase, orcein, and electron optical methods have been compared and used to identify HBsAg. HBCAg was demonstrated in sections by both immunoperoxidase and electron microscopy.

Material and methods

Liver biopsies from a group of 33 asymptomatic blood donors, 22 of whom had had at least two biopsies over periods of two to four years, were reviewed. In most of these biopsies (48/55) there was sufficient material for immunoperoxidase and orcein staining. The investigation of the blood donors has been described previously (Woolf et al., 1974; Tapp et al., 1976).

IMMUNOPEROXIDASE

Paraffin sections were stained using the type of sandwich technique described by Mason et al. (1975). The following sera were applied to the sections in turn:

(a) for HBsAg—normal porcine serum—specific anti HBsAg raised in rabbits (Hoechst)—antibody to rabbit serum raised in pigs (Mercia Diagnostics)—the peroxidase-anti-peroxidase complexes (Mercia Diagnostics);

(b) for HBCAg—normal porcine serum—human serum containing high titre anti HBCAg and devoid of anti HBs and antibody to e antigen—anti human IgG serum raised in rabbits—antibody to rabbit

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Received for publication 17 January 1977
Fig. 1  The cytoplasm of the majority of the cells in some lobules is stained. Immunoperoxidase × 145.

Fig. 2  Small groups of cells show positive cytoplasmic staining. Immunoperoxidase × 400.
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serum raised in pigs—the peroxidase-anti-peroxidase complexes.

The optimal dilutions for the anti HBs and anti HBc that produced least non-specific staining were found by titration. The specificity of the HBsAg staining was confirmed in control sections where staining was abolished when anti-HBs previously absorbed with HBsAg was used. Specificity of the HBcAg staining was demonstrated by treating control sections with normal human serum instead of anti HBc. The anti HBc serum was tested for anti HBs by haemagglutination and for anti e antigen by immunodiffusion.

In both (a) and (b) the peroxidase and hence the antigen was visualised by the development of the antibody-bound peroxidase using diamino benzidine.

ORCEIN
Paraffin sections were stained by the method described by Deodhar et al. (1975).

ELECTRON MICROSCOPY
A part of each biopsy was processed separately for electron microscopy, and ultrathin sections were stained with urinylacetate and lead citrate.

Results

IMMUNOPEROXIDASE STAINING FOR HBsAg
HBsAg staining was seen in the cytoplasm of hepatocytes in 34 of the 48 biopsies which were examined. The biopsies from 12 of the 33 carriers did not have demonstrable HBsAg in the first or subsequent biopsies. The amount of cytoplasm staining in the individual cells varied from almost the whole to only

Table

<table>
<thead>
<tr>
<th>Liver lesion</th>
<th>No. of biopsies</th>
<th>HBsAg staining</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Diffuse</td>
<td>Focal</td>
</tr>
<tr>
<td>Chronic aggressive hepatitis</td>
<td>7</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>Chronic persistent hepatitis</td>
<td>17</td>
<td>6 (35%)</td>
</tr>
<tr>
<td>Focal parenchymal necrosis</td>
<td>17</td>
<td>9 (53%)</td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>20 (42%)</td>
</tr>
</tbody>
</table>

Fig. 3 The two nuclei in the centre of the field are stained heavily. Other nuclei show lighter staining. Immunoperoxidase × 400.
small parts of the cell. The intensity of the staining varied from a dark brown to a pale yellow, and it is assumed that this is a reflection of the amount of HBsAg in the cytoplasm. Where there were lipid droplets in the cytoplasm these were unstained, giving rise to a foamy appearance of the cytoplasm. There was no evidence of HBsAg limited to the margins of the cells in these biopsies.

The number of cells which contained HBsAg varied in different biopsies, and two main groups could be identified. In the first, there was diffuse staining of sheets of cells affecting up to two-thirds of the liver lobule, while in the second considerably fewer cells were affected (Figs. 1 and 2). There was no particular distribution of HBsAg staining within the lobule in either the diffuse or the focally stained sections.

**HBsAg staining and liver lesions**

The type of HBsAg staining in biopsies showing

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**Fig. 4** There is marked dilatation of the endoplasmic reticulum and part of the cytoplasm contains very few mitochondria (upper part of photograph). EM × 5400.
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Diffuse staining was more common in livers with focal parenchymal necrosis and was seen also in two of the three biopsies showing cirrhosis. Focal staining was seen more commonly in livers showing chronic aggressive hepatitis. Biopsies with the histological lesion of chronic persistent hepatitis showed both patterns of HBsAg staining with equal frequency.

HBsAg staining in sequential biopsies
There was little variation in the pattern of HBsAg staining in the first and subsequent biopsies from each individual.

In these carriers, 2/4 of those having a focal distribution and 5/8 of those having a diffuse distribution of HBsAg showed some deterioration of the histological appearances in the second biopsy; this occurred in only 1/10 carriers in whom HBsAg was not demonstrated in the liver.

IMMUNOPEROXIDASE STAINING FOR HBcAg
Staining was seen only in the nuclei of the hepatocytes. Of 48 biopsies that were stained for HBcAg, in only eight, from four donors, could positively stained nuclei be identified with certainty. In these biopsies only relatively few nuclei stained, and the

![Fig. 5](https://example.com/fig5.png)

**Fig. 5** Round particles (single arrow) and filaments (double arrow) are seen within the dilated endoplasmic reticulum. EM x 53250.
Discussion

With the immunoperoxidase technique specific staining of 
 HBsAg and HBcAg was obtained. The morphological appearance of 
 HBsAg in sections stained by the immunoperoxidase technique is 
 similar to that described by other workers using this 
 method (Burns, 1975; Nayak and Sachdeva, 1975) or 
 immunofluorescent techniques (Akeyama et al., 
 1974; Ray et al., 1976). However, staining of the 
 peripheral part of the cytoplasm or of the cell mem- 
 brane described by the latter two groups of workers 
 was not seen in the present formalin-fixed material. 
 
The immunoperoxidase technique was more time-
 consuming than orcein staining but was more 
 sensitive and gave clearer staining. Orcein staining 
 was variable in quality because some commercial 
 orcein preparations stain better than others. 
 Afroudakis and his co-workers (1976) also found 
 that immunoperoxidase staining for HBcAg was more 
 sensitive than orcein, although this was not the 
 experience of Nayak and Sachdeva (1975). 
 
In ultrathin sections, tubular and circular struc- 
 tures were seen in the cytoplasm of hepatocytes in 
 biopsies which were positive for HBsAg with the 
 immunoperoxidase technique. Gerber and his co-
 workers (1974), using direct immuno-electron microscopy, 
 have shown that these structures contain 
 HBsAg determinants, and there is little doubt that 
 these bodies represent cytoplasmic HBsAg. Exam- 
 ination of the sections by electron microscopy is 
 laborious and only small areas of the liver can be 
 examined by this method. In view of the focal nature 
 of HBsAg in some sections it is clearly a less satis- 
 factory method for the quantitation of HBsAg in the 
 liver. 
 
Using the immunoperoxidase technique, HBsAg 
 was found in the cytoplasm of 71% of biopsies. How- 
 ever, the type of distribution of HBsAg in the biop- 
 sies varied, a focal distribution predominating in 
 those showing the histological lesions of chronic 
 aggressive hepatitis while a diffuse distribution 
 of HBsAg was seen more commonly in those showing 
 none or only minor histological abnormalities. A 
 similar distribution of HBsAg in patients with 
 chronic aggressive and persistent hepatitis has been 
 described by Ray et al. (1976). Portmann and his co-
 workers (1976) also found large numbers of positive 
 hepatocytes in chronic carriers showing only minor 
 histological lesions, and it would appear in general 
 that there is an inverse relationship between the 
 amount of HBsAg in the cytoplasm and the activity 
 of the disease in the liver. The relative amounts and 
 distribution of HBsAg and HBcAg in the liver have 
 been related to the different immunological responses 
 to hepatitis B virus infection (Gudat et al., 1975). 
 
In those carriers having two or more biopsies the 
 presence of demonstrable HBsAg in the initial 
 biopsy was more likely to be associated with some 
 deterioration in the histological appearances in the 
 second biopsy. This appeared to be so irrespective 
 of the amount of HBsAg in the liver or the initial
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histological diagnosis.

The immunoperoxidase technique was also used to demonstrate HBcAg in the nuclei of hepatocytes. There was a good correlation between the incidence of positive staining and the presence of the 20 nm core particles. It is probable that the increased sensitivity of the immunoperoxidase technique is responsible for the finding of two biopsies positive for HBcAg by the immunoperoxidase method in which core particles could not be found in the nuclei.

All the biopsies that were positive for HBcAg showed either chronic aggressive or chronic persistent hepatitis. These HBcAg positive biopsies came from four blood donors in whom significant numbers of Dane particles had been found repeatedly by electron microscopy of the serum and who are also persistently e antigen positive (Tapp et al., 1974).

References