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AUTOFLUORESCENT HEPATIC PARTICLES IN HB Ag SERO-POSITIVE PATIENTS

(Preliminary report)

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SUMMARY

This paper describes autofluorescent particles present in hepatic specimens of HB Ag sero-positive patients. They are demonstrable by fluorescent microscopy of hematoxylin-eosin sections. Their peculiar aspects and relation to HB Ag are discussed.

INTRODUCTION

Hepatitis B antigen (HB Ag) has been demonstrated in human tissues by different ways [4].

More recently, the availability of paraffin sections employing immunofluorescent, immunoperoxidase and elastic staining methods has been reported [1, 2, 6, 7].

This paper deals with the presence of autofluorescent hepatic particles which are demonstrable in hematoxylin-eosin sections and are similar to the HB Ag particles described by immunological procedures.

MATERIAL AND METHODS

Liver biopsies of HB Ag sero-positive patients with clinical and histologic diagnosis of viral hepatitis and control hepatic specimens were used. The latter included schistosomiasis, venous chronic congestion by heart failure, and nonspecific reactive hepatitis in children. Two necropsy cases of fatal viral hepatitis without serologic investigation for HB Ag were also studied (Table I). Formalin or ethanol-fixed, paraffin-embedded sections were rotineously employed.

Hematoxylin-eosin sections of these specimens were examined by both light and fluorescence microscopy using a Zeiss Large Fluorescence Microscope supplied with a partly transmitting reflector and the bright-field, phase-contrast, dark-field VZ condenser.

RESULTS

The particles have green autofluorescence. They were very numerous and regularly found in liver biopsies of HB Ag sero-positive patients and generally scanty or absent in the control specimens (Table I).

The pattern of distribution and the morphology of the autofluorescent particles clearly reproduced the previously reported immunofluorescent findings of HB Ag bearing human tissues [5, 6].

We have observed associated nuclear and cytoplasmic autofluorescence as well as dissociated forms, with large predominance or exclusiveness of nuclear or cytoplasmic particles.

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TABLE I

Autofluorescent particles in HB Ag sero-positive and control patients

Cases	C and N 1	C 2	N 3	S	Positive Total	Negative Total	Total No. of cases
HB Ag sero-positive patients	15 +++ (C) + (N)	20 +++	—	—	35	0	35
Schistosomiasis	1 +	1 +	—	—	2	10	12
Nonspecific reactive hepatitis	1 +	—	—	—	1	13	14
Chronic heart failure	4 +	2 +	1 +	1 +	7	4	11
Fatal acute viral hepatitis	—	2 +		2 +++	2	0	2
T O T A L	21	25	1	3	47	27	74

C — cytoplasmic autofluorescence N: nuclear autofluorescence
S — sinusoidal free particles

Grade positiviness is indicated by number of signal plus (+)

1 — Simultaneous presence of cytoplasmic and nuclear autofluorescence
2 — Almost exclusive presence of cytoplasmic particles
3 — Almost exclusive presence of nuclear particles

In hepatocytic cytoplasm, they generally corresponded to the hematoxylin-eosin aspect of ground-glass alteration (Fig. 1), and to some eosinophilic globules within cytoplasmic vacuoles. The positive nuclei were constantly enlarged by a vacuolar peculiar alteration with eosinophilic particles inside (Fig. 2).

In some cases, we have seen free and plentiful particles in the vascular lumen, including the sinusoids. This occurred mainly in massive hepatic necrosis, and was always accompanied by hepatocytic autofluorescence (Fig. 3).

There was no coincidence of the autofluorescence with lipochromes or other pigments.

DISCUSSION

It is accepted that HB Ag has tinctorial affinity for elastic dyes and the elastic tissue is strongly autofluorescent. Also, although an immunohistochemical study of the autofluorescent particles is lacking, the peculiar pattern of distribution and the clear-cut correspondence with the clinical and serological data, suggest an identity with the HB Ag.

The cytoplasmic autofluorescence reproduces exactly the aspects already described in the immunofluorescent studies of the HB Ag particles [5, 6], except by the occasional presence of autofluorescent eosinophilic globules with a clear halo around.

Only recently, Huang has demonstrated nuclear HB Ag particles in paraffin sections by immunohistochemical methods [6]. The vacuolar alteration with eosinophilic particles inside which was seen by us in hematoxylin-eosin sections as corresponding to the autofluorescence, is frequent in viral hepatitis, as well as passive chronic hepatic congestion and in diabetes mellitus [3], but was not mentioned by HUANG [6] and by GUDAT et al. [5], who have correlated fluorescent and cytomorphologic findings.

The autofluorescence was also present as numerous free, extra-cellular, apparently circulating particles, in viral hepatitis with massive necrosis but not in hepatic necrosis due to heart failure. To our knowledge, there is no previous report of similar particles. This may be a point of disagreement to the idea of inverse relationship between cell damage and amount of HB Ag, referred by some Authors [4].

Finally, although we cannot be sure about the identity between HB Ag and the autofluorescent particles, the latter are undoubtely related to HB antigenemia and to viral hepatitis. Furthermore, extensive studies concerned with liver diseases are feasible using this simple method.

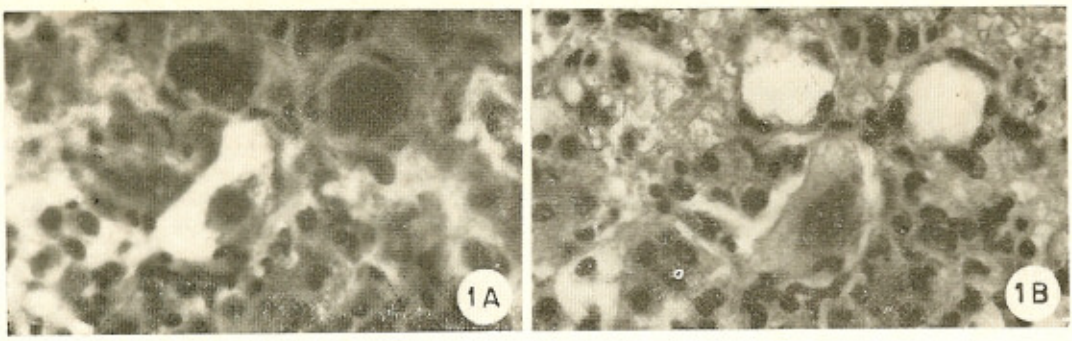

Fig. 1 — A) Cytoplasmic autofluorescence. Hematoxylin-eosin stain. Fluorescence microscopy. 400 X. B) Same field, under light microscopy. Note ground-glass aspect of the corresponding hepatocytic cytoplasm. Hematoxylin-eosin stain. Light microscopy. 400 X.

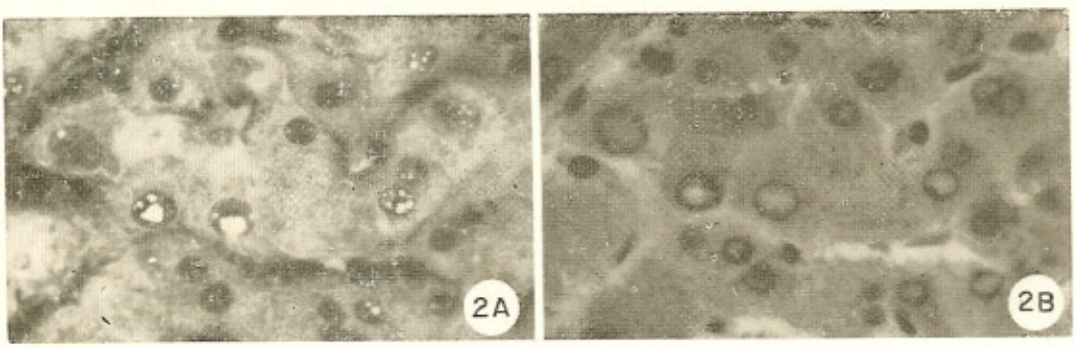

Fig. 2 — A) Nuclear autofluorescence. Hematoxylin-eosin stain. Fluorescence microscopy. 400 X. B) Same field, under light microscopy. Note the corresponding nuclear enlargment by vacuolar alteration. Hematoxylin-eosin stain. 400 X.

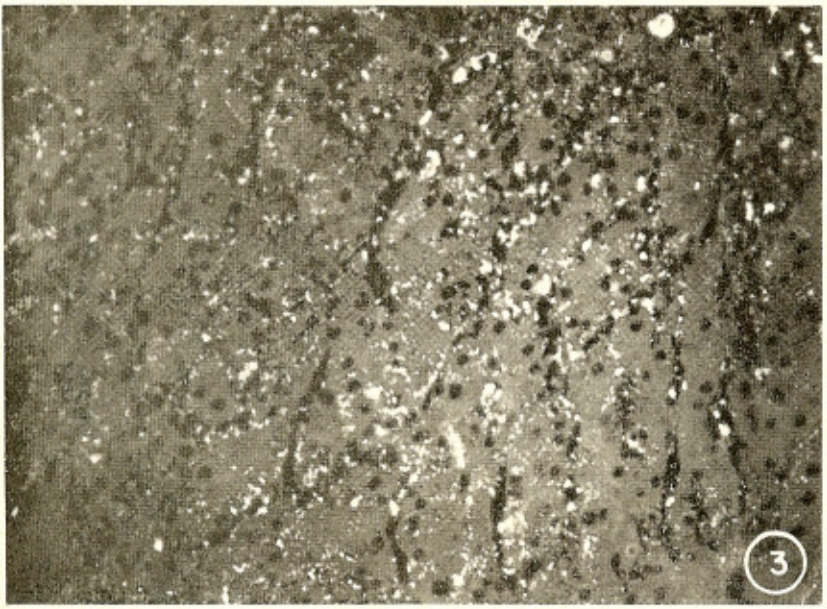

Fig. 3 — Numerous sinusoidal autofluorescent particles; necrotic area in fatal viral hepatitis. Hematoxylin-cosin stain. Fluorescence microscopy. 160 X.

RESUMO

Partículas hepáticas autofluorescentes em pacientes com antígeno de hepatite B no soro

(Nota Prévia)

Os Autores descrevem partículas autofluorescentes presentes em tecido hepático de pacientes com antígeno da hepatite B (HB) Ag no soro. Sua demonstração foi feita examinando-se cortes corados pela hematoxilina-eosina e examinados em microscópio de fluorescência.

As características das partículas e sua relação com o HB Ag são discutidas.

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