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Morphometric Investigations on the Portal Tracts of the Liver, the Differentiation of Variable Progression in Chronic Persistent Hepatitis

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Summary. Morphometric investigations were carried out on the portal tracts of the liver in different forms of chronic hepatitis. The investigation groups each contained 25 liver biopsies, which were subdivided into cases with normal liver, a subsiding acute virus hepatitis, three different forms of chronic persistent hepatitis (CPH) and chronic aggressive hapatitis type IIa (CAH IIa). Determinations of the volume and surface of the portal tracts and their components enabled three forms of CPH (type Ia, Ib, Ic) to be characterised. Preliminary clinical and semiquantitative histological investigations were correlated with a significant difference in the histological characteristics and prognosis. HB_sAg-positive and HB_sAg-negative cases showed no significant morphologically detectable differences in all groups investigated. Morphometry is suitable for investigation of pathological changes in liver tissue, especially the portal tracts.

Key words: Chronic persistent hepatitis – Variable progression – Portal tracts – Morphometric analysis

Introduction

Although acute virus hepatitis is a diffuse disease of the hepatic parenchyma (Altmann 1969), alterations in the area of the portal tracts are most prominent in chronic hepatitis. The distinction of chronic persistent hepatitis (CHP) from chronic aggressive hepatitis (CAH), which is important for prognosis and therapy, is essentially based on alterations in form, size, cell content, connective tissue content and other features of the portal tracts, together with the interactions and structural changes of the contiguous liver parenchyma due to these alterations.

The classification of chronic hepatitis into CPH and CAH undertaken by de Groote et al. ("European Association for the Study of Liver" 1968) has been widely applied. According to the definition by de Groote et al. (1968)

* Dedicated with reference to Prof. Dr. C. Froboese to his 90th birthday

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CPH is not an entity comparable to the various progress forms of CAH. The definition formulated in America by the "International Association for the Study of Liver" (Fogarty International Center Proceedings 1976) describes certain form variants for CPH. This is also emphasized in the most recent publication of Bianchi et al. (EASL 1977) after re-examination of the criteria drawn up in 1968. However, the two classifications do not include the manifestations of borderline forms of acute and chronic hepatitis. The problem of assigning these borderline forms to categories is manifested in a bewildering diversity of terms ranging from acute persistent to protracted subacute, subchronic, non-healing and residual progression forms and progression forms in which there was suspicion of chronicity.

The typing drawn up by Bianchi et al. (1978) concerns acute and chronic forms of virus hepatitis B only and is hence not representative for all forms of virus hepatitis.

Our study group (Lüders et al. 1978; Volmer et al. 1979) has been able to demonstrate the presence of different progression forms of persistent hepatitis. This was achieved by systematic morphological investigations with semiquantitative analysis of 96 patients with HB_sAg-positive and HB_sAg-negative persistent hepatitis. The observation period was at least one year with an average of three to a maximum of 12 years; at least two and average of three, and in occasional cases up to five biopsies were made in the cases examined. These investigations led to the definition of three clearly demarcated histological types with different prognosis.

Chronic Persistent Hepatitis

Type Ia. A mainly *lobular*, subsiding virus hepatitis.
Type Ib. A mainly *portal* chronic inflammatory cell infiltration with sharply delimited portal tracts without fibrosis and without or with only slight intralobular inflammation.
Type Ic. Portal-septal fibrosis with mononuclear-cell infiltrates and a patchy periportal inflammatory infiltrate as well as moderate intralobular parenchymal reaction.

Type Ic can pass into a chronic aggressive hepatitis; this course is highly improbable for types Ia and Ib. In agreement with Vido et al. (1975), Thaler et al. (1978) and Poulsen (personal communication), the frequency of such transitions in our investigation material was about 10%.

Checking of our results by morphometric methods was therefore indicated. As far as we know, morphometric investigations had not been carried out so far on portal tracts of human liver.

Material and Methods

1. Material

The investigation was carried out on 175 liver biopsies from the years 1969 to 1977, which had been taken with a Menghini needle of 1.8 mm diameter under pinpointed laparoscopic control. Each biopsy had a minimum length of 1.5 cm^1.

1 The biopsies derive exclusively from the investigation material of the Föhrenkamp Clinic of the Bundesanstalt für Arbeit in Mölln. We are grateful to the head physician of the hospital, Priv.-Doz. Dr. H. Henning, for providing the clinical data

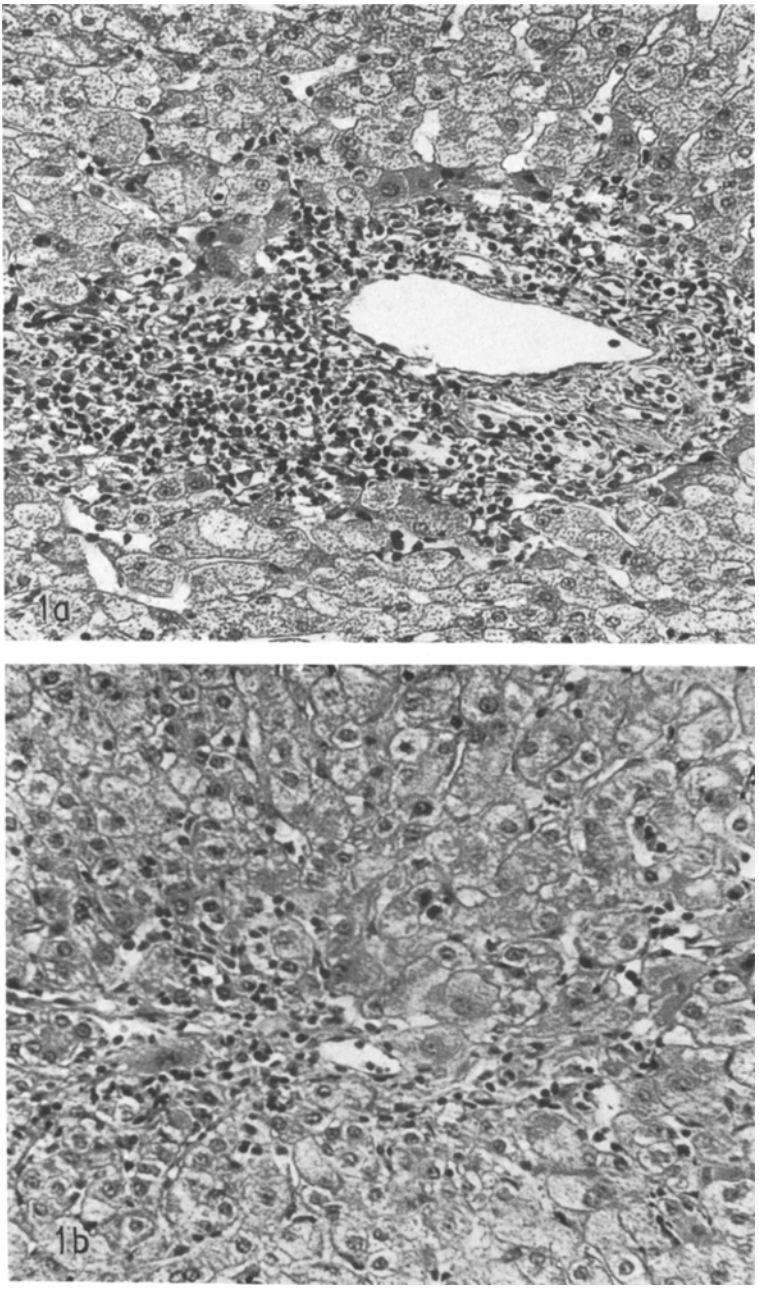

Fig. 1a, b. Chronic persistent hepatitis, type Ia (CPH Ia). Indistinctly limited portal tract with mononuclear inflammatory infiltration, spilling over. HE, ×160 (**a**). Liver parenchyma with proliferated sinusoidal cells and some hepatocellular necroses. HE, ×160 (**b**)

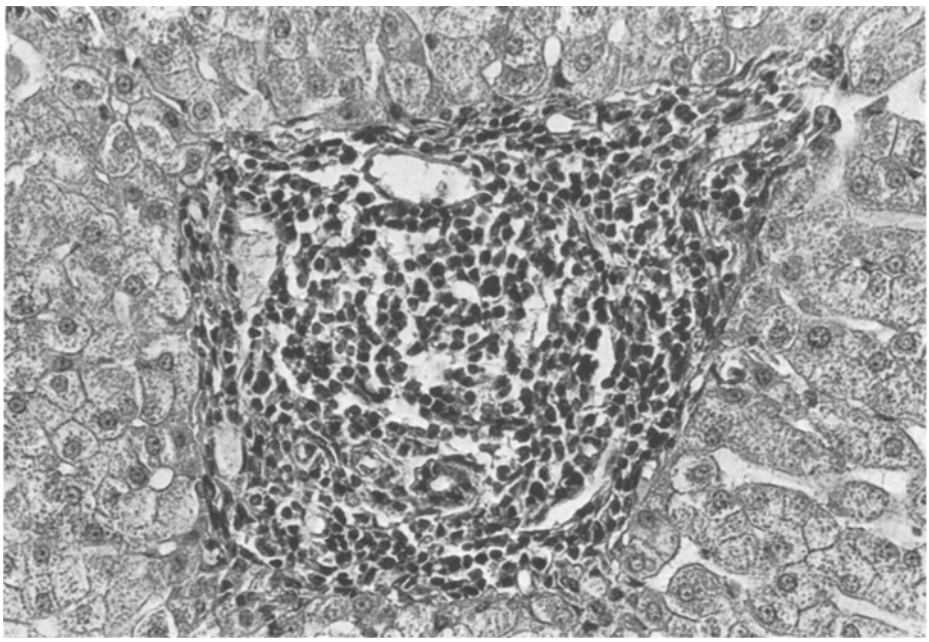

Fig. 2. Chronic persistent hepatitis, type Ib (CPH Ib). Rounded and sharply limited portal field, dense uniform lymphocytic infiltration. No portal fibrosis, no intralobular activity of macrophages. HE, ×400

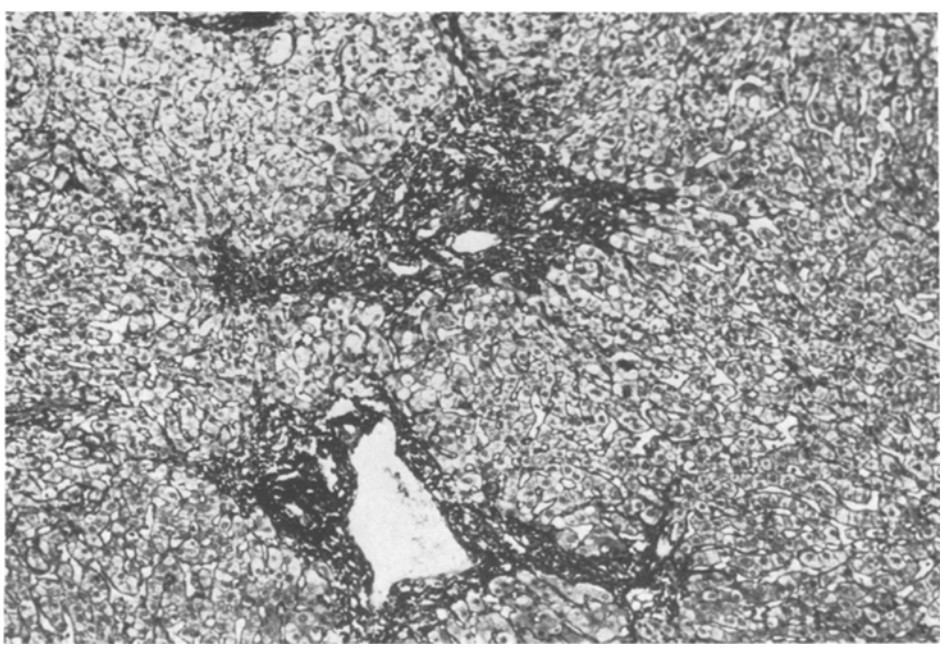

Fig. 3. Chronic persistent hepatitis, type Ic (CPH Ic). Enlarged portal tracts with fibrosis and indented surface, almost no intralobular mesenchymal activity. Gomöri, ×63

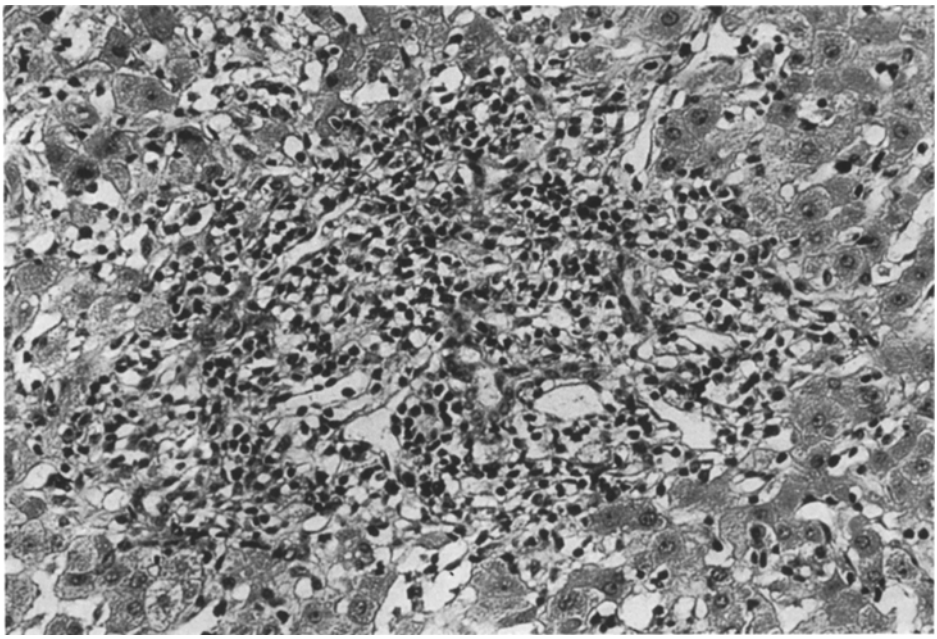

Fig. 4. Chronic aggressive hepatitis, type IIa (CAH IIa). Small portal tract with dense mononuclear infiltration, destruction of the limiting plate. Periportal inflammation, some piecemeal necroses. HE, ×160

They were fixed in 5% formalin, embedded in paraffin and stained in 4–5 μ thick sections with HE, Azan or Mallory stain, Turnbull reaction to iron and if necessary with silver staining according to Gomori and with aldehyde thionine.

HB_sAg and antiHB_s were demonstrated by radioimmunoassay, and HB_eAg and antiHB_e were determined by means of immunodiffusion according to Ouchterlony.

The patients were subdivided into seven groups each containing 25 liver biopsies:

A. normal liver (NL).
B. subsiding active hepatitis (AAH).
C. chronic persistent hepatitis, type Ia (CPH Ia).
D. chronic persistent hepatitis, type Ib (CPH Ib).
E. chronic persistent hepatitis, type Ic (CPH Ic).
F. chronic persistent hepatitis, type Ic, with transition in chronic aggressive hepatitis (CPH Ic→CAH).
G. chronic aggressive (active) hepatitis, type IIa (CAH IIa).

2. Methods

a) Histology. Histological evaluation of the liver biopsies was performed independently by two investigators according to the histological criteria of the international nomenclature (de Groote et al. 1968, Bianchi et al. 1977; IASL 1976). The subdivision of CPH into three forms with different progress (type Ia, Ib and Ic) is based on a development of the definition of de Groote et al. (1968), Bianchi et al. (1977) and the IASL (1976) on the basis of the investigation by Lüders and Volmer (1978 and 1979).

The histological investigations were supported by continuous monitoring of blood chemistry and by surveys of the clinical history.

"Non-specific reactive hepatitis" could be excluded in all groups by differential diagnosis (Becker 1974; Seifert et al. 1975), as could the presence of liver damage due to drug poisoning, which can simulate acute or chronic virus hepatitis (Popper and Greim 1973; Lüders et al. 1975).

Table 1. Clinical data of the groups

	Number of biopsies (patients)	Average age	Sex		HB_sAg +	Beginning of disease		
			f.	m.		acute	chronic	unknown
NL	25 (25)	43	12	13	–	–	–	–
AAH	25 (20)	42	13	12	8	17	–	3
CPH Ia	25 (20)	48	13	12	7	12	6	2
CPH Ib	25 (19)	43	8	11	1	11	8	–
CPH Ic	25 (18)	42	8	10	9	10	8	–
CPH Ic→CAH	25 (9)	36	1	8	12	5	4	–
CAH IIa	25 (25)	47	9	16	12	11	6	8

The HB_sAg-positive cases were specially grouped together and compared with the others. Subdivision into hepatitis A and hepatitis non-A, non-B was not possible.

b) Histometry. For quantitative measurements of *volumes* and *surfaces*, we chose the point counting procedure (Glagoleff 1933). By means of a projecting microscope, a projection image of the histological sections of the liver biopsies was shown and covered with a grid plate with a square line raster (calibrated grid distance 5 mm, test line length 5,120 mm, registration with the counting and storage instrument MOP KM II). The diagram (Fig. 5) illustrates the single components of the histometric model.

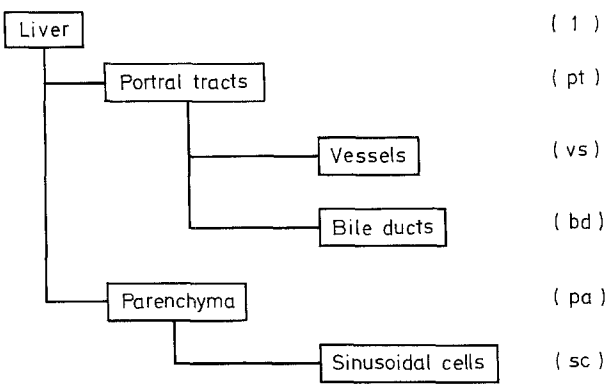

Fig. 5. Histomorphometric diagram

To determine the *volume density* (volume proportion) of the structures in the liver, we used the equation:

$$V_{Vi} = \frac{P_i \times 100}{P_T} (\%)$$

(V_{Vi} = volume density, P_i = number of score points of the structure, P_T = number of test points of the grid plate).

The *volume density* of the following structural components were determined:

1. portal tracts per liver tissue $V_{V(pt/l)}$
2. vessels per portal tracts $V_{V(vs/pt)}$
3. bile ducts per portal tracts $V_{V(bd/pt)}$
4. sinusoidal cells per liver parenchyma $V_{V(sc/pa)}$

Because the size of the portal tracts sometimes varied appreciably between the test groups (e.g., small volumes with NL, large volumes with CAH), the volume density of the portal tracts of the groups CPH Ic→CAH and CAH IIa were measured optionally at a magnification of ×25, all other groups were measured using an enlargement of × 40. Four measurement areas were measured per biopsy. Each measurement area contained 4–5 portal tracts on average (about 16–20 portal tracts per biopsy, about 400–500 portal tracts per group). Since the liver did not

always completely fill the optical picture at the given magnifications, the number of points covering the liver tissue was measured as the number of test points (P_T) and related to the number of score points of the portal tracts (P_i).

The volume proportion of the vessels and the bile ducts of the portal tracts was determined on four portal fields of each biopsy at a magnification of $\times 160$, and that of the sinusoidal cells on four measurement areas of the liver parenchyma of each biopsy at a magnification of $\times 1{,}000$.

To determine the surfaces of the portal tracts and the portal bile ducts we measured the *surface density*. The surface density S_{Vi} of a structural component is defined as the surface of this component per unit volume of the sample (Weibel and Elias 1967). The formula of Tomkieeff (1945), Smith and Guttmann (1953), Henning (1956) and others yielded

$$S_{Vi} = f \times \frac{I_i}{L_T} \left(\frac{mm^2}{mm^3} = mm^{-1} \right)$$

(I_i = number of penetration points on the surface contours of the component, L_T = length of test lines of the grid plate, f = factor dependent on the structural component).

The size of factor f is dependent on the object. Sitte (1967) defined different object classes according to the arrangement of the components inside the object and their measurements. The liver is classified as a symmetrical unlimited object. The portal tracts arborize throughout the liver and into the periphery (Bolck and Machnik 1978). Because they have no general direction and their boundary surfaces do not run parallel to an axis, they can be classified as limited symmetrical objects. The factor for this object is $f = 2.0$.

To determine the surface density four portal tracts of every biopsy (100 per group) were measured. In order to improve the local measurement of these delimited objects statistically and to avoid a methodological error which might have resulted from randomly oriented arrangement of the portal tracts, measurements were performed with three different azimuths at each measurement point: 1. with a vertical measurement scale; 2. with a measurement scale set 19° from vertical; 3. with a horizontal measurement scale.

The mean of the three measurements was calculated and used as measurement value.

The following measurements were determined:

5. surface density of the portal tracts per volume of the liver tissue $S_{V(pt/l)}$
6. surface density of the portal bile ducts per volume of the portal tracts $S_{V(bd/pt)}$

The surface density of the portal tracts were determined on four portal tracts of each biopsy (100 per group) at a magnification of $\times 160$, and that of the bile ducts on four portal tracts of each biopsy (100 per group) with a magnification of $\times 400$.

c) Statistical Methods. The cases of our investigation groups corresponded to random samples from a basic collective. Four measurement areas were measured per biopsy, so that 100 determinations were available for all variables of every investigation group. The mean value per biopsy was calculated from the measured single values of each histological variable and from these mean values (25 biopsies per group), the median value M was calculated for each test group. The median M was tested for standard error s.

The values determined for the individual groups were tested for significant differences with the U-test according to Wilcoxon, Mann and Whitney (Sachs 1978). The differences of the median values, for which the probability was two-sided $\leqq 5\%$ ($2\alpha \leqq 0.05$), was found to be significant.

d) Criticism of the Method. A 1 cm long liver biopsy of 1.8 mm diameter obtained with a Menghini needle had a volume of 25.4 mm^3. Beneke (1878) found a liver volume averaging 1.581 cm^3 for 20 to 50 year old men. A liver biopsy with the specified dimension then corresponds roughly to 1/63,000 of the entire liver tissue. Despite this infinitesimally small proportion a liver biopsy contains a representative amount of liver tissue and provides good results (Menghini et al. 1956; Federlin and Sandritter 1961), but with constraints on the evaluation of processes which are not evenly distributed over the liver, e.g., ascending cholangitis or hepatic metastases (Wagoner et al. 1950).

Morphometric measurement of the extralobular tissue is not without its problems. In diseases with severe portal and periportal connective tissue development, the proportion of connective tissue in the biopsy is mostly under-represented because of its consistency: when biopsies are taken, it is more difficult to deteach from this tissue than the softer liver parenchyma. This disadvantage could only be partly compensated for, firstly by investigating only biopsies with many complete

or almost complete portal tracts and secondly, by studying cases without advanced stages of parenchymal transformation by septal fibrosis. Thirdly, every test group contained 25 liver biopsies, so that individual differences in the degree of the inflammatory activity could be taken into account.

The preparations for morphometric investigations were stained with Azan or Mallory stains, these most sharply contrasted the contours of the enlarged portal tracts. This had the disadvantage that piecemeal necrosis without fibrosis could not always be identified. The cellular infiltrates appeared much more clearly with HE stain, however the outer boundary of the portal tracts is unevenly blurred and the measurements were not as exact.

The biopsies were 1.8 mm in diameter and had an average length of 2.4 cm (1.5–3.3 cm). 16–20 portal tracts (400–500 per test group) were measured per biopsy in the calculation of the volume density of the portal tracts. 4 portal fields per biopsy (100 per test group) were measured for all the remaining histological variables. The volume density of the portal tracts varied from test group to test group and it was desirable to have as many portal tracts as possible in the field of observation. Finally, the points of the test grid had to fall on the components with the same probability. For these reasons the volume density of the portal tracts of the groups CPH Ic→CAH and CAH IIa were measured using a 25× enlargement, all other groups were measured at 40× magnification. This caused 1 to a maximum of 5 score points of the grid to fall on each portal tract. The calculation of the volume density $V_{V(pt/l)}$ was obtained in this fashion for each test group (e.g., for CPH Ia 1284, for CPH Ic 1734 and for CAH IIa a total of 1715 score points were obtained).

The standard measurement error of our countings was calculated by repeated measurement of 20 randomized preparations. This gave an average value of 4.40% (0 to 11.10%). The measurements of the vessels and bile ducts in the portal tracts of CPH Ic→CAH and CAH IIa showed the greatest deviations as these structures are often covered or unevenly compressed by dense cellular infiltrates.

The progression of the morphometric analysis was controlled by continual checking of all measurements. This was accomplished by taking the mean of the previous measurements (including the value being tested) per liver biopsy over the number of the measurement areas (Meßanleitung, C. Zeiss). This value approaches the arithmetic mean or median with a closer approximation the more measurement areas are included.

It was found that after investigating 10–12 liver biopsies (40–48 measurement areas) that a value for the volume density $V_{V(pt/l)}$ of the portal tracts would be arrived at which was not very different from the median value for all 25 liver biopsies of a group (100 measurement areas).

A meaningful morphometric investigation requires a uniform test collective with around 10–12 liver biopsies per group. A morphometric analysis of single or too few biopsies is pointless.

Due to the rarity of this illness, the 25 liver biopsies of the group CPH Ic→CAH were taken from only 9 patients. The danger of giving false weight to these findings was minimal, because, according to the control curve, 10–12 biopsies per group will produce a meaningful result.

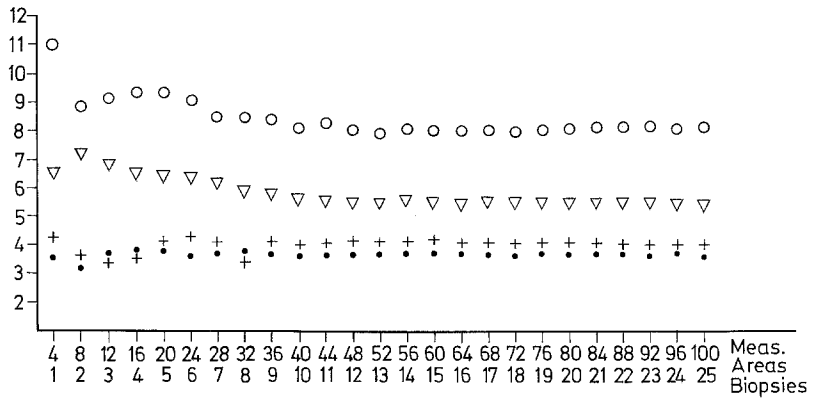

Fig. 6. Control of measured values. Volume density of the portal tracts $V_{V(pt/l)}$ (e.g.: NL●, CPH Ia+, CPH Ic▽, CAH IIa ○)

Results

The Portal Tracts

The median for the volume density (volume percent) of the portal tracts per liver tissue $V_{V(pt/l)}$ increased from 3.80% in the normal liver to 7.90% in chronic aggressive hepatitis type IIa. The values for the groups "subsiding acute hepatitis" (AAH) and "chronic persistent hepatitis" type Ia (CPH Ia) did not show a statistical difference ($V_{V(pt/l)}=4.10\%$ and 4.20%). If one compares the different progress forms of CPH, an increase is found from CPH Ia (4.20%) via CPH Ib (5.00%) to CPH Ic (5.90%). The differences between CPH Ia and CPH Ib are significant ($2\alpha \leqq 0.02$), that between CPH Ib and CPH Ic is not and that between CPH Ia and CPH Ic is highly significant ($2\alpha \leqq 0.01$).

The volume density $V_{V(pt/l)}$ of all CPH groups, even type Ic, clearly differs statistically ($2\alpha \leqq 0.01$) from the value for group CAH IIa ($V_{V(pt/l)}=7.90\%$). The median value ($V_{V(pt/l)}=7.18\%$) for the group "chronic persistent hepatitis with transition into chronic aggressive hepatitis" (CPH Ic→CAH) is between that of CPH Ic and CAH IIa (as expected) and differs to a highly significant extent ($2\alpha \leqq 0.01$) from the types Ia, Ib and Ic of the CPH, but *not* from CAH IIa.

If one subdivides the 25 liver biopsies of the group CPH Ic→CAH in the early stage ($n=12$) and later control biopsies ($n=13$), there is a volume density of the portal tracts of 6.10% for the initial phase $V_{V(pt/l)}$, and 7.85% for the late phase $V_{V(pt/l)}$. These values are significantly different ($2\alpha \leqq 0.01$)

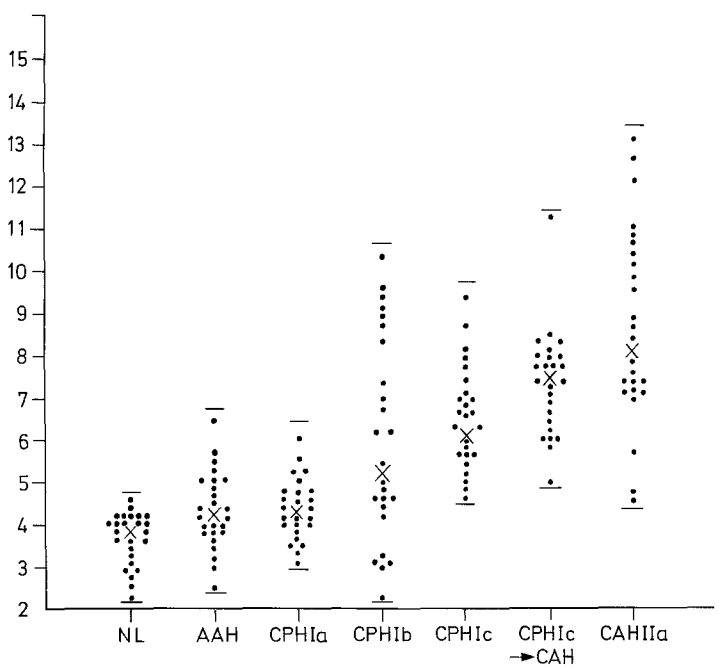

Fig. 7. Volume density of portal tracts $V_{V(pt/l)}$ (Median of biopsy●, Median of total group ×)

from each other and $V_{V(pt/l)}$ of the early stage corresponds to the same measurement figure for the group CPH Ic with $V_{V(pt/l)}=5.90\%$. $V_{V(pt/l)}$ of the late stage is of the same order as the corresponding value for the CAH IIa with 7.90%.

The surface density $S_{V(pt/l)}$ confirms and complements the significant differences between the individual investigation groups found in the volume measurement. *In addition*, it permits a separation between CPH Ib and CPH Ic, the portal tracts of which differed non-significantly in area size. The surface density of the portal tracts in CPH Ic is significantly larger ($2\alpha \leqq 0.02$) however, than the same characteristic for CPH Ib.

The alterations in the portal tracts in AAH and CPH Ia, of which the volume density $V_{V(pt/l)}$ was the same, revealed differences in the surface measurement. The surface density $S_{V(pt/l)}$ was significantly larger ($2\alpha \leqq 0.02$) in CPH Ia with 1.60 than in AAH with $S_{V(pt/l)}=1.42$. The slight fibrosis discernible histologically and due to the persistent inflammatory process, together with the indistinct outer demarcation of the portal tracts in CPH Ia led to a marked elongation of the surface contours compared to AAH.

The value of the group CPH Ic→CAH for the surface determination is between those of CPH Ic and CAH IIa; on average, it is significantly larger than those of the three progress forms of CPH, but does not differ from that of CAH IIa.

For the early stage of CPH Ic→CAH, the same applies for the surface as for the volume of the portal tracts: $S_{V(pt/l)}=2.29$ corresponds to the value

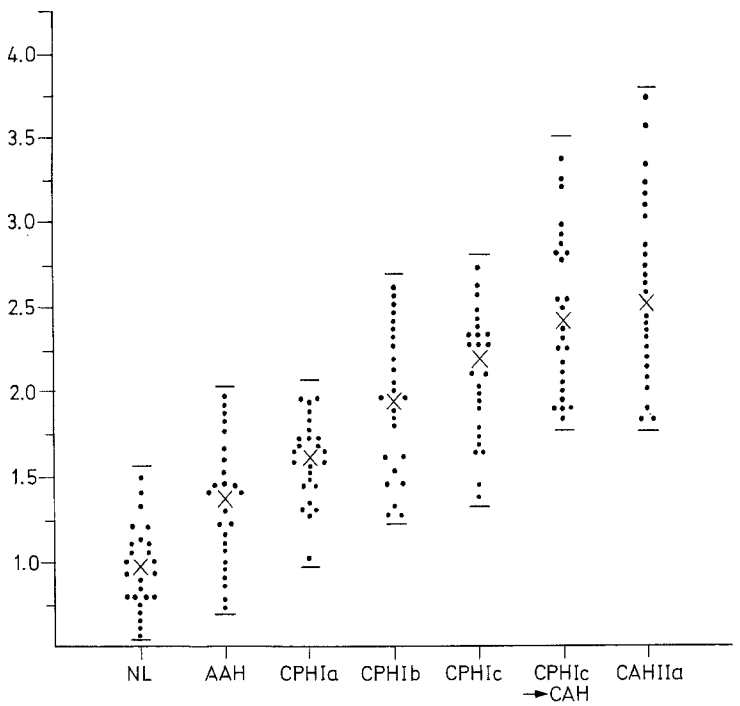

Fig. 8. Surface density of portal tracts $S_{V(pt/l)}$ (Median of biopsy●, Median of total group ×)

of CPH Ic ($S_{V(pt/l)}=2.20$); the surface density of the cases which represent a late stage of CPH Ic→CAH ($S_{V(pt/l)}=2.62$) is in the region of CAH IIa ($S_{V(pt/l)}=2.52$).

The Portal Vessels

The average proportion of vessels in the portal tract $V_{V(vs/pt)}$ in normal liver is 28%. It is thus markedly higher ($2\alpha \leqq 0.01$) than in all other groups. With increase of the volume proportion of the portal tracts in liver tissue in the various inflammatory clinical pictures, the space taken up by the vessels decreases and reaches its lowest value in CAH IIa with 8.30%.

The Portal Bile Ducts

The volume density of the portal bile ducts $V_{V(bd/pt)}$ in relation to the portal tracts of healthy livers averages 7.60%. It is thus significantly higher than in other groups. A definite distinction of the variously progressive forms of CPH is not possible on the basis of $V_{V(bd/pt)}$. The median values are between 3.60% (CPH Ib) and 4.70% (CPH Ia), and 4.00% in CPH Ic. The differences are not statistically significant.

If one relates the volume density of the bile ducts to the entire liver tissue, median values varying between 0.20% and 0.28% result for the groups AAH to CAH IIa. These thus do not display any appreciable differences and are in the region of the percentage of 0.28% determined for normal liver.

The Sinusoidal Cells

In the normal liver, the volume density of the sinusoidal cells averages $V_{V(sc/pa)}=0.45\%$. AAH ($V_{V(sc/pa)}=1.85\%$) and CPH Ia ($V_{V(sc/pa)}=1.85\%$) show the highest value compared to all other groups. The mesenchymal activity in CPH Ia is greater than in the group CPH Ib ($2\alpha \leqq 0.02$). The cases of the group CPH Ic→CAH show a smaller value compared to the three forms of CPH ($V_{V(sc/pa)}=1.40\%$), but can be distinguished statistically only from CPH Ia and CPH Ic; there is no arithmetically detectable difference from $V_{V(sc/pa)}$ of CAH IIa.

Comparison Between HB_sAg-Positive and HB_sAg-Negative Cases of the Same Groups

Differences in the morphological characteristics between HB_sAg-positive and HB_sAg-negative cases were also tested for their significance only in the groups CPH Ic→CAH and CAH IIa, since these groups contained a sufficiently large number of liver biopsies with HB_sAg-positive patients.

On average, the volume density of the portal tracts $V_{V(pt/l)}$ was greater in the HB_sAg-negative cases of all groups than in the HB_sAg-positive cases. An exception was found in the group CPH Ic→CAH, where the converse was found. No difference was statistically significant.

There were no significant differences between the surface density of the portal tracts $S_{V(pt/l)}$ in HB_sAg positive and HB_sAg-negative cases. There was a similar situation for the volumes and surfaces of the portal vessels and bile ducts, and for the sinusoidal cells in all groups.

Table 2. Medians of histological indices (all groups)

Indices		NL	AAH			CPH Ia			CPH Ib	CPH Ic			CPH Ic→CAH			CAH IIa		
		G	+	−	G	+	−	G	G	+	−	G	+	−	G	+	−	G
$V_{V(pt/l)}$ (%)	M	3.8	3.9	4.4	4.1	4.1	4.2	4.2	5.2	5.7	6.2	5.9	7.4	7.0	7.2	7.6	7.9	7.9
	s	±0.08			±0.13			±0.13	±0.75			±0.25			±0.23			±0.3
$V_{V(vs/pt)}$ (%)	M	28.0	20.5	14.2	16.7	14.7	15.0	15.5	11.8	10.6	12.2	11.8	9.9	8.7	8.9	8.5	6.8	8.3
	s	±0.50			±1.55			±0.85	±1.45			±0.85			±0.25			±0.9
$V_{V(bd/pt)}$ (%)	M	7.6	5.4	5.3	5.3	5.0	3.7	4.7	3.6	4.0	3.9	4.0	3.1	3.3	3.3	3.5	3.4	3.5
	s	±0.33			±0.28			±0.43	±0.30			±0.23			±0.25			±0.2
$V_{V(sc/pa)}$ (%)	M	0.45	2.0	1.7	1.85	1.6	1.8	1.85	1.45	1.8	1.6	1.70	1.4	1.4	1.40	1.1	1.5	1.2
	s	±0.00			±0.15			±0.05	±0.10			±0.10			±0.10			±0.1
$S_{V(pt/l)}$ (mm^{-1})	M	0.90	1.4	1.3	1.42	1.7	1.6	1.60	1.89	2.3	2.2	2.19	2.4	2.4	2.40	2.3	2.8	2.5
	s	±0.06			±0.10			±0.05	±0.09			±0.05			±0.12			±0.2
$S_{V(bd/pt)}$ (mm^{-1})	M	0.66	0.9	0.8	0.86	0.9	0.9	0.89	0.87	0.8	0.8	0.80	0.8	0.8	0.80	0.9	0.8	0.8
	s	±0.03			±0.02			±0.05	±0.05			±0.04			±0.07			±0.1

+ = HB_sAg positive cases, − = HB_sAg negative cases, G = Total group

Early stage:

$V_{V(pt/l)}$: 6.10

$S_{V(pt/l)}$: 2.29

Late stage:

$V_{V(pt/l)}$: 7.85

$V_{V(pt/l)}$: 2.62

Discussion

The values for the normal liver tissue are for comparison and have not been previously determined in human liver. Pfuhl (1932) calculated a proportion of "interlobular space" in the total liver tissue of 4.60% for adults. However, his results are based on determinations in a *single* liver, so that a comparison with the value of $V_{V(pt/l)}=3.80\%$ for the volume density of the portal tracts which we determined in 25 liver biopsies is not directly admissible.

The determination of the proportions of volume and surface of the portal tracts confirm the presence of three differently progressing forms of CPH which show significant differences from each other. All three forms of CPH, especially CPH Ic, shows *significant* differences from CAH IIa and thus can be clearly delimited from it.

In preliminary investigations (Lüders et al. 1978; Volmer et al. 1979), we characterized *CPH Ia* as a form of CPH "with the histological appearance of a lobularly pronounced, subsiding virus hepatitis". This typing is confirmed in the morphometric analysis, which on average showed the same volumes of the portal tracts and an equal volume proportion of the sinusoidal cells for CPH Ia and AAH; only the surface of the portal tracts was larger in CPH Ia.

The groups AAH and CPH Ia are comprised of cases of a subsiding virus hepatitis, in various stages of regression with the precise *difference* that in CPH this process persisted for at least *one year* (up to 12 years). The type Ia of CPH corresponds to the "persistent viral hepatitis" of Ishak (1973) and to the "unresolved viral hepatitis" of Peters (1975), and most of the cases of persistent acute hepatitis described by Hübner (1978).

The morphological alterations of CPH Ia resemble the clinical picture of "chronic lobular hepatitis" (CLH) described by Popper and Schaffner (1971) in many cases. In contrast to Bianchi et al. (EASL 1977) but in agreement with the IASL (1976) we do *not* believe that chronic lobular hepatitis is an entity, according to the information so far available. Apart from the fact that CLH is a new, purely descriptive anatomical term with which (as shown by experience) hepatologists working clinically have difficulties, by definition CLH is a (chronic) persistent liver inflammation with the appearance of a lobular pronounced hepatitis and should be designated and classified accordingly (Schmid 1979). The "akut rezidivierte persistierende Hepatitis" described by Wildhirt (1978) is also likely to belong to this progression form of CPH (Korb 1979).

The histomorphological alterations of *CPH Ib* with prominent portal manifestation correspond to the elementary criteria of CPH laid down by de Groote et al. (EASL, 1968). CPH Ib is to be separated from CPH Ic on the basis of morphometry only by virtue of its different surface shape. The marked enlargement of the surface of the Glisson tracts in CPH Ic arises from the indented and irregular outline; this is a manifestation of the periportal activity with focal destruction of the limiting plate. CPH Ib displays rounded or triangular-shaped, sharply delimited portal tracts.

Classification of *CPH Ic* in the chronic persistent hepatitis category is not

simple. It is largely consistent in its appearance with the "chronic septal hepatitis" of Gerber and Vernace (1974). The authors interpret this form of liver inflammation as a "regressing form of chronic aggressive hepatitis"; however, the clinical picture is alleged to occur de novo without prior therapy with immunosuppressives.

The cases of CPH Ic mostly have a long course, extending over years, with fluctuations in the degree of inflammatory activity. In most cases, there is healing in the form of portal-septal fibrosis after a period of years. The assignment to CPH hence appears admissible and reasonable. The borderline situation of CPH in relation to CAH is determined by a labile equilibrium between inflammatory activity and local resistance, however, so that transition into chronic aggressive hepatitis can occur. This transition is documented morphologically in that the early stage of CPH Ic→CAH display measurement data which are in the range of values for CPH Ic, while those of the late stage corresponds to the values of CAH IIa. The signs of aggression occur irregularly, mostly after a longer course, but in some cases after short duration of the disease.

CPH Ia CPH Ib CPH Ic CPH Ic→CAH CAH IIa

borderline region.

The Portal Vessels

The significant differences between the variably progressive forms of CPH and when these are compared with CAH, revealed by measurement of volume density and surface density of the portal tracts, are reflected in the evaluation of the vascular portion of the portal fields $V_{V(vs/pt)}$. The significance of the differences is less pronouced than in the histological characteristics discussed above.

If one converts the volume proportion of the vessels per portal tract to the entire liver tissue, a median value between 0.61% and 0.69% results for all groups from AAH to CAH IIa. This comparison confirms that a substantial increase of vessel volume does not occur in chronic hepatitis. This correlates with the impression that the inflammatory process is determined by cellular infiltration and a proliferation of connective tissue gained from the histological investigations, whereas new growth of vessels (granulation tissue) does not play a role.

The Portal Bile Ducts

Determination of the volume density of the bile ducts $V_{V(bd/pt)}$ does not permit any distinction between the different progression forms of CPH. The relation of $V_{V(bd/pt)}$ to the entire liver tissue and the determination of the surface area of the bile ducts show that no morphometrically detectable increase in the bile duct structures has occurred for any of the groups investigated.

The calculation of the volume density of the bile ducts and the proliferated ductules has proved to be an important characteristic for differentiation between cases with chronic non suppurative destructive cholangitis (primary biliary cirrhosis) and chronic aggressive hepatitis (non-published investigations).

The Sinusoidal Cells

The number of sinusoidal cells is a measure of the mesenchymal activity of the hepatic parenchyma. The results involving the sinusoidal cells have to be interpreted with caution, since this structural component is very small.

The AAH and the CPH Ia showed the highest values for this component. The cases of CPH Ib displayed the lowest mesenchymal activity of all three progression forms of CPH. The cases of the group CPH Ic→CAH show a smaller value for $V_{V(sc/pa)}$ compared to the other forms of CPH; there is no difference from $V_{V(sc/pa)}$ of CAH IIa. The early stage of cases CPH Ic→CAH does not differ with regard to $V_{V(sc/pa)}$ when compared with the later stage of this disease.

Comparison Between HB_sAg-Positive and HB_sAg-Negative Cases of the Same Groups

The biopsies from HB_sAg-negative patients showed a slight or moderately increased inflammatory activity compared with the HB_sAg-positive patients. This result corresponds to the experience that the size of the portal fields and the degree of inflammatory activity in the region of the periportal zones does not permit any definite inferences to be drawn with regard to the presence of a HB_sAg-positive or HB_sAg-negative chronic hepatitis.

The focal HB_cAg type of chronic persistent hepatitis B, according to Bianchi et al. (1978) frequently showed transition into a chronic aggressive hepatitis. Our group of chronic persistent hepatitis with transition to a chronic aggressive hepatitis (CPH Ic→CAH) contains, like the other groups of CPH, HB_sAg-positive and HB_sAg-negative cases which did not display any significant morphometric difference from each other with regard to the degree of inflammatory activity. Since a determination of HB_cAg in the nucleus of the hepatocytes could not be performed, a comparative morphometric analysis and differentiation of the forms of hepatitis B postulated by Bianchi et al. (1978) was not possible in our material.

A serological subdivision of the HB_sAg-negative cases of our groups could not be performed. Assignment to hepatitis A or hepatitis non-A, non-B is not possible on a histological basis. It can be assumed that the HB_sAg-negative cases of our groups can probably be placed with the hepatitis non-A, non-B because there is no known transition of an acute virus hepatitis A into a chronic form in the literature (Rakela et al. 1978; Mathiesen et al. 1980). Hepatitis non-A, non-B frequently shows a relatively mild chronic course, corresponding to the cases of our type Ic of CPH (Rakela and Redeker 1979; Berman et al. 1979).

Conclusions

The present study is based on a histomorphometric analysis of 175 liver biopsies subdivided into seven histologically different groups. The object of the analysis was to evaluate the pathological changes in the Glisson tracts and their components systematically, by means of volume and surface determinations.

The results permit the following conclusions to be drawn:

1. The morphometric volume and surface determinations of the portal tracts confirm the presence of three differently progressive forms of CPH which show significant differences from each other. The types Ib and Ic of CPH can only be separated morphometrically on the basis of a different surface shape.

2. The three forms of CPH, especially type Ic, show significant differences from CAH IIa and can be clearly delimited from it. The cases of the group CPH Ic→CAH differ significantly from CPH Ic as a total group in terms of morphometry, but not from CAH. In the early stage of CPH Ic→CAH, the histological alterations on the portal tracts are largely identical with those of type Ic of CPH. A transition into a chronic aggressive hepatitis is highly improbable for the types Ia and Ib of CPH.

3. The cases of CPH Ic and CPH Ic→CAH comprised HB_sAg-positive and HB_sAg-negative cases, which did not show any statistical differences histomorphometrically in the degree of inflammatory activity. The transition of a CPH to a CAH appears to be independent of the virus type causing the liver inflammation.

4. Morphometry is a useful and readily applied method for investigation of pathological alterations of the liver tissue, especially in the region of the portal tracts. Its value is in comparison or in the analysis of case groups. It requires a certain number of biopsies (about 10–12, Volmer 1979), since the measurement results are approximate values, the accuracy of which must be checked statistically.

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