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Detection of HBs antigen in routine paraffin embedded liver tissue by enzyme-labelled antibody technique

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Abstract

HB surface antigen (HBs Ag) was detected using the enzyme-labelled antibody technique on routinely processed liver biopsy material fixed in Bouin's fixative and embedded in paraffin. Of 85 examined specimens, 45 cases were HBs Ag positive by both the immunofluorescent test and the enzyme labelled antibody technique. The remaining 40 cases were negative by both techniques. The specificity of HBs Ag detected by the enzyme-labelled antibody technique was confirmed by the blocking test using guinea pig specific HBs antibody. The results indicate that the enzyme-labelled antibody technique may be useful for detecting HBs Ag on routine paraffin sections.

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—— BRIEF NOTE ——

DETECTION OF HBs ANTIGEN IN ROUTINE PARAFFIN EMBEDDED LIVER TISSUE BY ENZYME-LABELLED ANTIBODY TECHNIQUE

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Abstract: HB surface antigen (HBs Ag) was detected using the enzyme-labelled antibody technique on routinely processed liver biopsy material fixed in Bouin's fixative and embedded in paraffin. Of 85 examined specimens, 45 cases were HBs Ag positive by both the immunofluorescent test and the enzyme-labelled antibody technique. The remaining 40 cases were negative by both techniques. The specificity of HBs Ag detected by the enzyme-labelled antibody technique was confirmed by the blocking test using guinea pig specific HBs antibody. The results indicate that the enzyme-labelled antibody technique may be useful for detecting HBs Ag on routine paraffin sections.

Two kinds of hepatitis B antigens (HB Ags), viz., HBc Ag and HBs Ag, are detectable in frozen liver sections by immunofluorescent technique. Recently, Ray and Desmet (1) described the possibility of applying the immunofluorescent method to Bouin-fixed paraffin embedded liver tissue. However, the immunofluorescent specimens were difficult to preserve for long periods of time and detailed histological characterization of the dark field was difficult. Nakane and Kawaoi (2), however, reported success in conjugation of peroxidase without inactivation of specific antibody. In the present study we employed the enzyme-labelled antibody technique on routinely processed paraffin embedded biopsy material.

Forty-five previously collected liver biopsies with immunofluorescent positive HBs Ag were selected. The specimen were fixed in Bouin's fixative (saturated aqueous picric acid, 16 ml; formaldehyde, 6 ml; and glacial acetic acid, 1.5 ml) and embedded in paraffin. As control material, 40 patients with immunofluorescent negative HBs Ag were selected. The enzyme-labelled antibody technique and immunofluorescent test were both applied to the paraffin sections for detection of HBs Ag. Rabbit antibodies to HBs Ag

(Behringwerke Institute and our own preparation) diluted 4-fold and peroxidase-labelled anti-rabbit IgG (Dakopatts Lab. and our preparation) as indirect

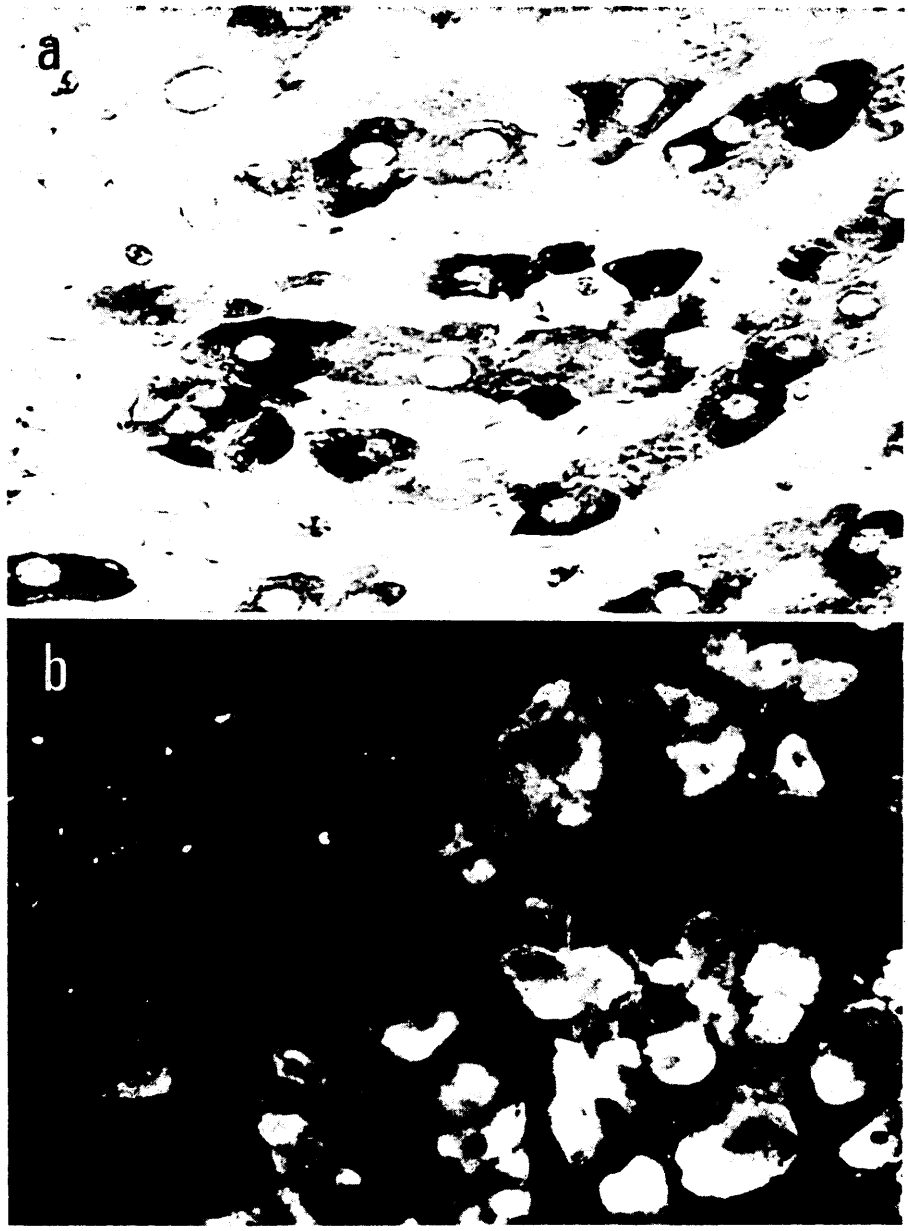

Fig. 1. Biopsy specimen from a patient with chronic aggressive hepatitis. **a**, Positive peroxidase activity of HBs Ag in hepatocytes. Paraffin section. ×200. **b**, Similar fluorescent HBs Ag pattern and intensity in the cytoplasm by immunofluorescent method. Frozen section. ×160.

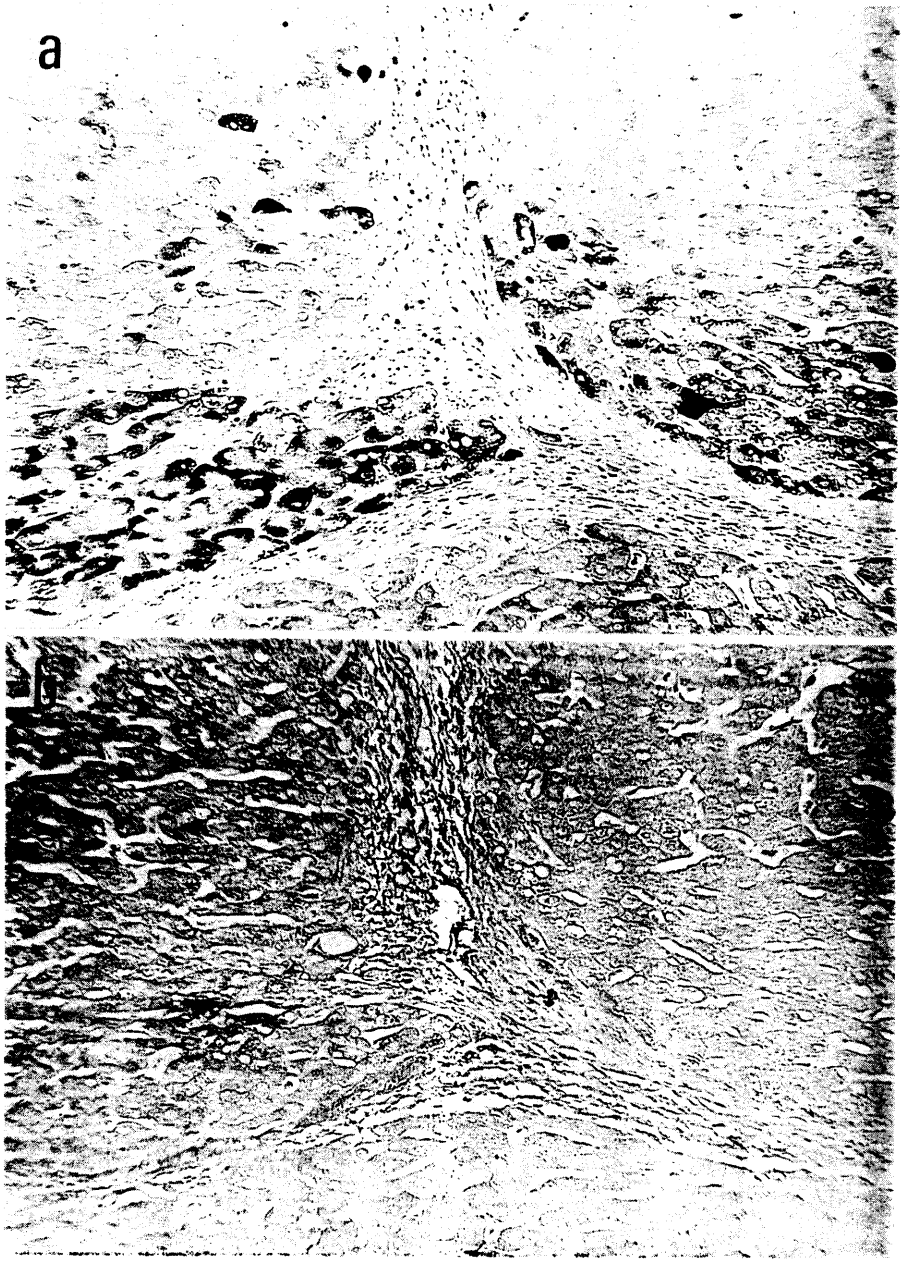

Fig. 2. Biopsy specimen from another case of chronic aggressive hepatitis. **a**, Common pattern of HBs Ag in liver lobules. Paraffin section stained by peroxidase-labelled antibody technique. ×100. **b**, Identical section by the blocking test shows negative HBs Ag. ×100.

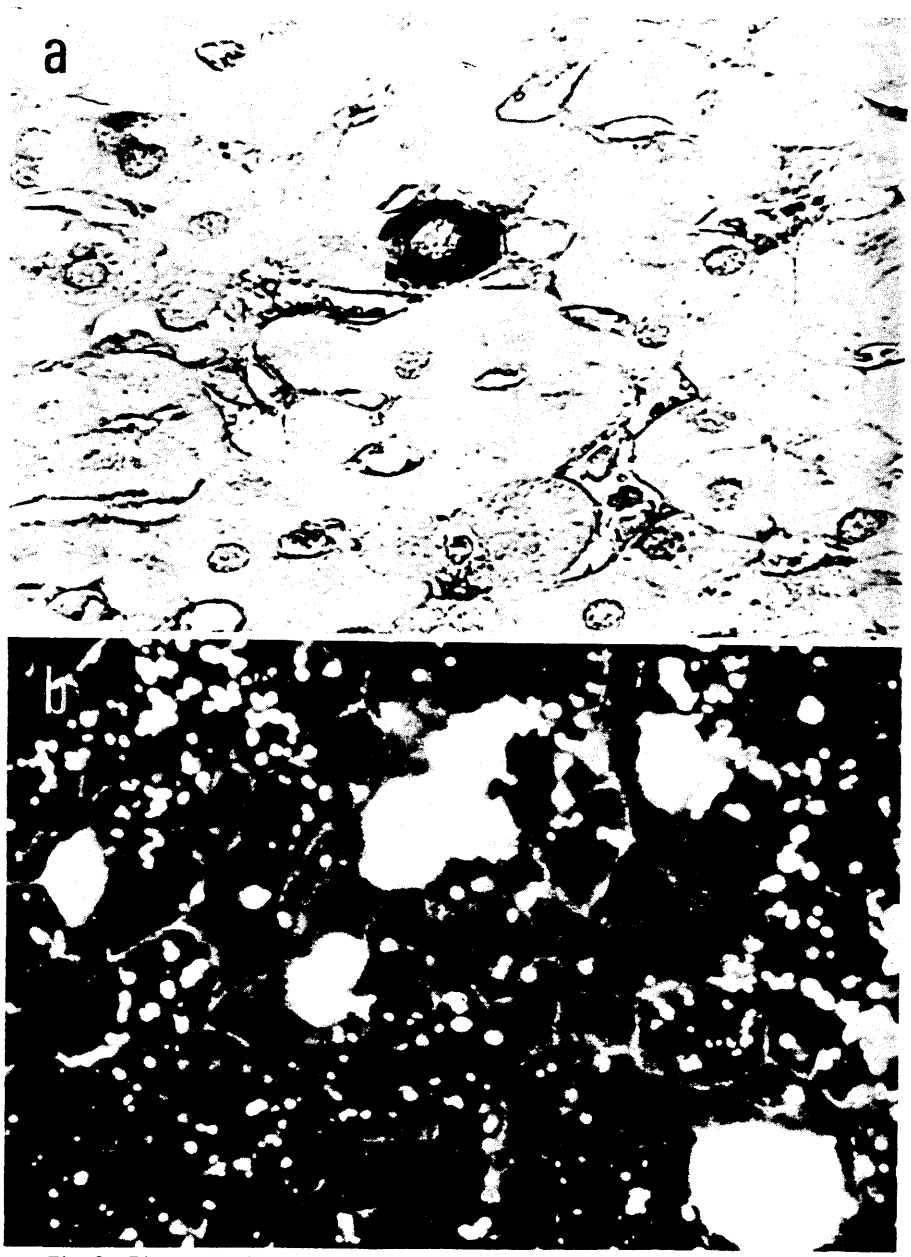

Fig. 3. Biopsy specimen from an asymptomatic carrier. a, Pattern of hepatocyte membrane, Disse's lumen or sinusoidal capillary wall. Paraffin section by peroxidase-labelled antibody technique. ×400. b, Frozen section of the same case shows similar fluorescent HBs Ag findings. Fixed in cold acetone. ×400.

method were used in the enzyme-labelled antibody technique. Each preparation was incubated at 37°C for 30 minutes.

All 45 cases positive to HBs Ag by the immunofluorescent test were positive by the enzyme-labelled antibody technique (Fig. 1, 2, 3). All 40 control cases were negative by both techniques. Furthermore, the specificity of HBs Ag detected by the enzyme-labelled antibody technique was confirmed by the blocking test using guinea pig specific HBs antibody (Fig. 2). This new technique was as sensitive and specific as the immunofluorescent method, and it was more sensitive and specific than other staining methods (3).

Furthermore, in a study of Bouin-fixed paraffin embedded liver tissue collected 10 years ago, it was possible to clearly stain HBs Ag. A study of the same material with the immunofluorescent complement technique enabled us (4) to detect HBc Ag, but the antigenicity was lost by Bouin-fixation. These findings suggest that the enzyme-labelled antibody technique may be useful for the detection of HBs Ag on routine paraffin sections.

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