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Hepatic Morphologic Findings and Viral Antigens in Acute Hepatitis B

A Longitudinal Study

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Summary. In the course of a prospective clinical study, 26 liver biopsies were taken in 24 previously healthy patients with an uncomplicated acute hepatitis B (AHB) and a subsequent complete recovery (UR group). Serum transaminase levels were used to distinguish four AHB stages, as the variability in duration made time dependent staging less appropriate. As a reference, a second group of 22 liver biopsies from 7 patients from the same prospective clinical study who eventually developed chronic hepatitis (CH group) was included. Due to fluctuations of transaminase levels in this group the stages two and three could not be separated, stage four was not reached within the follow-up period of median 5 years. Histopathological criteria and the distribution patterns of hepatitis B surface (HBsAg) and core (HBcAg) antigens were studied.

Piecemeal necrosis and bridging hepatic necrosis were a frequently occurring feature in the liver biopsies of the UR group taken during the stage of peak transaminase levels. In this respect, no differences could be demonstrated between the UR group and the CH group. The occurrence of these features in AHB thus appears to have no prognostic value as risk factor for chronic liver disease. In contrast, diffuse cytoplasmic ('ground glass') HBsAg localization was only found in stage 2/3 of the CH group.

It is concluded that apart from ground glass cells, care must be taken to interpret any histopathological feature in AHB as risk factor of progression to chronicity without reference to the duration of illness. The possible significance regarding the host's immune mechanisms of some features that may persist during chronic liver disease is discussed.

Key words: Acute hepatitis B – Liver biopsy – Viral antigens – Prognosis – Chronicity.

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Introduction

A liver biopsy in patients with acute hepatitis may reveal histological patterns associated with a substantial risk of progression to one of the forms of chronic liver disease (Bianchi et al. 1971, 1977; Dietrichson et al. 1975; Ware et al. 1975; Scheuer 1977). These histopathological signs of chronicity resemble forms of chronic active hepatitis (CAH) and include piecemeal necrosis of periportal liver cells (PMN) and bridging hepatic necrosis of liver acini (BHN). However, both patterns may be followed by complete recovery, and may thus offer a risk of over-diagnosis of CAH (Peters 1975; Scheuer 1977).

In the liver of patients with persistent or chronic hepatitis B viral infections the viral surface (HBsAg) and core (HBcAg) antigens are readily detectable in many cases, using immunofluorescence (IF) or immunoperoxidase (IP) methods (Gudat et al. 1975; Huang 1975; Ray et al. 1976a, b). Distribution patterns of these antigens in the liver parenchyma have been recognized and were related to the host's immune mechanisms (Gudat et al. 1975; Ray et al. 1976a, b; Yamada et al. 1978). In only a proportion of liver biopsies in patients with acute hepatitis B can HBsAg or HBcAg be detected (Gudat et al. 1975; Ray et al. 1976b; Portmann et al. 1976; Huang and Neurath 1979). The lower occurrence in uncomplicated acute hepatitis B (AHB) is probably related to the diminishing activity of viral replication in combination with viral elimination at the time the diagnosis becomes apparent and a biopsy is taken: generally only after peak transaminase levels in the serum have been reached (Hoofnagle et al. 1978). A distribution pattern of HBsAg throughout the liver cell cytoplasm ('ground-glass' cells) or in relation to liver cell membranes may indicate possible transition to chronic liver disease (Ray et al. 1976b).

Liver biopsies of patients from the onset of AHB onwards have been investigated in the course of a prospective clinical study (Niermeijer and Gips 1977). From the total number of biopsies two groups have been formed and are presented here; a group of biopsies from patients with a clinically uncomplicated AHB with a subsequent complete recovery, versus a reference group of biopsies from patients who eventually developed various froms of chronic hepatitis B. A comparison is made between these groups, especially with regard to the histopathological and viral antigenic distribution patterns associated with a substantial risk of progression to chronicity.

Material and Methods

Material. The material consists of 48 liver biopsies from 31 patients who were all followed for three to seven (median 5) years in the course of a prospective clinical study of hepatitis B. The clinical, biochemical and serological data of the whole series were presented in previous papers (Niermeijer and Gips 1977; Niermeijer et al. 1977, 1978).

According to the clinical follow-up, two groups were formed from the total number of biopsies. One group of 26 liver biopsies were taken in 24 previously healthy members of hospital and laboratory personnel, who contracted a hepatitis B infection during a temporary hepatitis B viral contamination of a haemodialysis unit. As blood samples from this personnel were regularly taken and analysed, the infection was detected relatively early in the disease and well before the elevation of serum transaminases. All these patients recovered completely. This group of liver biopsies covered

all AHB stages and was designated as the uncomplicated and completely recovering AHB group (UR group). The reference group consisted of 22 liver biopsies from seven patients, of whom four were addicated to the intravenous use of illicit drugs. All developed chronic liver disease in follow-up. In 5 of them AHB was diagnosed on admission; in the 2 others AHB was diagnosed elsewhere initially, they were referred with clinically persistent disease. In these 7 patients without historical data of chronic liver disease, the clinical diagnosis could be established early in the acute disease before peak transaminase serum values were reached. This group of liver biopsies was designated as the HBsAg seropositive chronic hepatitits group (CH group).

Serum glutamic pyruvic transaminase levels (SGPT, normal value to 30 U) were used to establish the stage of AHB in which the biopsies were taken: stage 1 before peak SGPT, stage 2 from peak to 450 U, stage 3 between 450 U and 45 U, and stage 4 after SGPT falling below 45 U. Due to the fluctuations of SGPT levels in the CH group the stages 2 and 3 could not be separated, stage 4 was not reached within the follow-up period.

Methods. Percutaneous liver biopsies were performed using a 1.6 mm diameter Menghini needle. A small part of the biopsy was immediately frozen in $-100°$ C freon-22, and stored at $-70°$ C; from 12 biopsies the frozen material was too small or no longer available at the time the IF studies were done. The larger part of the biopsy was fixed in 4% formaldehyde, embedded in paraplast and used for light microscopy (LM) and for IP studies. In two cases small tissue fragments were fixed in 2% phosphate-buffered glutaraldehyde, postfixed in 1% phosphate-buffered OsO_4, embedded in epon and used for electron microscopy (EM).

Light Microscopy. 4 μm thick paraplast sections were stained with haematoxylin and eosin (H&E), Gomori's reticulin staining, periodic acid-Schiff reaction (PAS) following diastase digestion, azan, Perl's prussian blue method for iron and orcein. In a double-blind study, all sections without any additional information were classified using a uniform, precoded protocol. In each biopsy 52 pathological alterations of the liver parenchyma and 12 pathological items of the portal tracts were graded from 0 to 4 (absent, sparse, intermediate, moderate and conspicuous), using where possible the criteria defined by Bianchi et al. (1971, 1977).

Electron Microscopy. Ultrathin sections were cut from selected areas of the epon-embedded material on a LKB microtome with a diamond knife, stained with 0.3% lead citrate and uranyl acetate and viewed in a Philips EM 300.

Antisera. Commercially obtained rabbit anti-HBs (RaHBs) serum (Behring), guinea pig anti-HBs (GpaHBs) serum following immunization with pooled HBsAg/adw resp. −/ayw positive sera (Houthoff and Houwen 1976) and human high titre anti-HBc serum (HaHBc) with HBsAg but without detectable anti-HBs were used. Anti-human activity of the animal antisera was removed by immunoadsorption (Houwen et al. 1975); no anti-nuclear or anti-liver antibody was detectable in the human antiserum by IF methods. Specificity of these antisera was checked in the human antiserum by IF methods. Specificity of these antisera was checked with the indirect IF methods (described below) on normal liver and on liver with EM proved core and/or surface particles. Checking was performed with and without prior absorption of the sera with partly purified HBsAg, and with and without omission of the specific antiserum, which was substituted by normal serum. The HaHBc serum was compared with chimpanzee anti-HBc test serum (NIH, Bethesda, Md), using an indirect IF method with liver sections of a patient with many core particles but no demonstrable IgG in liver cell nuclei and very low anti-HBc serum titers. The peroxidase-antiperoxidase (PaP, from rabbit) complexes were kindly provided by Dr. S. Poppema (Groningen). The other antisera used were purchased from Nordic (Tilburg, The Netherlands).

Immunofluorescence Methods. 4 μm cryostat sections of the snap-frozen material were used for the detection of IgG, HBsAg and HBcAg. Incubations were carried out at 37° C for 60 min., unless specified otherwise. Between the steps the sections were thoroughly washed in phosphate-buffered saline (PBS) for 15 min. The steps consisted of: (1) for IgG: normal goat serum (NGS, diluted 1:20), rabbit anti-human (RaH) IgG (1:100), FITC-conjugated goat anti-rabbit immunoglobulins (GaR Ig, 1:80). (2) For HBsAg: NGS (1:20), RaHBs (1:60), FITC-conjugated GaR Ig (1:80). (3) For HBsAg, second serum: normal rabbit serum (NRS, 1:20), GpaHBs (1:10), FITC-

conjugated RaGp Ig (1:50). (4) For HBcAg: NRS (1:20), HaHBc (1:20), FITC-conjugated RaH Ig (1:40).

Immunoperoxidase Methods. 4 μm paraplast sections were digested (Huang et al. 1976) with 0.1% trypsin (Sigma type I) in tris-HCl buffer with pH 7.6 for 60 min at 20° C, thoroughly washed in PBS for 12 h and pretreated with 0.3% H_2O_2 in methanol for 30 min at room temperature. The sections were then used for antigen detection. The incubation with the specific antiserum was carried out at 4° C for 16 h (Huang et al. 1976). The steps consisted of: (1) for IgG: NGS (1:10), RaH IgG (1:20), GaR Ig (1:15), PaP (1:20) and finally the diaminobenzidin reaction (DAB) for 5 min to demonstrate the peroxidase activity. (2) For HBsAg: NGS (1:10), RaHBs (1:10), GaR Ig (1:15), PaP (1:20) and DAB. (3) For HBcAg: NRS (1:10), HaHBc (1:15), RaH Ig (1:20), GaR Ig (1:15), PaP (1:20) and DAB.

Results

Histopathology

A selection of the pertinent data in the histopathological grading is given in Table 1. From both the severity of an item and the number of biopsies showing it in each stage, a general picture of the AHB stages and of the gradients in severity between subsequent stages was recorded.

UR Group. Hepatocytic polymorphy, spotty necrosis, small lymphohistiocytic aggregates and prominent Kupffer cells with diastase resistent PAS positive material were always present throughout the first three stages; to a lesser extent, lymphohistiocytic aggregates also occurred in stage four. Gradients in these items were not obvious during the first three stages.

In stage one, a large number of acidophilic bodies, many mitotic figures in liver cells and a sparse and predominantly lymphocytic infiltrate in the portal tracts and liver parenchyma were present (Fig. 1).

In stage two, lymphocytic infiltrates in the portal tracts and parenchyma were prominent with a variable number of neutrophils and a moderate number of degenerating liver cells (both acidophilic and ballooning) throughout the parenchyma. Lymphocytic spillover from the portal tracts into the adjacent liver parenchyma was always an obvious feature (Fig. 2). In most cases, degenerating liver cells in the limiting plates surrounded by lymphocytes were present, fulfilling all criteria of PMN (Fig. 2). In some cases, appreciable focal collapse of reticulin fibers or portovenous collapse strands with the appearance of BHN were observed (Fig. 3).

In stage three, ballooning degeneration of liver cells and small amounts of bile pigment in bile canaliculi or in liver cells of the acinar zone three were regularly present. Many of the portal tracts were enlarged to a variable extent, sometimes with small fibrotic septa extending into the adjacent liver parenchyma. Lymphocytic spillover was less conspicuous and PMN was generally absent during this stage. BHN was not observed.

Stage four showed residual changes, consisting of enlarged portal tracts and some small fibrous septa. In one of the cases with BHN in stage two, fibrous septa interconnecting portal tracts with terminal hepatic venules and an otherwise normal architecture were observed in a second biopsy during stage four (Fig. 4). Two cell thick liver plates were regularly present in all

Table 1. Histopathological grading of liver biopsies in acute and chronic hepatitis B. 24 patients (26 biopsies) had AHB with an uncomplicated recovery (UR group); 7 patients (22 biopsies) had AHB and eventually developed chronic hepatitis B (CH group). In every biopsy, each item was graded from 0 to 4 (absent, sparse, intermediate, moderate and conspicuous). The numbers in the table refer to the *number of biopsies* showing grades 3 and 4 for the items indicated by 1), while for the other items the number of biopsies showing their presence (grade 1 to 4) is given. The stages of acute and chronic hepatitis are based on SGPT levels: stage 1 before peak SGPT, stage 2 from peak to 450 U, stage 3 between 450 U and 45 U, and stage 4 from 45 U to normal

Histopathological criteria in acute hepatitis B	Uncomplicated recovery (UR group) 24 patients				Development of chronic hepatitis B (CH group) 7 patients	
Stage related to SGPT level	1	2	3	4	1	2/3
Duration of stage: median (range) in weeks	4 (3–5)	2 (1–8)	5 (3–12)	2 (0–5)	4 (3–7)	(4wk–7yr)
Number of biopsies	6	8	9	3	3	19
Hepatocytic mitosis[1]	4	5	3	0	1	4
Degeneration:						
acidophilic[1]	4	3	1	0	2	6
ballooning[1]	1	3	6	0	2	11
Inflammation:						
parenchymal lymphocytes[1]	3	6	4	0	3	6
parenchymal neutrophils[1]	0	3	4	0	1	1
portal lymphocytes[1]	3	8	4	0	3	17
portal neutrophils[1]	0	1	1	0	1	1
portal spill over[1]	3	8	1	0	3	13
Necrosis:						
spotty[1]	3	4	4	1	2	5
piecemeal necrosis	1	6	1	0	1	10
bridging hepatic necrosis	0	2	0	0	0	3
Regeneration:						
2 cell plates[1]	0	0	5	2	0	13
nodular	0	0	0	0	0	9
Reticulin fibers:						
focal collapse[1]	0	3	0	0	0	4
fibrosis[1]	0	1	3	2	0	14
Bilirubin	0	2	6	1	0	0
HBcAg in hepatic nuclei	4	6	5	1	1	14
HBsAg:						
focal cytoplasmic	3	5	5	1	0	2
diffuse cytoplasmic	0	0	0	0	0	9
cell membrane related	0	0	0	0	0	5

three acinar zones. Liver cell degeneration and inflammation were absent or inconspicuous during this stage.

CH Group. In the biopsies from stage one, the distribution of histopathological changes was comparable to that in the UR stage one. During stage 2/3 a considerable variation in pathological alterations between the biopsies was present, ranging from some inactive fibrosis without other changes to prominent

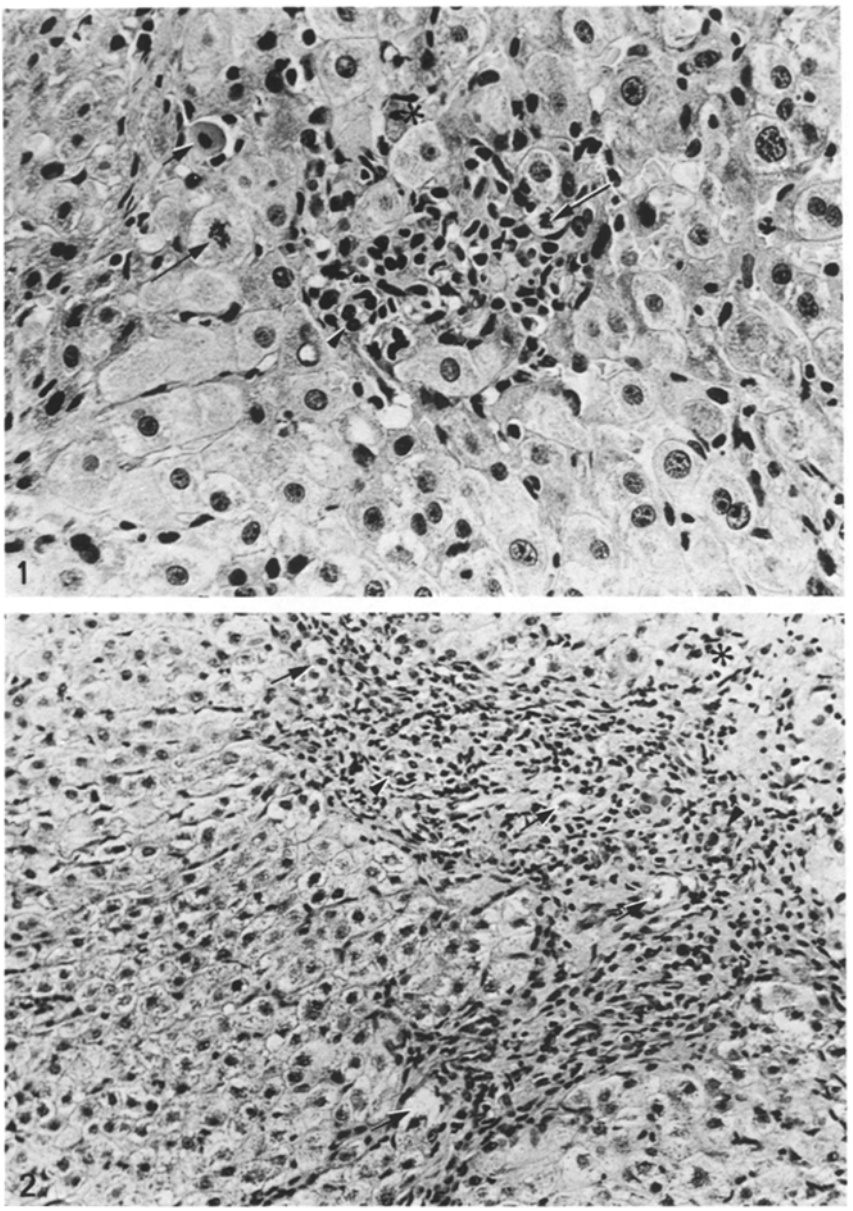


Fig. 1. AHB, uncomplicated recovery, stage 1. An acidophilic body (*short arrow*), liver cell mitoses (*long arrow*) and prominent Kupffer cells (*asterisk*) are present in the liver parenchyma. Note bile ductule (*arrowhead*) in edge of a portal tract. The inflammatory infiltrate in the portal tract and parenchyma is scarce. H&E, ×500

Fig. 2. AHB, uncomplicated recovery, stage 2. An enlarged portal tract with bile ducts (*arrowhead*), lymphocytic spillover (*asterisk*), irregular portal boundaries and piecemeal necrosis of hepatocytes (*arrow*). H&E, ×250

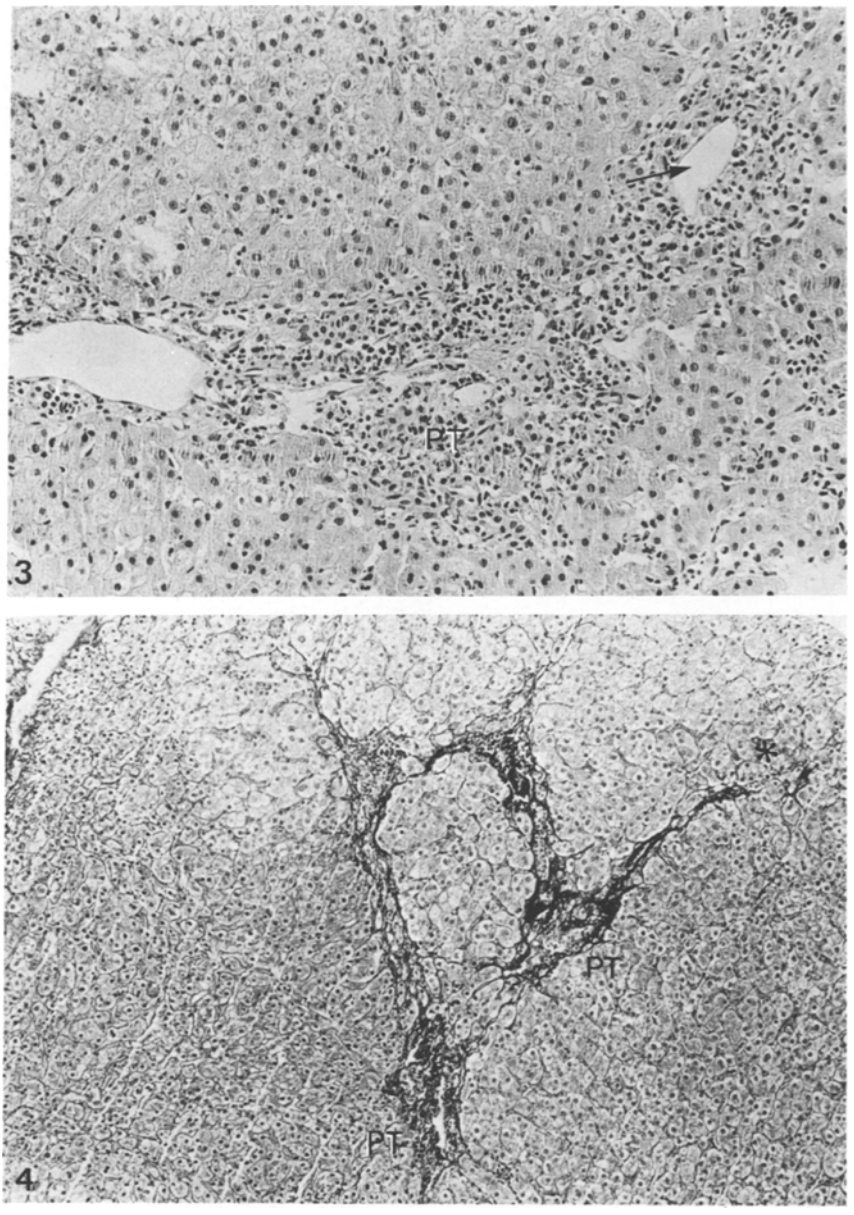


Fig. 3. AHB, uncomplicated recovery, stage 2. Bridging hepatic necrosis: a strand of necrotic liver cells interconnecting a portal tract (*PT*) with a terminal hepatic venule (*arrow*). H&E, ×200

Fig. 4. AHB, uncomplicated recovery, stage 4. Posthepatitic scarring with small fibrous septa interconnecting portal tracts (*PT*) with each other and with the acinar zone 3 (*asterisk*). Gomori's reticulin, ×100

Table 2. Evaluation of the prognostic value of some histopathologic parameters in liver biopsies taken during the first six months of hepatitis B. As stage 4 (as based on SGPT levels) was not reached in the CH group, biopsies taken during this stage in the UR group were excluded. In case of more than one liver biopsy per patient during the first 6 month period, only the first biopsy has been included. The two-sided test of Fisher (fourfold contingency tables) with normal approximation and continuity correction was used. Grading of the items and stages of hepatitis are the same as those in Table 1. The numbers in the table refer to the *number of biopsies* showing grades 3 and 4 for the items indicated by 1), while for the other items the number of biopsies showing their presence (grade 1 to 4) is given. The P_d values indicated by 2) may be interpreted as a high probability that for these parameters both groups of samples are from the same population, i.e. that the parameter is equally distributed over both groups

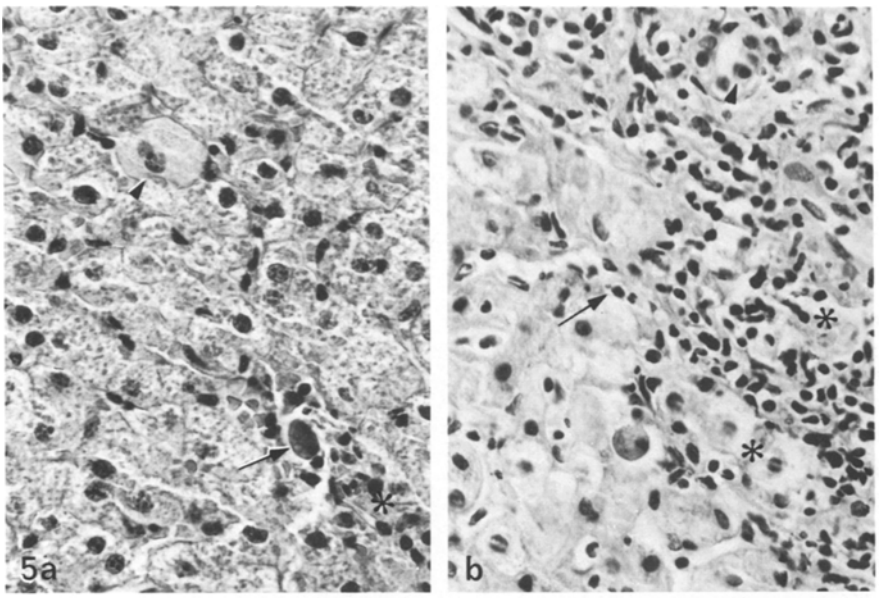
Patient group	UR	CH	
Stages, related to SGPT level	1+2+3	1+2/3	
Number of biopsies (1 biopsy/patient)	23	6	
Spotty necrosis [1]	11	3	$P_d=0.7188$ [2]
Piecemeal necrosis	8	3	$P_d=0.8336$ [2]
Portal spill-over of lymphocytes [1]	12	4	$P_d=0.8650$ [2]
Bridging hepatic necrosis	2	0	$P_d=0.8728$ [2]
Diffuse cytoplasmic HBsAg	0	1	$P_d=0.4592$

PMN with BHN and nodular regeneration. Ground glass hepatocytes staining positively with orcein occurred in one or more biopsies of five patients (nine biopsies); their scattered distribution throughout the parenchyma corresponded to the occurrence of liver cells with diffuse cytoplasmic localization of HBsAg (Figs. 5 and 7). This feature was never observed in the biopsies of the 17 patients of the UR group during the stages two and three ($P_d=0.0007$, fourfold contingency tables). In five patients of the CH group, one or more liver biopsies were taken during the first six months after the onset of SGPT elevation. In Table 2, some of the histopathological variables in the first biopsy during this period are compared with those of the biopsies from the UR group during the stages one to three.

Immune Cytochemistry

The use of the rabbit and guinea pig antisera for the detection of HBsAg gave comparable results in all cases. For the detection of HBcAg, the results with IF and IP methods were comparable in all cases. For HBsAg, the diffuse and focal cytoplasmic localizations in hepatocytes were comparable with IF and IP methods; the cell membrane related localization of HBsAg was not reproducible with the IP methods. With the IF methods, a fully comparable localization in liver cell nuclei was present for HBcAg and IgG in most cases, some of the UR cases from stage one had HBcAg but no detectable IgG in nuclei. With the IP methods IgG could not be demonstrated in the nuclei. The combined results for HBsAg and HBcAg detection are given in Table 1.

In many of the biopsies of the UR group, focal cytoplasmic HBsAg or nuclear HBcAg was detectable in a few scattered hepatocytes (Fig. 7a/c). The



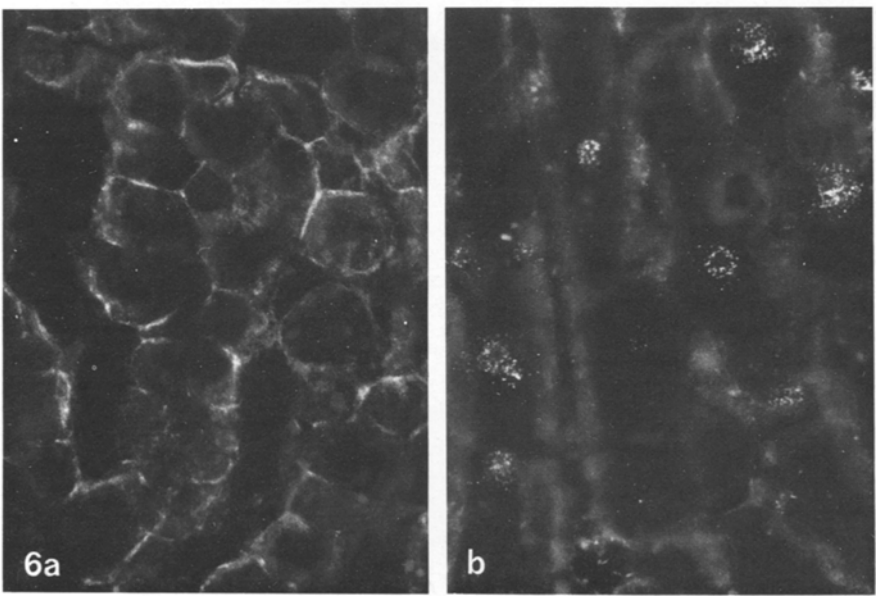


Fig. 5a and b. Eventual development of chronic hepatitis B, stage 2/3, biopsies taken within 6 months after onset of hepatitis. **a** Groundglass hepatocyte (*arrowhead*), acidophilic body (*arrow*) and focal lymphocytes (*asterisk*). **b** Part of enlarged portal tract with bile duct (*arrowhead*), lymphocytic spillover (*arrow*), regressive changes in hepatocyte indicative of piecemeal necrosis (*asterisk*) and acidophilic body. H&E, × 500

Fig. 6a and b. Chronic hepatitis B, stage 2/3. **a** Localization of HBsAg near liver cell membranes. IF, rabbit anti-HBs serum, × 400. **b** HBcAg localization in and around liver cell nuclei. IF, human anti-HBc serum, × 400

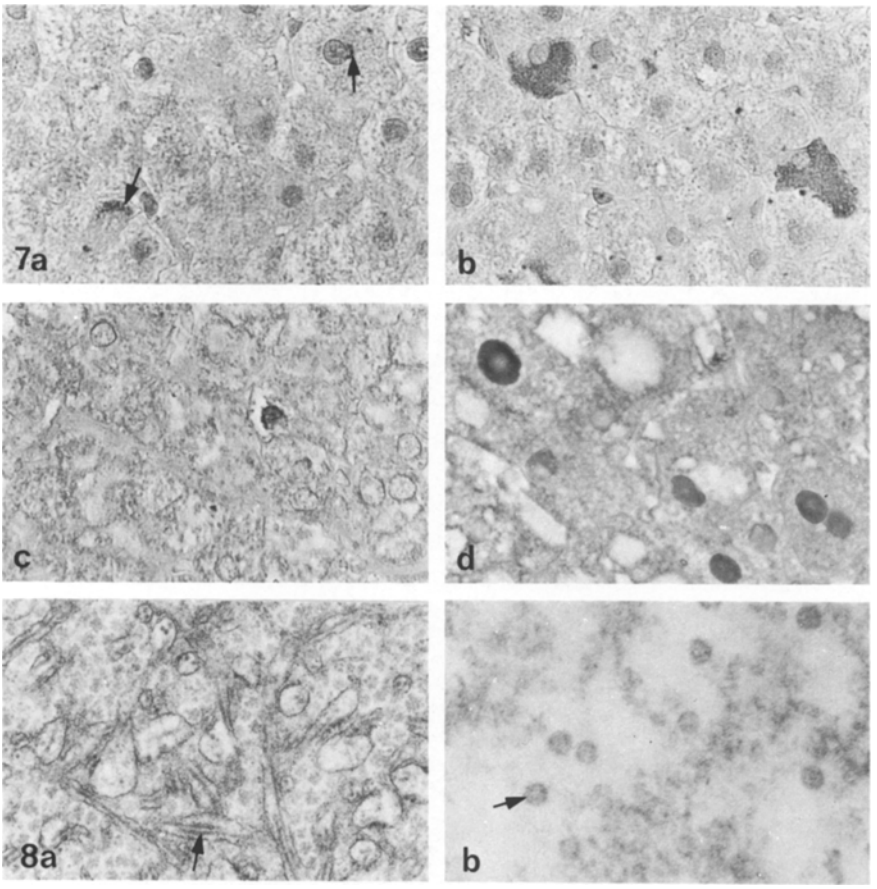


Fig. 7. a AHB, uncomplicated recovery, stage 3. In two lifer cells small focal accumulations of HBsAg are present (*arrow*). IP, rabbit anti-HBs serum, ×500. **b** Chronic hepatitis B, stage 2/3. Groundglass cells with diffuse cytoplasmic HBsAg. IP, rabbit anti-HBs serum, ×500. **c** AHB, uncomplicated recovery, stage 3. Solitary liver cell nucleus with HBcAg. IP, human anti-HBc serum, ×500. **d** Chronic hepatitis B, stage 2/3. High proportion of HBcAg containing liver cell nuclei. IP, human anti-HBc serum, ×800

Fig. 8a and b. Chronic hepatitis B, stage 2/3. **a** Surface particles (*arrow*): threadlike structures in the hyperplastic and dilated smooth endoplasmic reticulum of a liver cell. EM, ×32,500. **b** Core particles: 23 nm noncoated spheres (*arrow*) in the nucleus of a liver cell. EM, ×162,500

number of positive hepatocytes for each HBsAg or HBcAg diminished during subsequent stages; during the UR stages three and four one or two positive hepatocytes in two or more sections of a biopsy were listed as positive in Table 1.

Diffuse cytoplasmic and/or membrane related HBsAg was only found in scattered hepatocytes in the CH biopsies from stage 2/3 (Figs. 6, 7). Large groups of hepatocytes with diffuse cytoplasmic HBsAg were not present in any of the biopsies. The percentage of HBcAg containing liver cell nuclei in

the positive CH biopsies from stage 2/3 varied from 5% to approximately 40% (Figs. 6b, 7d).

Electron Microscopy

In both cases studied, intracisternal filaments in a proliferated and hyperplastic endoplasmic reticulum and rather numerous non-coated cores in nuclei were found in some hepatocytes (Fig. 8).

Discussion

The material presented here mainly differs from that in comparable studies (Bianchi et al. 1971, 1977; Dietrichson et al. 1975) by the presence of biopsies from all stages of AHB in a prospective set-up, with special emphasis on the pathology of uncomplicated AHB at all stages of the disease. Although the boundaries between subsequent stages were selected clinically, the stages appeared to coincide well with the respective changes in morphological pattern. To discern these patterns the stages based on SGPT levels were preferred, as in time dependent staging the variability in duration of AHB makes a choice of stage boundaries imprecise in individual cases.

From the results in the UR group generalisations about the course of liver changes during self limited AHB may be made. At first, acidophilic bodies and liver cell mitoses are prominent, scattered hepatocytes contain some HBsAg or HBcAg, and inflammation is scarce. There follows a full-blown acute hepatitis with an increase in inflammatory infiltrate, liver cell polymorphism and hepatocytolysis. Apart from the landmarks of full-blown acute hepatitis, this stage of highest transaminase levels is characterized by lymphocytic spill over from the portal tracts and PMN in the limiting plates; BHN may be present too. Subsequently, these features disappear and acidophilic bodies, mitotic figures and viral antigens become relatively less prominent. Next, the inflammation and degeneration gradually subside with disappearance of the viral antigens, finally leaving only residual changes. This course of events shows that liver cell regression and regeneration occur concomitantly and not after each other; their interrelation underlines the importance of an unimpaired regenerative capacity to obtain complete restitution of the parenchymal architecture, as has been pointed out by Peters (1975).

The presence of PMN and/or BHN has generally been associated with a high risk of transition to chronicity, although their presence in some cases of uncomplicated recovery has been observed incidentally (Scheuer 1977). In particular, in BHN during acute hepatitis recent studies suggest that the prognosis is much better than formerly believed and a relatively unimpaired recovery is not at all rare in these patients (Spitz et al. 1978; Theodor and Niv 1978). The main result of this study is the finding that in patients with an uncomplicated acute hepatitis B and subsequent complete recovery, BHN and PMN are frequently occurring features. Their transitory presence during the second stage

appears to contribute to the irregular portal tract enlargement that is frequently observed in the later stage of acute hepatitis. Taking the absence of improvement for at least six months after the onset of hepatitis as a defining criterion of chronicity (IASL 1976), indications in a liver biopsy of possible progression to chronicity should preferably be present before this time. The relevant biopsies in the CH group were taken separately (table 2) and compared with those from the first three stages of the UR group, i.e., those from cases that proved to have self limited disease in follow-up. Portal spill over of lymphocytes, PMN and BHN were not more frequently present in the former group, and according to these variables both the CH and UR group appeared to be samples from the same population. Hence, the presence of these features in a liver biopsy from patients with AHB can not be interpreted as a landmark of chronicity in individual cases, nor as a statistical risk factor for the CH group as a whole.

Literature data about the number of biopsies and the number of hepatocytes per biopsy with demonstrable viral antigens in AHB vary considerably (Gudat et al. 1975; Houthoff et al. 1975; Ray et al. 1976b; Huang and Neurath 1979). Apart from the sample size and the number of sections used, this variation may depend on the stage of AHB at the time of biopsy. In the UR group with self limited disease, the rapidly decreasing number of hepatocytes containing focal cytoplasmic HBsAg or nuclear HBcAg after the second stage, and their "spotty" distribution throughout the liver parenchyma, is in agreement with literature data (Gudat et al. 1975; Hoofnagle et al. 1978). Orcein-positive groundglass hepatocytes, filled with cytoplasmic HBsAg, were not observed in any of the biopsies from the UR group. However, they were present in the CH group (six patients), even before six months duration (three cases) and once in a first liver biopsy at 14 weeks duration. Thus, groundglass cells became more prominent with longer duration of the AHB in this group. This is in accordance with literature data about the association between groundglass cells and chronic hepatitis B.

In taking these data together, a basic difference in histopathology between uncomplicated AHB and cases eventually becoming examples of chronic liver disease is not exemplified by the presence of any histopathologic feature per se, but by its transitory presence in the early stages, and hence its disappearance in due course, during self limited disease. PMN, BHN and the number of hepatocytes with viral antigens decrease rapidly after the second stage of self limiting disease and were virtually absent after the 16th week of SGPT elevation in the UR group. On the other hand these features remained, appeared or became more prominent with time in the biopsies from patients eventually developing chronic liver disease (CH group). Thus, the prognostic significance of these features as risk factors for progression to chronic disease relates to the duration of SGPT elevation and may very well be meaningful at the time liver biopsies are generally considered diagnostically useful, i.e., after three months duration of AHB. The transitory presence of these histopathological features is comparable to the transitory occurrence of various serological findings during the early stages of self limited AHB, as for instance HBeAg, viral DNA polymerase and anti-HBc of the IgM class (Niermeijer et al. 1978a, b; Froessner et al. 1978). For both these histopathological and serological variables, their

persistence over longer periods makes them markers of chronic hepatitis B. The persistence of the early histopathological changes of self limited AHB during chronic disease may be interpreted as the incomplete host response to the presence of viral particles. The continuation of a stage two picture with piecemeal necrosis leads to chronic active hepatitis with incomplete viral elimination. The continuation of a stage one picture with acidophilic bodies and nuclear HBcAg without prominent inflammation may be present in patients with chronic hepatitis B viral infection during immunosuppressive therapy (Yamada et al. 1978); immunosuppression from the onset of AHB onwards thus appears to prevent the proper attack of viral particles and hence the progression of disease to a full-blown acute hepatitis with viral elimination.

In conclusion, from our results it may be postulated that in AHB the lack of progression to subsequent stages of disease with the persistence of histopathological features of earlier stages indicates a risk of progression to chronicity. The appearance of groundglass hepatocytes (checked by orcein staining or HBsAg localization) can further substantiate this progression to chronicity. Without reference to the duration of AHB the finding of PMN, BHN or quite a number of hepatocytes with viral antigens may be a feature of self limited disease and has in itself no prognostic value as risk factor for chronic liver disease.

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