Immunofluorescent detection of α_1 -antitrypsin in paraffin embedded liver tissue

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SYNOPSIS Alpha₁-antitrypsin was detected by indirect immunofluorescence in frozen sections of liver biopsies from patients with clinically and biochemically proven α_1 -antitrypsin deficiency. The antigen could also be demonstrated in those liver specimens of the same patients which were fixed in Bouin's fluid and embedded in paraffin. The cellular localization and the brightness of the fluorescence were the same in both frozen and paraffin sections. Four additional biopsies from three other patients were selected on the basis of PAS-positive diastase-resistant inclusions reported in the hepatocytes. All these biopsies showed bright fluorescence in the cytoplasm of the liver cells although one of the biopsies was stored for as long as eight years. Specific fluorescence was constantly found in the periportal hepatocytes with varying degrees of positivity. No fluorescence was observed in the six control biopsies from patients with various other liver diseases. These findings prove that paraffin embedded specimens are suitable for immunofluorescent detection of α_1 -antitrypsin and that a retrospective study on old paraffin blocks is possible.

Hepatitis B antigen (HBAG) can be detected by immunofluorescence in frozen sections of liver tissue (Hadziyannis *et al*, 1972; Ray *et al*, 1974). Recently, we described the possibility of applying this immunofluorescent method on Bouin-fixed paraffin embedded liver tissue; this proved to be more sensitive and more specific (Ray and Desmet, 1975) than other staining methods with histological dyes (Shikata *et al*, 1974). These findings encouraged us to apply the immunofluorescence method for the detection of other antigens on paraffin sections. During our studies on hepatitis we came across liver biopsies of patients with proven α_1 -antitrypsin deficiency (AATD), and in each case α_1 -antitrypsin could be demonstrated in frozen liver sections. In order to investigate the applicability of the method on paraffin sections, we performed the immunofluorescent test on routinely processed paraffin embedded biopsies of proven cases and also of suspected cases whose biopsies were stored for a long time.

Materials and Methods

Seven liver biopsies fixed in Bouin's fixative (1.2%

w/v; saturated aqueous picric acid 75 ml, formaldehyde 25 ml, and glacial acetic acid 5 ml) and embedded in paraffin, obtained from six patients, were selected for this study. Three patients had biochemically and clinically proven α_1 -antitrypsin deficiency. Frozen tissue specimens from the biopsies of two of these three cases were available for study. The remaining four cases were selected from the files on the basis of reported periodic acid-schiff (PAS)-positive diastase-resistant inclusions observed in the hepatocytes during routine liver biopsy examination. Six additional liver biopsies (frozen specimens and paraffin sections) were taken as controls from patients with a variety of hepatic diseases. All the paraffin blocks were randomly coded, mixed, and recut for haematoxylin-eosin, PAS with and without diastase digestion, orcein, and immunofluorescent staining.

IMMUNOFLUORESCENT METHOD

The indirect immunofluorescent technique was applied on both frozen and paraffin sections for the detection of α_1 -antitrypsin. Specific anti- α_1 -antitrypsin (Behringwerk, Brussels) and fluorescein (FITC) conjugated antirabbit globulin (Organon Teknika, Antwerp) were used. The procedures for deparaffinization, processing, and reading of the

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Biopsy	Patient		Date of Biopsy	Immunofluorescence on		PAS after Diastase Digestion	Liver Histology
	Age	Sex		Frozen Section	Paraffin Section		
Cases with proven AATD							
1	21 yr	M	Feb. 1975	+	+	+	Cirrhosis Periportal cholestasis Cirrhosis with acute hepatitis
2	2 m	M	May 1973	+	+	+	
3	59 yr	F	Jan. 1973	NA	+	+	
Cases with PAS-positive inclusion in the biopsies							
4	21 yr	F	Feb. 1967	NA	+	+	Cirrhosis Periportal steatosis Periportal steatosis Periportal and mid-zonal steatosis
5	67 yr	M	June 1971	NA	+	+	
6a	60 yr	M	May 1972	NA	+	+	
6b	61 yr	M	Jan. 1973	NA	+	+	
Controls							
7-12	28-67 yr	{ 3 M 3 F	Jan. 1973 Jan. 1975	—	—	—	Miscellaneous

Table Results of immunofluorescence test

NA = not available

biopsies for immunofluorescence were the same as previously described for the detection of HBAG in liver tissue (Ray and Desmet, 1975).

The following control tests were used: incubation of the slides with anti-human IgG, anti HBAG from

rabbit, normal rabbit serum, and normal human serum instead of using specific anti- α_1 -antitrypsin. Further controls included application of FITC-conjugated heterologous antiglobulin instead of species specific antiglobulin antisera.

Fig 1 Paraffin section (biopsy 5): Shows cytoplasmic globules of varying size, mostly in the periportal hepatocytes (PAS after diastase $\times 325$)

Results

The results of immunofluorescence and histology of the liver of the positive cases are summarized in the accompanying table. Alpha₁-antitrypsin was detected in the two available frozen specimens and in all the paraffin sections from the cases with established α_1 -antitrypsin deficiency. It was also found in the four biopsies selected because of the presence of PAS-positive inclusions in the parenchymal cells. Of the seven positive biopsies, three were diagnosed as cirrhosis, and the other four biopsies showed periportal macro and micro vesicular steatosis. PAS-positive, diastase resistant globules in the periportal hepatocytes were present in all the positive biopsies (fig 1). Most of the hepatocytes in the periphery of cirrhotic nodules contained large amounts of lipofuscin granules.

ALPHA₁-ANTITRYPSIN IN THE FROZEN SECTIONS

In the frozen sections specific fluorescence for α_1 -antitrypsin was seen in the cytoplasm of hepatocytes as bright green fluorescent globules of different size, sometimes single but mostly multiple (fig 2). Most fluorescence was observed in the periportal zones. The periphery of the globules was relatively more fluorescent than the centre. In some cirrhotic nodules roughly 90% of the hepatocytes were positive; there was no clear-cut gradient in the population of positive cells towards the periphery or the centre of the cirrhotic nodules. No fluorescence was detected in the control slides nor in the control cases.

ALPHA₁-ANTITRYPSIN IN THE PARAFFIN SECTIONS

Specific fluorescence for α_1 -antitrypsin was detected

Fig 2 Frozen section (biopsy 1): Brightly fluorescent cytoplasmic globules in the hepatocytes of proven AATD patient ($\times 245$)

Fig 3 Paraffin section (biopsy 1): Shows a similar pattern and intensity of fluorescent globules in the cytoplasm as in fig 2. ($\times 245$)

in serial paraffin sections of both proven (fig 3) and suspected (fig 4) biopsies of AATD. The intensity of the fluorescence and its cellular localization were almost the same as in the frozen sections. In the paraffin sections the fluorescent globules were relatively smaller in size and more granular. The fluorescence was often brighter in the periphery than in the centre of larger globules. The histological localization of the fluorescent globules in the hepatocytes was easily observed and more precise. No specific fluorescence was observed either in the control slides or in the six biopsy specimens used as controls. The orcein stain for the detection of HBAg (Shikata *et al*, 1974) was performed on serial paraffin sections and showed dark brown fine granules in the cytoplasm of the hepatocytes in the area of the cirrhotic nodules where lipofuscins were present. Occasionally the PAS-positive globules stained faintly.

Fig 4 Serial paraffin section of biopsy 5 (processed more than three and a half years after collection): Shows strong cytoplasmic fluorescence in the periportal hepatocytes. These cells correspond (indicated by arrows) to the fluorescent hepatocytes in fig 1. ($\times 245$)

Discussion

Alpha₁-antitrypsin was detected in the frozen liver specimens of the patients with clinically and biochemically proven α_1 -antitrypsin deficiency. The antigen was also detected in the paraffin sections of all three patients. The localization and distribution patterns were similar in frozen and in paraffin sections. The absence of specific fluorescence in all control tests and in the six control biopsies confirmed the specificity of the immunofluorescent reaction. These findings indicate that the fluorescence observed in the other four 'suspected' biopsies with PAS-positive diastase-resistant inclusions is specific for α_1 -antitrypsin, which remains detectable in biopsies stored for as long as eight years.

A recent immunohistochemical study (Palmer *et al*, 1974) reports the failure to demonstrate α_1 -antitrypsin in formalin fixed paraffin embedded liver tissue by immunofluorescence, although it could be detected by the immunoperoxidase method in the same biopsy specimens. These immunofluorescent findings are contradictory to our results. The discrepancies may be due to differences in methodology.

This study confirms the specificity and the validity of performing immunofluorescent tests on routinely processed paraffin sections for the detection of α_1 -antitrypsin in liver biopsies. The advantages of using paraffin sections over frozen specimens were discussed in detail previously (Ray and Desmet, 1975); not the least is the possibility of performing retrospective studies on older biopsies. The immunofluorescent method has the advantage of higher specificity and apparently higher sensitivity over the histochemical PAS reaction.

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