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Detection of HBsAg Containing Cells in Liver Biopsies by Different Stains and Classification of Positively Reacting Ground-Glass Hepatocytes*

F. Borchard and V. Gussmann

Institute of Pathology (Head: Professor Dr. W. Hort), University of Düsseldorf, Düsseldorf, Federal Republic of Germany

Summary. The diagnostic significance of orcein, aldehydthionine, and chromotrope anilinblue stains for the demonstration of HBsAg containing hepatocytes was investigated in 602 unselected liver biopsies. Five types of specifically stained ground-glass hepatocytes (GGH) were distinguished: Type I showed a positive staining reaction of the cytoplasmic periphery (marginal GGH), type II a diffuse staining of the total cytoplasm (diffuse GGH). Type III contained round or oval globular positive cytoplasmic masses (globular GGH). Type IV showed only very small round, drop-like or sickle-shaped positive structures (spotty GGH). The GGH with fatty changes were designated as type V.

In all carriers and patients with minimal hepatitis GGH, mostly type I and II, appeared in extensive clusters within the lobules. In chronic persistent hepatitis, there were moderately numerous, partly grouped, partly disseminated ground-glass hepatocytes of type II and III. In chronic active hepatitis there were only a few GGH of type IV. In acute viral hepatitis, there were no typical GGH, however, positively stained phagocytes were seen. The intracellular antigen localization and the intralobular distribution of GGH are considered to be the result of an immune reaction.

Single so-called 'metabolic' GGH sometimes showed similar pictures. However, they could usually be distinguished from virus containing GGH because of their granular cytoplasmic structure and a lower staining intensity in the applied stains. Among the three stains the orcein stain yielded the best results. In some cases with HBsAg-positive chronic active hepatitis virus infection could not be proved by means of staining.

Key words: HBs-Ag – Stains – Ground-glass hepatocytes – Classification of GGH – Unselected liver biopsies.

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Send offprint requests to: Prof. Dr. F. Borchard, Pathologisches Institut der Universität, Moorenstraße 5, D-4000 Düsseldorf, Federal Republic of Germany

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Introduction

In liver biopsies of patients with infectious or toxic diseases Klinge and Bannasch (1968) found several cells with light foamy or finely-granular cytoplasm. It was suggested this change produced by the proliferation of the smooth endoplasmic reticulum, was the result of non-specific toxic damage to liver cells, even in viral hepatitis. The same cells were called ground-glass hepatocytes (GGH) because of their homogeneous cytoplasm constituents and could be identified by immunofluorescence microscopy as HBsAg containing cells in viral hepatitis (Hadziyannis et al., 1973). Systematic investigations with different stains showed good results for orcein and aldehydthionine in detecting virus containing GGH (Shikata et al., 1974). Other authors favour the chromotrope anilinblue stain (Bianchi, personal comm., 1976) or the resorcin stain (Krutsay, 1977) for the identification of GGH in routine preparations.

The diagnostic yield of the orcein reaction was first tested by Deodhar et al. (1975) in 97 seropositive patients. Aldehydthionine staining was thought to be specific for HBsAg containing GGH in similar reports (Vogel et al., 1974; Winckler et al., 1976). However, Thomsen et al. (1976) described non-specific staining results in so-called 'metabolic' GGH.

This investigation was planned in order to demonstrate the diagnostic value of modified orcein and aldehydthionine stains and of the chromotrope anilinblue stain. At the same time, the incidence, structure, and distribution of GGH in viral hepatitis and non-specific results in other diseases would be described.

Materials and Methods

Between November 1975 and February 1976, 602 consecutive unselected paraffin blocks of liver biopsies from the University Institute of Pathology, Düsseldorf, were investigated. 48 cases had to be excluded because of scanty tissue or technical mistakes during the biopsy or the preparation. The diagnoses of the other liver biopsies are listed in Table 1.

Three sequential serial sections of the biopsies were treated synchronously with the three stains (as listed below) in order to prevent different staining results because of different ages of the solutions used. In addition, there were available from the routine daily work for every case two H.E., one Elastica-van-Gieson stain, one silver impregnation (Gordon and Sweet), one Pearls' iron stain, and one orcein stain. The sections were examined for GGH by both investigators independently. Granular positive results were noted as well as other positive cell reactions and staining artefacts. In each case with a positive or doubtfully positive result the serological HBsAg data were asked for when lacking. For various reasons it was not possible to gain the serological data of all the cases.

The 4–5 μ thick sections of paraplast or paraffin blocks were treated according to the following three methods.

1. Orcein Stain According to Shikata et al. (1974), as Modified by Scheuer (personal comm., 1976). Solution A: 5% potassium permanganate 9.5 ml (fresh); 3% sulphuric acid 5 ml; aqua bidest. to 100 ml. – Solution B: Orcein (British Drug Houses) 1 g; 70% alcohol 100 ml; conc. alcohol until pH 1–2 is reached.

Working method: Deparaffinised sections: 1) 10 min to potassium permanganate – sulphuric acid (=solution A); 2) 10 min in 2% oxalic acid, wash in tap water; 3) 4 h or more at room temperature in orcein (=solution B); 4) Differentiate in 1% HCl in 70% alcohol; 5) Dehydrate, mount.

Table 1. Histopathological diagnoses and serological findings in 602 consecutive liver biopsies

Diagnosis	(*n*)	HBsAg - pos.	HBsAg - neg.	HBsAg data not available
Acute viral hepatitis	22	7	11	4
AHTC[a]	4	2	1	1
Minimal hepatitis	4	4	–	–
Chronic persistent hepatitis	6	6	–	–
Chronic active hepatitis (with/without cirrhosis)	13	7	5	1
Chron. act. hepatitis/PBC and PBC	6	1	2	3
Cirrhosis of unsettled aetiology	9	–	1	8
Fibrosis	5	–	1	4
Alcoholic liver (with/without cirrhosis)	173	1	1	171
Fatty liver	106	–	4	102
Haemosiderosis/Haemochromatosis	14	–	–	14
Pigmentation	46	–	–	46
Drug lesions	12	–	2	10
Non-specific reactive hepatitis	58	–	7	51
Cholestasis/Cholangitis	29	–	4	25
Miscellaneous	14	–	1	13
Normal liver	33	–	1	32
Excluded material	48	–	–	48

[a] AHTC = Acute viral hepatitis with possible transition to chronic active hepatitis

2. Aldehydthionine Stain According to Klinge (personal comm., 1976). Solution A: Thionin (Merck 1421) 500 mg; 70% ethanol 91.5 ml; paraldehyde (Merck 7165) 7.5 ml; conc. HCl 1 ml; filtrate; solution has to ripe 10–14 days. – Solution B: Azocarmin G (Merck 1593) 100 mg; aqua dest. 100 ml; boil, cool to 50° C and add 1 ml of acetic acid.

Working method: Deparaffinised sections to aqua dest.; 1) 3–7 min potassium permanganate (300 ml $KMnO_4$ in 100 ml 0.3% H_2SO_4); 2) 15 min tap water; 3) merge several times to decolouration in 3% $Na_2S_2O_5$ (never more than three object glasses at once); 4) 10–15 min tap water; 5) 2 min 70% alcohol; 6) staining in solution A (aldehydthionine) for 2 h, possibly over night; the duration of staining has to be adapted to the age of the solution; 7) Microscopic control by a coloured rat pancreas (B-cells deep blue) or by HBsAg-positive liver biopsy; 8) 2–3 times rinse in tap water; 9) 1 min nuclear staining in hematoxylin according to Boehmer; 10) rinse with tap water; 11) 1–2 min counterstain in azocarmin (=solution B) at 60° C; 12) rinse in tap water; 13) Dehydrate in alcohol. Xylol. Eukitt.

3. Mallory-Trichrome Stain According to Bianchi (personal comm., 1976) = Chromotrope Anilinblue Stain. Solution A: Weigerts iron hematoxylin. – Solution B: 1% aqueous phosphormolybdenic acid. – Solution C: Chromotrope anilinblue = Chromotrop 2 R (Chroma) 4 g; Anilinblue 1.0 g; 2 N HCl 200 ml; Anilinblue has to be dissolved in HCl by slight warming. After cooling add chromotrope. In this solution the pH is between 0.9 and 1.3; control with the pH-meter is necessary.

Working method: Deparaffinised sections to aqua dest. 1) 5 min nuclear staining in Weigerts iron hematoxyline (=solution); 2) rinse and differentiate in 0.5% HCl; 3) 10 min rinse in running tap water; 4) for 2 min corrosion in 1% phosphormolybdenic acid (=solution B); 5) bring quickly to water; 6) 8 min in chromotrope anilinblue (=solution C); 7) rinse quickly; dehydration (96% alcohol); 8) Xylol. Eukitt.

Results

A. Normal Staining Artefacts, and Non-Specific Staining Results

The orcein stain yielded constant results if the oxidation solutions were freshly prepared. This staining method can be considered to be optimal when elastic fibres of the portal tracts appear brown-black and liver cells faintly brownish. In the chromotrope anilinblue stain the results were also constant. The cytoplasm appeared greenish with occasional red-green metachromasia. The nuclei were black. The aldehydthionine stain showed very different intensities of colour, even in sections stained in parallel so that it had to be repeated in control sections.

Artefacts appeared in the marginal parts of the biopsies in form of a dark brown rim in the orcein stain and a blue rim in the aldehydthionine stain. The chromotrope anilinblue method sometimes showed an increased red metachromasia. In very small biopsies, these marginal zones sometimes were confluent so that a definite interpretation of the staining results was not possible, in particular in the orcein or aldehydthionine stains. Changes of this kind have to be regarded as compression artefacts caused during liver puncture. Non-specific positive results were seen in the orcein and aldehydthionine stain in granulocytes and mast cells. Pigment deposits, mainly lipofuscin and bile granules, appeared a more intense brownish colour in the aldehydthionine stain. Similar interactions of staining and natural pigments were lacking in the chromotrope anilinblue stain.

B. Several Types of Specifically Stained Ground-Glass Hepatocytes

In the HBsAg-positive cases, we could distinguish five different types of ground-glass hepatocytes (GGH): *Type I* (Fig. 1 a–d) liver cells were those with a homogeneous cytoplasmic rim (*marginal GGH*). In these cells the position of the nucleus is normally central, but sometimes there is a translocation to the biliary pole of the cell. The peripheral cytoplasmic content is stained dark brown by the orcein stain and bluish in a pink background by the aldehydthionine stain. In the chromotrope anilinblue stain, these areas appear homogeneously grey, whereas the perinuclear cytoplasmic areas are stained more intensely green. This type of GGH usually appears together with diffusely positive cells.

Type II (Fig. 1 e–h) of GGH was found especially in minimal hepatitis and HBsAg carrier status. These cells showed a homogeneous structure of the whole cytoplasm (*diffuse type of GGH*). The fine-granular or vesicular substructure of the cytoplasm is due to a proliferation of the smooth endoplasmic reticulum. The nucleus in these cells sometimes shows a central or more usually a peripheral position towards the biliary pole. The cytoplasm is stained by orcein and aldehydthionine in a more intense brownish or bluish tone. The chromotrope anilinblue stain distinguishes these cells because of a reduced colour and a homogeneous greyish tint. GGH of the diffuse type may show a varying intensity of colour.

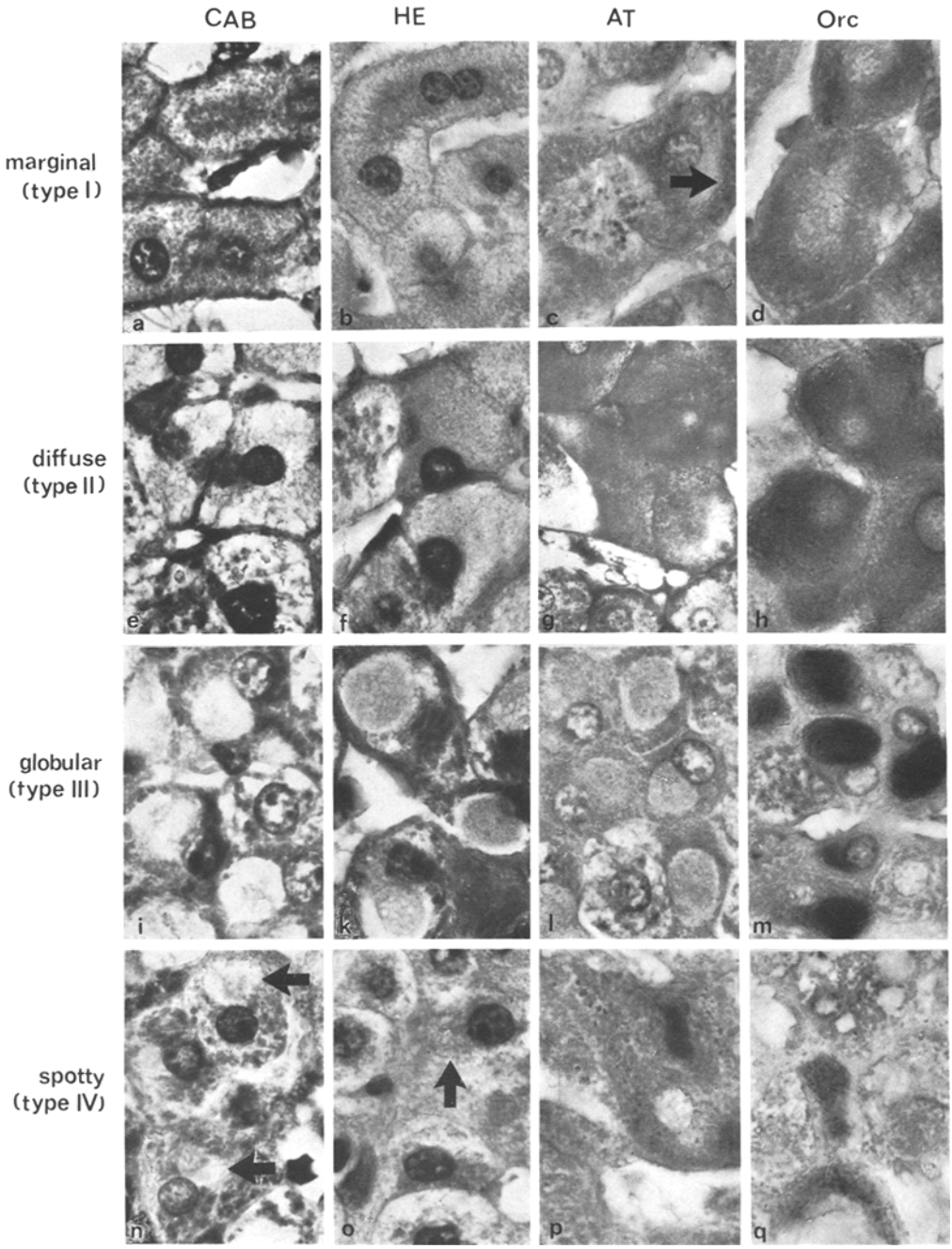

Fig. 1 a–q. Four types of ground-glass hepatocytes in different stains. In the horizontal lines the first one corresponds to marginal GGH (type I; **a–d**), the second one to diffuse GGH (type II; **e–h**), the third line to globular GGH (type III; **i–m**), and the fourth one to spotty GGH (type IV; **n–q**). The vertical lines show, from left to right, the stains applied: Chromotrope anilinblue (*CAB*), hematoxylin-eosin (*H.E.*), aldehydthionine (*AT*), and orcein (*Orc*) (all figures ×1,000)

Type III of GGH (Fig. 1i–m) cells had a round or oval, intensely stained cytoplasmic area, which is lighter in the H.E. preparation and the chromotrope anilinblue stain (*globular GGH*). This area, however, is more intensely stained by the orcein and the aldehydthionine reaction. Usually, the nucleus is displaced into the cell periphery by these cytoplasmic constituents. These forms were usually seen in chronic persistent hepatitis. This type is identical to the so-called 'inclusion body type' by Shikata et al. (1974) and Sakurai and Miyaji (1977).

Type IV of GGH (Fig. 1n–q) shows small roundish, sometimes sickle-shaped or drop-like structures which may be localized in a somewhat granular fashion in the perinuclear area (*spotty GGH*). These particles usually escape one's notice in H.E. specimens and are scarcely detected in the chromotrope anilinblue stain. By means of orcein or aldehydthionine, however, they may be detected by their brown or bluish colour. This type is mostly seen in chronic active hepatitis.

Type V of GGH (Fig. 3a), was mainly found in diabetes mellitus and earlier described as *GGH with fatty changes* (Borchard and Gussmann, 1977). Among liver cells with severe fatty changes these cells, also with fatty changes, appear clearly distinguished from the fine-vesicular changes that sometimes may be seen in the diffuse type of GGH. They could not be detected with certainty in H.E. sections or in chromotrope anilinblue stain. However, the orcein stain showed these cells clearly by their intense staining reaction.

C. Incidence of Various Forms of Ground-Glass Hepatocytes in Different Types of Hepatitis

1. Minimal Hepatitis or Carrier Status. In four cases of minimal hepatitis the incidence of ground-glass hepatocytes (GGH) was 15, 45, 50 and 75%. Within the liver tissue the orcein or aldehydthionine positive cells were mainly arranged in groups and only few isolated positive hepatocytes could be seen. The cytoplasmic staining of the GGH mostly corresponded to type I or II (cf. Fig. 1a–h), sometimes also to type III (Fig. 1i–m). The orcein stain gave good results, the aldehydthionine reaction an adequate staining technique as well. On contrast the chromotrope anilinblue stain showed a predominance of type II hepatocytes, since type I cells could hardly be detected because of slight light-dark-contrast. Figure 2a shows the typical distribution of ground-glass hepatocytes in minimal hepatitis.

2. Chronic Persistent Hepatitis. In 6 cases of HBsAg-positive chronic persistent hepatitis the ground-glass hepatocytes (GGH) were seen with an incidence of 15, 20, 35, 40 and 65%. Within the lobules, they were seen as isolated cells in cases with a low per cent positivity, but in higher incidence cases groups of such cells were seen. The distribution within the cytoplasm of the liver cells corresponded in every case to type II and III. With an incidence of more than 40%, GGH was combined with a predominance of type III cells. The results of the orcein stain were fairly well in keeping with those of the aldehydthionine stain. The chromotrope anilinblue stain – in some cases with a low

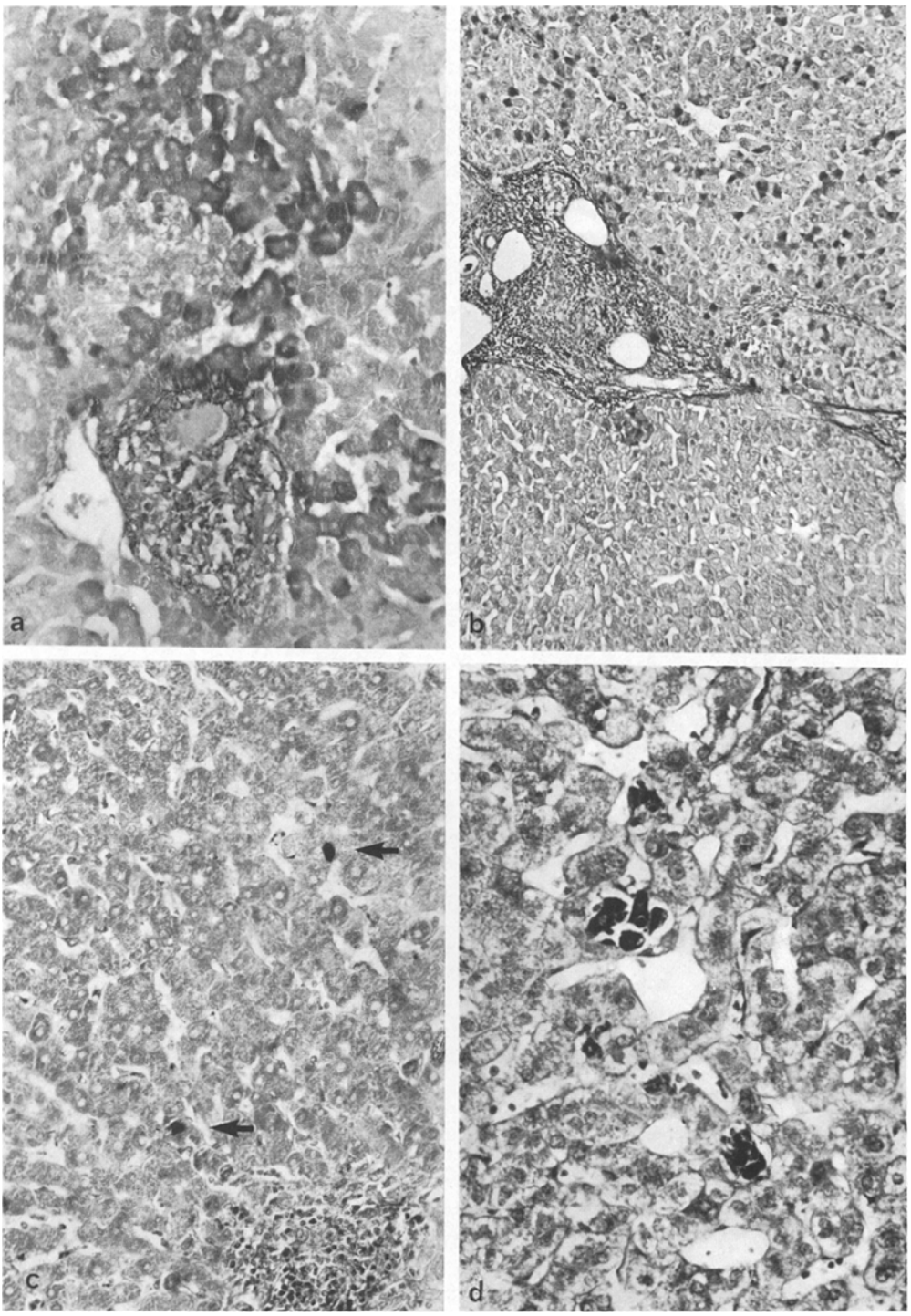

Fig. 2. **a** Groups of GGH of type I and II in minimal hepatitis (Orcein ×120). **b** Disseminated arrangement of GGH of type II and III in chronic persistent hepatitis. (Orcein ×36). **c** Isolated GGH of type III and IV (/) in chronic active hepatitis. (Orcein ×66). **d** Positively stained Kupffer cells in acute viral hepatitis. (Aldehydthionine ×250)

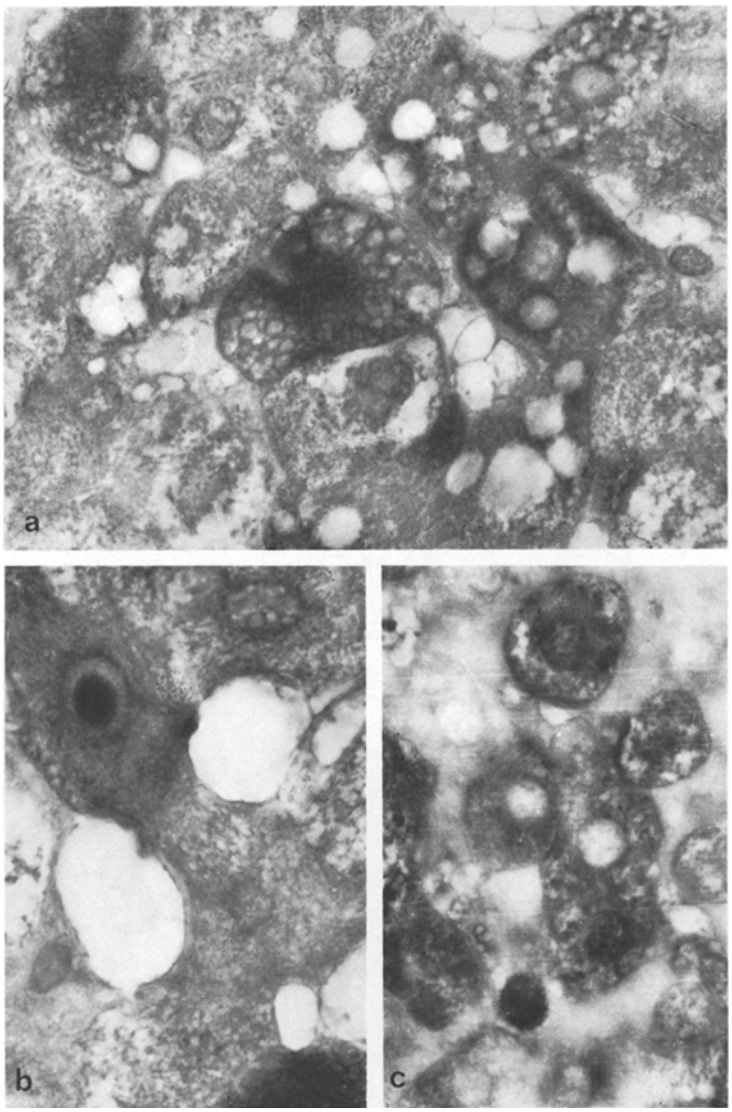

Fig. 3. a GGH with fatty changes (type V) in diabetes mellitus (Orcein ×1,000). **b** Positive staining reaction of the nucleus in single hepatocytes. (Orcein ×1,000). **c** Coarse-granular positive cells in HBsAg-negative alcoholic liver disease. (Orcein ×1,000)

incidence of HBsAg-positive cells – yielded questionable or even negative results. In the other cases, the incidence seemed to be slightly reduced and could not be judged properly.

Four of six cases with chronic persistent hepatitis showed fatty changes of various degree and GGH partly surrounded by liver cells with fatty changes. Some fatty changes could also be seen in the GGH cytoplasm (type V of GGH) (Fig. 3a).

Table 2. Positive staining results in 13 cases of chronic active hepatitis

	HBsAg-positive $n=7$	HBsAg-negative $n=5$	No serological test $n=1$
Orcein positive	4	2[a]	0
Aldehydthionine positive	4	2[a]	0
Chromotrope anilinblue positive	2	0	0

[a] Granular positive hepatocytes

3. Chronic Active Hepatitis. There were 7 cases of HBsAg-positive chronic active hepatitis. Despite seropositivity the orcein and aldehydthionine stain was negative in 3 cases. In five cases the chromotrope anilinblue stain did not show virus containing cytoplasmic areas. Two of the four cases with positive orcein and aldehydthionine staining reactions showed GGH of type IV, which appeared without any correlation to piecemeal necroses. Sometimes, in the orcein and aldehydthionine stain, there was a finely-granular perinuclear staining, which was sometimes difficult to assess.

In addition, we found a dark staining of several liver cell nuclei in the orcein stain in one case, and in two other cases, there were a very few nuclei of the same kind (Fig. 3b). In one further case, this reaction was found in a routine specimen prepared using older solutions.

Staining experiments for the detection of HBc by the orcein stain could not be reproduced. In the aldehydthionine preparations such changes in the nuclei were not found in any case.

4. Acute Viral Hepatitis. In 18 of 22 cases with viral hepatitis – mainly biopsied after a short interval following the outbreak of disease – we saw small groups of Kupffer cells in the central lobular area which contained coarse-granular orcein or aldehydthionine positive material (Fig. 2d). Further light microscopical analysis reviewed that these Kupffer cells sometimes contained iron or ceroid pigment. In 5 of the 22 cases in the orcein stain and in 2 of 22 cases in the aldehydthionine reaction single cell necroses with a homogeneous or granular cytoplasm were seen. They were stained intensely by both stains. Sometimes, these cells showed evidence of ingestion of bile. Further clarification of these characteristics was not possible because of the applied staining method, but they were felt to be non-specific. The chromotrope anilinblue stain showed no similar findings.

5. Acute Viral Hepatitis With Possible Transition to Chronic Active Hepatitis. In all 4 cases with signs of this disease the same positive Kupffer cell aggregates were found as described for acute viral hepatitis. In 3 cases we obtained the serological results for HBsAg. In the 2 seropositive cases acute inflammatory changes in mild chronic active hepatitis could not be excluded on morphological grounds, if the clinical findings were not taken into account. In these cases very few isolated positive GGH of type IV (Fig. 1n–q) could be identified

Table 3. Positive staining results in 4 cases of acute viral hepatitis with possible transition to chronic active hepatitis

	Serological finding HBsAg	Orcein		Aldehydthionine		Chrom. Anilinblue	
		hepato-cytes	Kupffer cells	hepato-cytes	Kupffer cells	hepato-cytes	Kupffer cells
1	pos.	GGH+	+	GGH+	+	GGH?	–
2	pos.	gran. pos. necroses	++	gran. pos. necroses	++	–	–
3	neg.	(+)	++	GGH+	+	–	–
4	?	GGH+	++	–	++	–	–

GGH = ground-glass hepatocytes

Table 4. Non-specific coarse-granular positive staining results in 12 cases with so-called 'metabolic' ground-glass hepatocytes

Diagnosis	Orcein		Aldehydthionine	
	gran.pos. hepatocytes	(Kupffer cells)	gran.pos. hepatocytes	(Kupffer cells)
Alcoholic liver	5[a]	(2)	4	(7)
Drug lesions	2	(1)	0	(0)
Non-specific reactive hepatitis	0	(2)	2	(2)
Fatty liver (diab.mell.)	4[a]	(1)	2	(0)
Granulomatous hepatitis	1	(0)	1	(0)

[a] In each case single hepatocytes with fine-granular changes

by means of the orcein and aldehydthionine stain. In the third serologically negative case, there was a positive granular staining reaction which was interpreted as the result of non-specific damage to liver tissue.

Table 3 gives a summary of the results in acute hepatitis with possible transition to chronic active hepatitis.

D. Positive Staining Reactions in So-Called 'Metabolic' Ground-Glass Hepatocytes (GGH)

The initially listed pigments and artefacts excluded, there remained 12 cases with non-specific staining results. They mainly corresponded to GGH of type I or II (Fig. 1a–h), their cytoplasm, however, had a more granular substructure and was stained less intensely be orcein or aldehydthionine. We saw fine- or coarse-granular GGH which sometimes appeared together in the same part of a liver lobule. As there were other morphological indications of metabolic

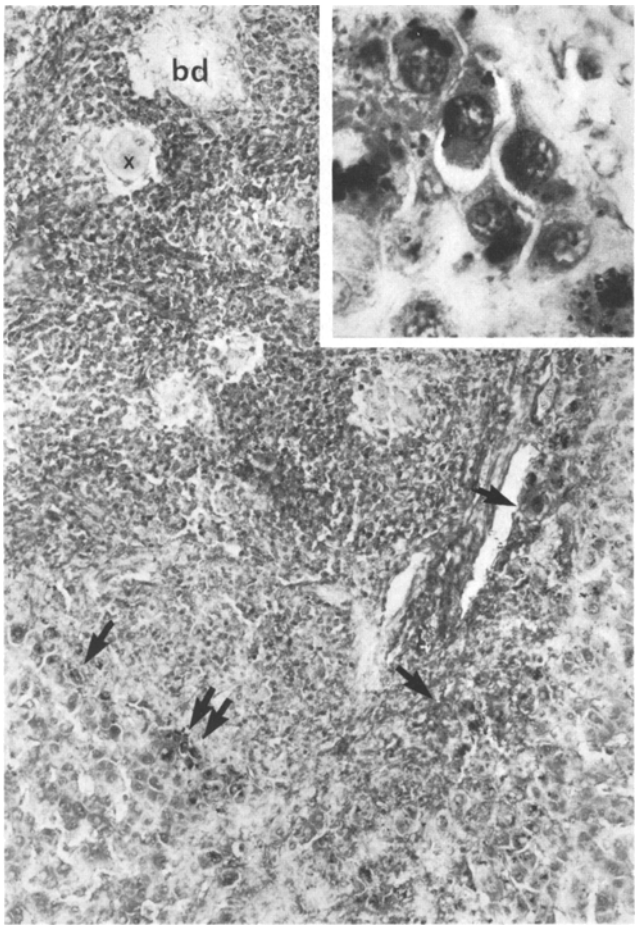

Fig. 4. Coarse-granular deposits in periportal hepatocytes in PBC. (Orcein ×160; inset ×1,000) (*bd*=bile duct; *x*=giant cell)

damages to the liver (e.g. fatty changes of Mallory bodies in alcoholism), these granular hepatocytes could usually be distinguished from specific homogeneous GGH in viral hepatitis. These types of GGH were also seen in drug lesions (e.g. reaction to antidiabetics and tuberculostatic drugs). In one case with homogeneous granular positive GGH there were morphological signs of alcoholism together with homogeneous positive GGH corresponding to a seropositive HBsAg finding. The chromotrope anilinblue stain was negative as in the other cases of this group.

The one case of granulomatous hepatitis did not correspond to primary biliary cirrhosis serologically or morphologically. In 5 cases with progressive PBC, which were not investigated in the current series, we found small orcein positive granules in the periportal hepatocytes (Fig. 4).

Discussion

Since the basic paper of Shikata et al. (1974) many groups have confirmed that HBsAg containing hepatocytes have an affinity to certain stains, especially to orcein and aldehydthionine (Bianchi and Gudat, 1975; Deodhar et al., 1975; Nayak and Sachdeva, 1975; Peters, 1975; Bartók et al., 1976; Bogomoletz, 1976; Klinge, 1976; Portmann et al., 1976; Ray and Desmet, 1976; Salaspuro and Sipponen, 1976; Sipponen, 1976b; Thomsen et al., 1976; Vogel et al., 1976; Winckler et al., 1976; Borchard and Gussmann, 1977; Sakurai and Miyaji, 1977; Turbitt et al., 1977; Cohen et al., 1978). Virus containing ground-glass hepatocytes (GGH) described by Hadziyannis et al. (1973) show enrichment of the surface antigen in the proliferated smooth endoplasmic reticulum. In consecutive sections it was demonstrated that those HBsAg containing cells demonstrated by immunofluorescent methods show a homogeneous staining reaction with orcein (Portmann et al., 1976). The mechanism of this technique and of the aldehydthionine stain is attributed to an attachment of the stain to disulfide bonds of the surface antigen, since prior esterification of these disulfide bonds is followed by a lack of positivity in viral GGH (Shikata et al., 1974). Proliferation of the smooth endoplasmic reticulum induced by toxic factors may also induce a granular positive staining of this cytoplasmic constituent (Thomsen et al., 1976; Borchard and Gussmann, 1977; Kostich and Ingham, 1977). Neither stain represents a typical viral stain, since staining experiments in juvenile viral warts did not intensify the colour of inclusion bodies. The virus containing cytoplasmic areas are not usually stained by chromotrope anilinblue. Even if colour intensity in the orcein and aldehydthionine stain corresponds roughly with the presence of virus, such a semiquantitative estimation cannot be made in the chromotrope anilinblue stain.

In spite of the relatively small number of seropositive patients, typical cellular expression of viral antigen and a characteristical lobular distribution was described in our biopsies. A few of the types of GGH have been mentioned earlier (Hadziyannis et al., 1973; Shikata et al., 1974; Bartók et al., 1976; Thomsen et al., 1976; Bianchi and Gudat, 1977; Borchard and Gussmann, 1977; Kostich and Ingham, 1977; Sakurai and Miyaji, 1977), but, to our knowledge, no such comprehensive classification has been proposed before.

There seems to be an agreement that in GGH of type I which we have called marginal GGH, the HBsAg expression through the cellular membrane is very pronounced. In type III (globular GGH) which was mainly found in chronic persistent hepatitis, there was a round focus of viral production in the middle of the cells. We think that this kind of viral localization does not occur in the later periods of the disease, as suggested by Shikata et al. (1974), but depends on the immune status of the patient (Bianchi and Gudat, 1977; Meyer zum Büschenfelde, 1975 and 1977) (cf. Fig. 5): In carriers an immuno-insufficiency predominates with a lack of elimination of surface antigen. In HBsAg-positive chronic active hepatitis antibodies against the surface antigen can be detected in a spotty distribution on the surface of the virus containing hepatocytes (Hopf et al., 1976). Possibly, in the marginal parts of the cytoplasm without viral particles in the GGH of type IV, there is an increased outflow and neutralisation by antibodies at the cytoplasmic membrane.

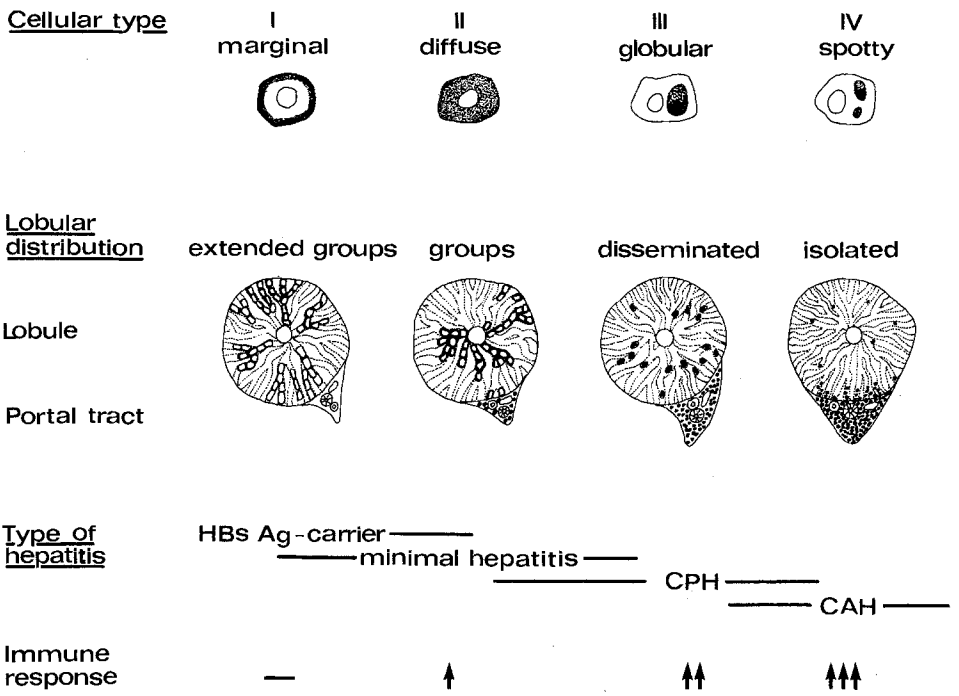

Fig. 5. Incidence of different types of groundglass hepatocytes, lobular distribution as compared with type of hepatitis and immune status

It is well-known, however, that in the inflammatory activity of hepatitis depends less on the presence of surface antigen, but much more on the intolerance of the organism to the viral core (Gudat et al., 1975; Gudat et al., 1976; Bianchi and Gudat 1977; Meyer zum Büschenfelde, 1977; Gudat et al., 1977). At present viral cores can only be detected by fluòrescence microscopy of PAP-method, a specific stain for these particles does not exist. The occurrence of orcein positive nuclear material in three of our cases with chronic active hepatitis was remarkable (Fig. 4b). One may speculate that modifications of the orcein stain might also be applied for the detection of the HBcAg.

The incidence and distribution of GGH within the lobules have been repeatedly discussed (Hadziyannis et al., 1973; Shikata et al., 1974; Deodhar et al., 1975; Klinge et al., 1975; Ray and Desmet, 1976; Portmann et al., 1976): In minimal hepatitis and in HBsAg-positive carriers 45% of the hepatocytes were transformed into GGH. Comparable figures in the literature gained partly with more specific immune methods lay between 20% (Portmann et al., 1976), 90% (Ray et al., 1976), and 80% (Bianchi and Gudat, 1977). The demonstration of positively stained GGH in every case of minimal hepatitis is in contrast to the statement of Klinge (1976) that half the cases with minimal hepatitis do not show stainable GGH. However, in our cases we cannot exclude falsely negative results for those cases without serological tests. The positive GGH in our cases of minimal hepatitis occurred mainly in groups with no predilection for certain parts of the lobular architecture (cf. Fig. 5). This observation is

in accordance with the findings of other authors (Hadziyannis et al., 1973; Shikata et al., 1974; Deodhar et al., 1975; Portmann et al., 1976; Ray et al., 1976; Thomsen et al., 1976; Hinkel et al., 1977; Sakurai and Miyaji, 1977). It has been suggested that the occurrence in groups of cells might be due to viral propagation from cell to cell without viral elimination (Portmann et al., 1976). In chronic persistent hepatitis, the hepatocytes in our cases correspond to type II and III and the distribution may already by disseminated (cf. Fig. 5). It is conceivable that this type of distribution corresponds to a reduced human tolerance to the surface antigen, preventing intracellular propagation of the virus.

In chronic active hepatitis, we found only very scarse positive GGH, in agreement with other authors (Desmet, 1975; Portmann et al., 1976; Ray et al., 1976; Thomsen et al., 1976; Bianchi and Gudat, 1977). Stains in this type of hepatitis only were falsely negative in half the cases, although the inflammatory hepatic lesions were definitely virus-associated from sero-positive findings. In our material the virus containing cells did not correlate with piecemeal necroses as suggested by Shikata (1973) and Deodhar et al. (1975). This finding can be explained by the agreement that the development of this severe form of hepatitis is dependant on a predominance of core particles (Gudat et al., 1975; Gudat et al., 1976; Ray et al., 1976; Bianchi and Gudat, 1977; Gudat et al., 1977; Meyer zum Büschenfelde, 1977; Endo et al., 1978). It has been suggested that the viral RNA-polymerase which is possibly identical with the e-antigen might be important (Arnold et al., 1975; Robinson and Lutwick, 1976; Poley, 1977). The assumption of a direct relationship between the surface antigen and the activity of hepatitis is disproved by our results.

In acute viral hepatitis we did not find positively stained GGH. This confirms the results of Desmet (1975), Gudat et al. (1975), Deodhar et al. (1976), Ray and Desmet (1976), Ray et al. (1976), Thomsen et al. (1976), Winckler et al. (1976), Bianchi and Gudat (1977), and Kostich and Ingham (1977). However, we sometimes noticed accumulations of orcein and aldehydthionine positive Kupffer cells near a few coarsely-granular positive single cell necroses, which have not been described before. We do not know whether these cells are identical with those described by Ray and Desmet (1976) which were shown, by fluorescent microscopy, to be virus containing. Elimination of the virus particles in these cases had not taken place. Moreover, in two cases the occurrence of single positive GGH was suggestive of a transition from acute viral hepatitis to a chronic type of hepatitis. This has also been described with immunofluorescent methods by Ray and Desmet (1976). As no surface antigen can be detected by specific immunological methods in the liver tissue in acute viral hepatitis, two interpretations of the positive staining in Kupffer cells are possible: It may be assumed that the staining is due to partially destroyed viral material which has lost its specific immunological reactivity. It could also be due to a non-specific staining of membrane layers of degenerated cytoplasmic lamellae. Since positively stained Kupffer cells could also be seen in other cases with necroses, the latter interpretation seems to be more probable.

Non-specific granular staining of hepatic cells with a proliferated endoplasmic reticulum in sero-negative cases is of special practical importance (Thomsen

et al., 1976; Borchard and Gussmann, 1977; Kostich and Ingham, 1977). These non-specific staining results were seen in the orcein stain and in the aldehydthionine reaction. The altered liver cells are usually less intensely stained and show a more granular structure of their cytoplasm, thus giving an additional hint that there is non-specific staining. Granular and faint staining results should be interpreted with caution; the more sensitive serological HBsAg findings should always be taken into account (Klinge, 1976; Thomsen et al., 1976; Sumithran, 1977). In contrast, a negative staining result, especially in hepatitis with high activity, does not speak against virus-associated lesions. We agree that the three staining reactions yield satisfactory results in HBsAg positive hepatitis with low activity, especially in minimal hepatitis and in chronic persistent hepatitis. These stains seem to be of special value in cases in which the GGH occur together with fatty changes as in type V cells and in cases with mixed aetiology.

In those cases with non-suppurative chronic destructive cholangitis in the stage of florid duct lesion (Scheuer, 1967) we did not find typical granular positive GGH as described by Sipponen (1976a, b) and Kostich and Ingham (1977). These particles correspond to copper containing lysosomes (Salaspuro and Sipponen, 1976). We found them only in other biopsies during later stages of this disease, in agreement with the results of Ludwig et al. (1978) (Fig. 4). Therefore the orcein stain is also of value for detecting copper in liver biopsies.

For methodical reasons, the orcein stain has proved to be the best method for semispecific detection of viral surface antigen. It has been routinely performed in our laboratories for three years.

In the short period of the current study, HBsAg was detected first by applied histochemical methods in 11 cases (40.7% of the sero-positive cases) and was later confirmed serologically.

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