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Morphological Lesions Detected by Light and Electron Microscopies in Chronic Type C Hepatitis

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HCV infection results in chronic hepatitis in most patients. The mechanisms determining liver damage and the events that lead to a high rate of chronic hepatitis remain unclear. In present study, an attempt was made to sum up data on lesions in the liver in the course of chronic type C hepatitis including those of our own cases, because that pattern is still a matter of debate. Cell lesions detected by light microscopy are characteristic but not specific and included inflammatory lesions of low or moderate intensity and a mild extent of fibrosis in the liver. The common and most characteristic trait of chronic HCV infection involves lesions in hepatocyte nuclei. These changes involved swelling, altered shape, hyperchromasia, disturbed nuclear chromatin structure, enlarged and frequently multiple nucleoli and lesions of nuclear envelope. Complexes of tubules or branching fibrils of 20 - 30nm in diameter were present in cell nuclei at electron microscope level. The nuclear lesions were accompanied in the same cells by changes in rough endoplasmic reticulum with long tubular structures or branching fibrills inside. Other cytoplasmic changes included mitochondrial lesions, numerous lipid vacuoles and free tubular structure of a highly osmophilic character. Cellular localisation of HCV proteins using immunocytochemical techniques remains to be a matter of studies. In most studies HCV proteins have been detected in the cytoplasm although some reports indicate nuclear localisation, especially of C protein. All our observations on morphological lesions in chronic type C hepatitis can generally confirm most of data of other authors, but the criteria of nuclear lesions defined at the ultrastructural level represent the original input of our studies. The studies using molecular biology techniques should be continued at the electron microscope level.

Introduction

The hepatitis C virus (HCV) was described for the first time in 1989 by the team of Chiron Corporation [27]. It was classified as belonging to *Flaviviridae* family [35]. HCV represents the principal cause of hepatitis previously described as non-A non-B cases and described in the seventies

as post-transfusion hepatitis [2]. The proportion of hepatitis with chronic transformation following this infection reaches up to 85% of cases, independently of patient's age [2, 22, 59, 82]. Chronic type C hepatitis leads to liver cirrhosis in at least 20% of patients within 20 years which follow the infection [17]. In the course of chronic type C hepatitis, however, the necrotic-inflammatory lesions seem to result in stroma reconstruction and early cirrhotic lesions more rapidly, particularly in young adults [41]. The risk of hepatocellular carcinoma development amounts to 1 - 5% after 20 years of the chronic process and increases each year by 1 - 4%, if liver cirrhosis has already developed. In general, HCV infection is regarded to be a more important risk factor for hepatocellular carcinoma development than that associated with HBV infection [30, 59].

Principal pathogenic mechanisms of chronic HCV infection

Although hepatitis C virus affects mainly hepatocytes, the tropism is controversial, and the mechanisms of cell entry remain unknown. HCV can replicate and persist also in other cells of human body, i.a., in peripheral blood mononuclear cells [18, 39, 49] and pluripotent hematopoietic CD34+ cells [74]. Pileri et al. demonstrated that human CD81 is sufficient for binding not only the envelope protein (E2) but also HCV particles. Given the wide distribution of CD81, these results imply that HCV can bind to a variety of cells other than hepatocytes [71]. HCV can induce both acute and chronic lesions in hepatocytes. In contrast to HBV infection, HCV infection results in chronic hepatitis in most patients [2, 22]. The lesions may lead to the development of primary cancer of the liver [30, 84]. The mechanism of carcinogenesis by HCV is poorly understood. HCV is a typical RNA virus, and thus there is no integration of the viral genome or a piece of genome into host chromosomes. In the development of hepatocellular carcinoma following HCV infection direct

involvement of HCV core protein has been suggested [65], though other studies do not support the hypothesis [52].

Pathogenesis of persistent HCV infection is dominated by specific defects of cell-mediated immune responses [55]. At the acute stage of the infection, CD8 lymphocytes recognise epitopes of structural and non-structural HCV proteins, including mainly NS3, NS4 and, to a lesser extent, NS5, but lymphocytes count in peripheral blood is low, which indicates that the cells accumulate first of all in the liver [23]. The cytotoxic lymphocytes, which infiltrate the liver, recognise HCV antigens in the context of MHC class I antigens [55]. A preferential presentation of selected HCV peptides may take place, with disturbed presentation of the other peptides. As shown by experiments on chimpanzees, the HCV protein epitopes may, in addition, exhibit variability and thus will not always be effectively recognised by CD8 lymphocytes [87]. In the chronic type C hepatitis, the ineffective cytotoxic response of CD8 lymphocytes is accompanied by a disturbed equilibrium of Th1 and Th2 helper lymphocytes. In this case, the disturbance involves prevalence of Th2 lymphocyte activity in the peripheral blood and of Th1 cells in the tissue [10, 58]. Infiltrates of lymphoid cells in the liver portal spaces have been found to contain high levels of CD20, CD4, CD8, LFA, ICAM-1 and VCAM-1 molecules [6]. In the liver-infiltrating helper lymphocytes, Th1 subpopulation prevailed, capable of IFN- γ secretion but unable to release IL-4 and IL-5 [10]. This may indicate activity of mechanisms, which support responses of cytotoxic CD8 lymphocytes. According to other hypotheses, the persisting intrahepatic prevalence of Th1 lymphocytes and of the released by them cytokine profile reflects compartmentalisation of cytotoxic response effectors in the liver, which fails to yield an effective reaction to viral antigens [60]. In chronic type C hepatitis, the inflammatory infiltrate contains also activated cells of myelomonocytic line [61]. A role of macrophages in the pathogenesis of chronic type C hepatitis has not been fully clarified [32, 61].

Chronic HCV infection is associated with several extrahepatic diseases, including most cases of type II and type III cryoglobulinemia, B lymphocyte proliferative disorders characterized by polyclonal B cell activation and autoantibody production [39, 42, 71]. Identification of the interaction between CD81 and HCV could help to elucidate the pathogenesis of HCV-associated diseases [71]. The mutations within HCV RNA (particularly within its envelope region) may also modify cellular tropism of HCV [17].

Standards in histopathological evaluation of liver biopsy in chronic hepatitis

Chronic hepatitis encompasses a syndrome of clinical, biochemical and pathomorphological signs and symptoms, which persist for at least 6 months [29]. Chronic hepatitis C

is defined as an inflammatory disease of the liver caused by hepatitis C virus, lasting 6 months or more, with the potential to progress to cirrhosis or associated with cirrhosis [46]. Morphological and aetiological classification of chronic hepatitis has been undergoing substantial modifications within the last 30 years [19, 64]. Application of terms such as chronic persistent hepatitis or chronic active hepatitis is no longer recommended, particularly in the case of chronic type C hepatitis, because the distinction is difficult in this type of infection: in several sites piecemeal necrosis may be patchy and subtle [8, 79]. In order to objectify histopathological appraisal of liver biopsies in chronic viral infections, attempts are made to establish optimum point systems to estimate severity of the morphological lesions [11, 19, 78]. The systems include separate appraisal of activity of the inflammatory process (*grading*) and evaluation of the advancement of fibrosis and stroma reconstruction (*staging*) in portal spaces and in the lobules. Both components of the necrotic-inflammatory process are rated in a four-point scale [78].

Morphological lesions detected by light microscopy in chronic type C hepatitis

Histopathological pattern of chronic type C hepatitis exhibits no characteristic traits. The respective lesions resemble those noted in another chronic viral hepatitis types, i.e., in chronic type B hepatitis. Most of the previously described non-A, non-B type hepatitis cases are identified at present as type C hepatitis. In such cases activity of inflammatory lesions and advancement of fibrosis are low, what leads to difficulties in qualifying type C hepatitis as chronic persistent or chronic active hepatitis [9, 21, 25, 59]. Inflammatory infiltrates are present in portal spaces, less frequently within the lobules, the latter usually accompanied by piecemeal necrosis of hepatocytes. Occasionally, the inflammatory infiltrate contacts the sites of piecemeal necrosis at the interface of lobules and portal spaces [8, 59]. The typical although not pathognomic traits of the morphological pattern in chronic type C hepatitis include presence of lymphoid follicles and/or accumulation of lymphoid cells in portal spaces or in lobules, damage to cells of bile duct epithelium, fatty degeneration of hepatocytes, presence of Mallory's bodies in the cytoplasm of hepatocytes [8, 21, 34, 57]. Hepatocyte transformation to giant cells can be observed, with higher number of cell nuclei, alterations in their structure and presence of numerous nucleoli [57]. The presence of lymphoid follicles or lymphocyte aggregates, mainly in portal spaces in HCV-infected patients, has been described also in another types of viral infections, e.g., in HBV infection [25].

In our studies on adults predominated inflammatory infiltrates consisting mainly of lymphoid cells and mono-

cytes. Sporadically, plasma cells were present. Intensity of inflammatory lesions was low (*grading* of 1 point) or moderate (*grading* of 2 points) in almost 66.7% of the patients [51]. The results have proven consistent with the respective literature data [2, 34, 62]. The extent of fibrosis (*staging*) was also low or moderate in most our patients. Cirrhosis was diagnosed in 10% of the study patients, in line with the literature data [17, 25]. In long-term studies on liver cirrhosis a significantly higher values of AF coefficient (AF=area of fibrosis related to the entire area of liver section) were detected in HCV-infected patients as compared to HBV-infected patients. Determination of the coefficient may represent a prognostic index, allowing to foresee the evolution of chronic type C hepatitis into liver cirrhosis [48]. Fatty degeneration of hepatocytes was a typical trait in 50% of our patients, including 20% with highly pronounced degeneration and presence of large lipid droplets in most of hepatocytes [51]. Other authors observed fatty degeneration of hepatocytes in 51.5% to 85% of patients infected with HCV [21, 57, 62, 79]. Only in 5 out of 30 our patients, cells undergoing lysis were relatively frequent. In the remaining patients only single such the cells were seen in all the biopsies. In individual cases, necrotic processes were expressed by degenerative lesions in cell nuclei and by mitotic figures. No evident morphological features of apoptosis could be detected. In all our patients changes were noted in cell nuclei, involving swelling, altered shape, hyperchromasia, disturbed nuclear chromatin structure, enlarged and frequently multiple nucleoli. The lesions affected also nuclear envelope (Figs. 1 and 2). As compared to our observations in HBV infection, in cases of HCV infection the empty nuclei were less numerous. In over one third of our HCV-infected patients binuclear hepatocytes were seen and sometimes hepatocytes contained even more numerous cell nuclei [1, 51]. The nuclear alterations did not develop in all cells of the biopsy but were restricted to certain regions. In these regions, application of the Brachet's technique permitted to detect RNA in the entire cell nuclei or in the sub-envelope region as well as in the cytoplasm, which might indirectly point to the presence of viral RNA in the hepatocytes (Fig. 3).

Studies on the histological pattern and progression of lesions in HCV-infected children were less frequent than those performed on adults [4, 14, 40, 41]. They demonstrated development of lesions similar to those found in adults: lymphocyte infiltrates, fatty degeneration of hepatocytes, formation of lymphoid cell aggregates in portal spaces and damage to epithelium of bile ducts. Also in children low activity of inflammatory-necrotic lesions prevailed [4, 24, 36, 41, 47]. In most cases, fibrosis affected portal spaces. Badizadegan et al. observed also cases of bridging fibrosis with altered cytoarchitecture of the liver and, not described before, frequent cases of pericellular fibrosis in the central portions of the lobule [4]. According to the authors, this

indicated advanced lesions (*staging*) despite the relatively short duration of the infection. In general, the fibrosis is thought to progress with the increasing duration of HCV infection and to reach its terminal stage in young adults [8, 41, 59]. Our studies on a child with acute liver insufficiency in the course of HCV infection demonstrated traits of pronounced cell necrosis and of fatty degeneration of hepatocytes. The typical cell complexes showed irregular cell nuclei of various size. This was frequently accompanied by chromatin margination, augmentation of nucleoli and altered pattern of nuclear envelopes. Lymphoid cells were spread all over the portal spaces, individual lymphoid follicles were located inside the lobules. Traits of epithelial injury in bile ducts were noted, with cholestasis [70].

Cellular localisation of HCV proteins using immunocytochemical techniques remains to be a matter of disputes and investigations. Envelope proteins (E1, E2) and non-structural proteins (NS2 - NS5) in most of cases were demonstrated inside the cytoplasm of infected cells [17, 31, 38, 43, 56]. In contrast, localisation of the core protein (C) remains controversial. In most of the detection systems, the protein has been detected in the cytoplasm although some reports are available indicating nuclear localisation of the protein, particularly when a truncated protein molecule is looked for [17, 56, 73].

Our immunocytochemical studies in adult patients with chronic type C hepatitis demonstrated structural (C) and non-structural (NS3) proteins in 9/10 patients. Microscopic analysis demonstrated the presence of the product of reaction for C and NS3 proteins mainly in cytoplasm of infected hepatocytes. Only in individual cells the nuclear pattern of C protein was noted. More cells with NS3 proteins and higher intensity of immunocytochemical reaction were observed in comparison to C protein (Fig. 4). Detection of C protein in our studies was possible only using the avidin-biotin-immunoperoxidase complex method (ABC technique) in combination with amplification of the signal with biotinylated tyramine (ImmunoMax technique) and microwave oven pretreatment for antigen retrieval [50].

Studies with the use of the classic *in situ* hybridisation aimed at detection of HCV RNA are limited by the low copy numbers of the viral nucleic acid molecules in chronic type C hepatitis in paraffin-embedded liver biopsies [20, 39, 54, 56]. Authors of several reports consistently concluded that detection of HCV RNA should be performed preferentially in freshly frozen adequate liver biopsies using signal amplification systems [39, 56, 67]. The available data indicate that also routinely processed specimens can be used for RT-PCR to demonstrate viral genome in the liver tissue even in patients seronegative for HCV RNA. The advantages as compared with tests performed on frozen tissue, are the lack of infectivity of the processed tissue and the histological evaluation antecedent to the molecular analysis allowing to

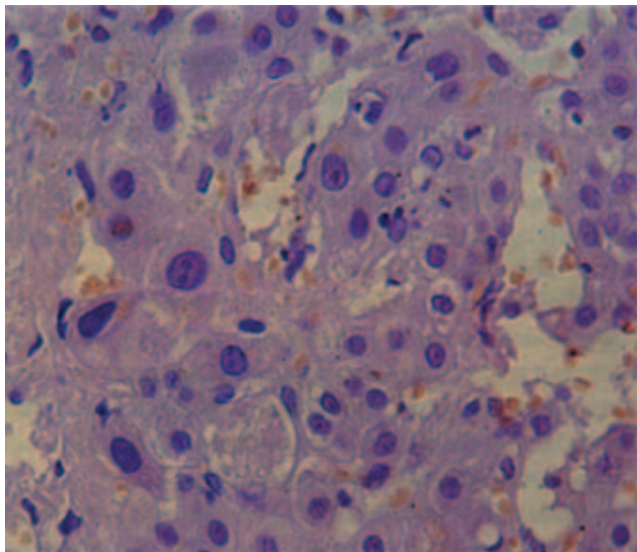


Fig. 1. Fragment of hepatic lobule in HCV infection. Note cells with differences in the size of cell nuclei, in chromatin distribution, in the number, size of nucleoli and in stainability of cell nuclei. HE. Orig. magn. 200x.

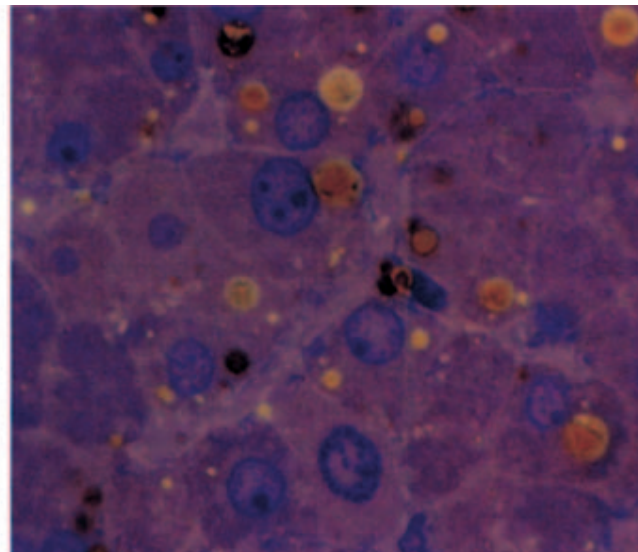


Fig. 2. HCV infection. Extensive differences in the size of cell nuclei, in chromatin distribution, in the number, size of nucleoli and in stainability of cell nuclei and fine lipid vacuoles. Semithin section, toluidine blue staining. Orig. magn. 1000x.

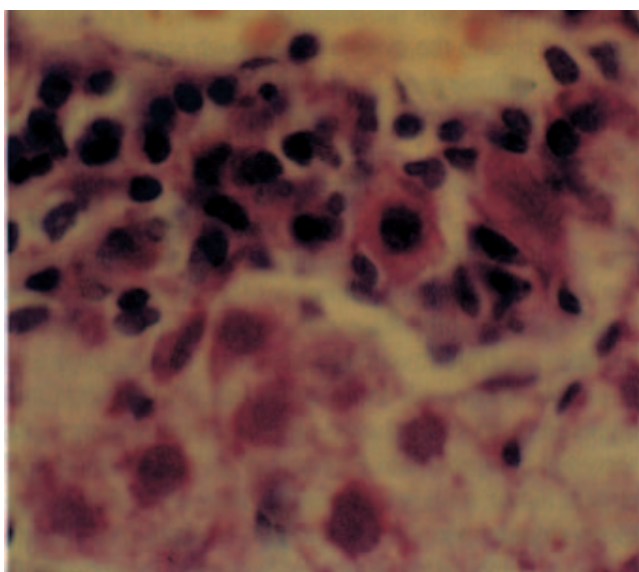


Fig. 3. HCV infection. Note cells of an inflammatory infiltrate (mainly lymphocytes and plasma cells). Individual hepatocytes contain RNA-positive material in the central and peripheral parts of cell nuclei as well as in vicinity of nuclear envelope. Brachet's method. Orig. magn. 1000x.

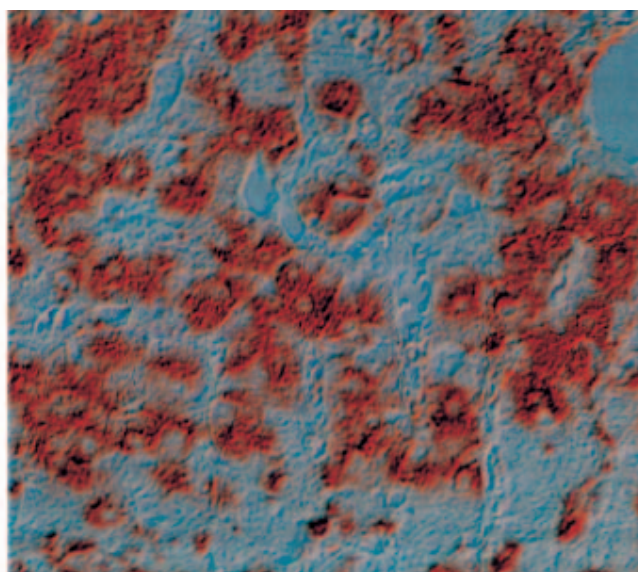


Fig. 4. Fragment of the hepatic lobule from HCV-infected patient. Immunocytochemical reaction localising NS3 protein in the cytoplasm of hepatocytes. ABC-ImmunoMax technique. Orig. magn. 200x.

exclude other diseases and unrepresentative or non-hepatic tissue [31]. Detection of HCV RNA using hybridocytochemical techniques and their varieties (e.g., *in situ* RT-PCR) indicated prevalence of a cytoplasmic localisation of HCV RNA [26, 39, 53, 56]. There are also few data available reporting the prevalence of nuclear signal of HCV RNA and proteins in hepatocytes, bile ductule cells and lymphocytes [86]. The obtained till now conflicting data provide no grounds for relating the number of HCV RNA positive cells to intensity of inflammatory lesions [26, 53, 68].

Morphological pattern of chronic type C hepatitis in electron microscopy

In this part of the presentation, morphological patterns of chronic hepatitis are given exclusively in serologically confirmed (anti-HCV positive) cases of human HCV infections, confirmed also using molecular techniques (HCV RNA positive). The patterns were related to ultrastructural patterns seen in selected models of cultured cells transfected with HCV. Review of literature on ultrastructural patterns observed in chronic hepatitis, previously defined as non-A, non-B hepatitis, has been omitted.

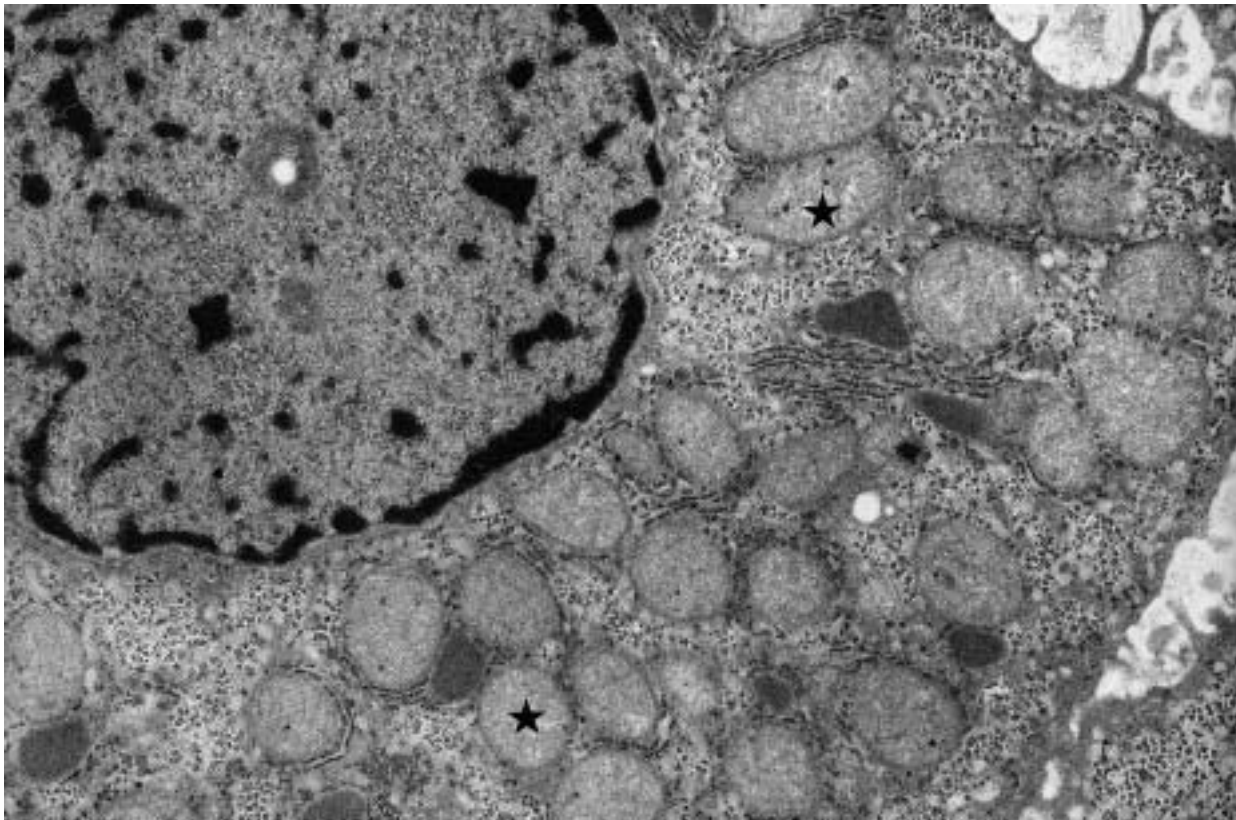


Fig. 5. Fragment of a hepatocyte from HCV-infected patient. Cell nucleus with normal chromatin arrangement. Mitochondria are enlarged, some of them lack cristae and Pallade's granules (asterisk). Magn. 17500x.

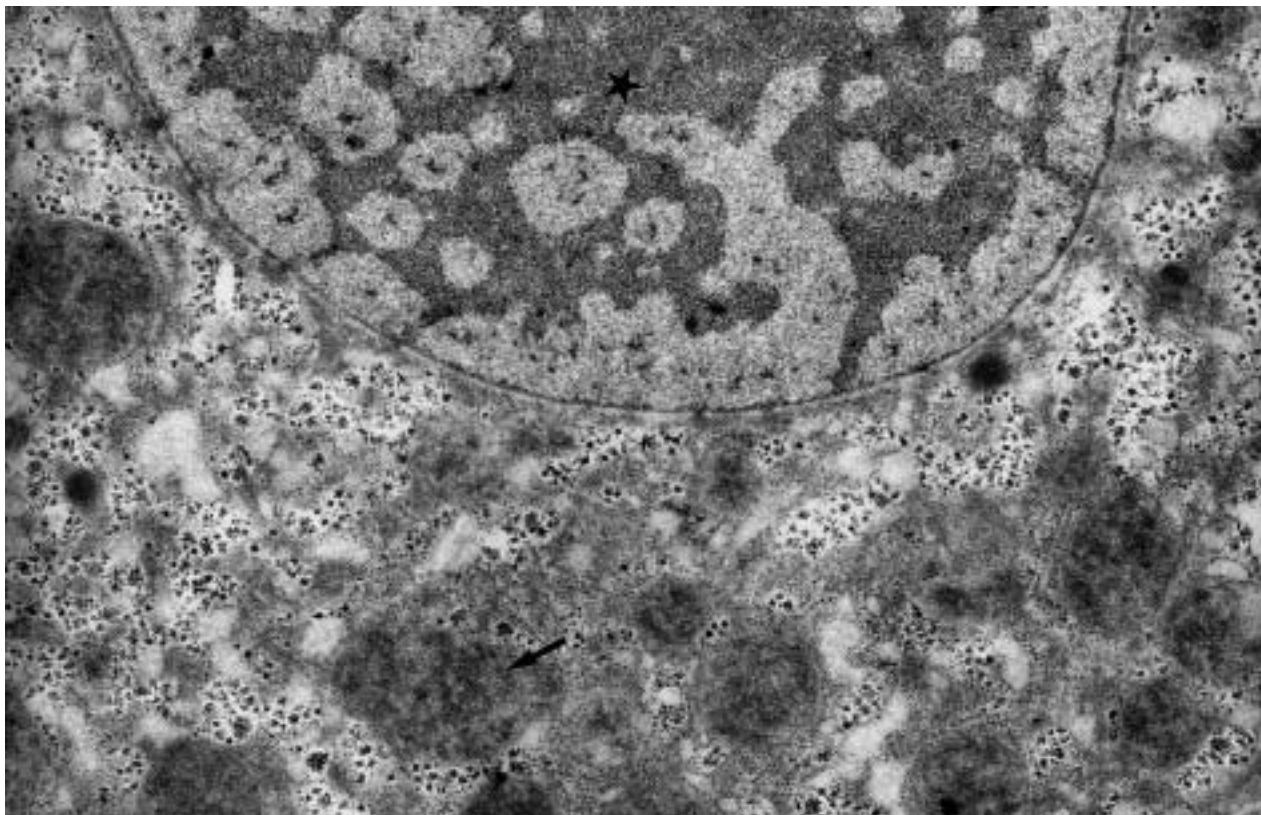


Fig. 6. Fragment of a hepatocyte of the region identical to that of Fig. 5. Note alterations of the chromatin - parts of sub-envelope chromatin resemble euchromatin, parts which usually are euchromatin-like are in this case dense (asterisk). The cell lacks normal mitochondria and the structures contain a dense material (arrow). Canals of endoplasmic reticulum (ER) with a diffuse outline of the membranes. Magn. 17500x.

The ultrastructural patterns in chronic type C hepatitis pertain to both cell nucleus and cytoplasm. A detailed analysis of literature on ultrastructural lesions in HCV infections has shown that the pattern remains to be a matter of debate. Extensive differences have been encountered in the observed lesions, particularly in cell nuclei. Sometimes, multiple cell nuclei were noted in hepatocytes, with irregular, frequently thickened outline of their envelopes [57, 85]. The other lesions in cell nuclei involved their lobulisation and presence of cytoplasmic inclusions. The nuclear chromatin underwent condensation and margination. In such cases it showed a tendency to form heterochromatin-resembling systems, including overlapping and interweaving sets of tubules of 20 - 30nm in diameter [5, 16]. In regions with less pronounced condensation, branching tubules were encountered. Presence of such structures used to be linked to HCV activity in hepatocytes. Cell nuclei with such lesions frequently contained also multiple enlarged, active nucleoli with a lucid zone around them [57]. Other authors were of the opinion that the tubular alterations showed no relation to the viral genetic material and were not typical for HCV infection [16]. Normal cell nucleus structure was sometimes noted despite the presence of cytoplasmic lesions [33, 83]. Cytoplasmic changes observed in electron microscopy in HCV infection are more uniform in many types of cell lines infected *in vitro* and in human or chimpanzee hepatocytes infected *in vivo* [3, 37, 45, 63, 77, 81]. The lesions included dilation of numerous endoplasmic reticulum (ER) cisternae, presence of individual virus-like particles or their aggregates in the cytoplasm or in dilated ER, tubular structures in ER, semicircular shape of ER [3, 15, 28, 37, 45, 81, 83]. Only few authors did not observe viral particles within hepatocytes [37]. Ultrastructural lesions affected also mitochondria with destruction of mitochondrial membranes which sometimes lost their bilayered structure. This suggested the cause of disturbed oxidation of fatty acids and the resulting processes of fatty degeneration of hepatocytes [65, 66]. As compared to small lipid droplets, the large ones were 2.5-fold more frequent and they shifted cell nucleus and cytoplasmic structures to the periphery [7, 57, 66]. At the surface of hepatocytes a reduced number of microvilli was detected. The ultrastructural lesions of hepatocytes failed to correlate with inflammatory activity, evaluated under a light microscope [5]. The most common cytoplasmic lesions detected by electron microscope in chronic HCV infection are listed in Table 1.

In our studies, the lesions described in the literature and concerning hepatocyte nuclei and cytoplasm were detected in all adults infected with HCV [1, 12, 51]. Occasionally, multiple cell nuclei were detected in hepatocytes, with irregular and frequently thickened outline of their envelopes. Nuclear chromatin showed a focally loosened structure (Figs. 5 and 6). In the less dense regions, complexes of tubules were present, bound to each other and manifesting

diameter of 20 - 30nm, or of branching fibrils (Figs. 7 and 8). The tubule complexes showed a tendency to form heterochromatin-resembling structures. In the system of tubules, the overlapping and interweaving was gradually increasing toward nuclear envelope. Under light microscope, such arrangement could be defined as a subcapsular condensation of dense chromatin. Cell nuclei with such lesions frequently manifested multiple and enlarged active nucleoli with a less dense zone around them. Within the enlarged cell nuclei elevated numbers of peri- and interchromatin granules were detected. The area of loose chromatin was also widened. In the perinucleolar region, at the interface between dense and loose chromatin or close to the nuclear envelope in other cells, fibrillar-tubular structures of high density were seen. Occasionally, the tubular structures were branched and formed a network of various condensation extent, which in two infected patients transgressed nuclear envelope and protruded directly to the cytoplasm (Figs. 7 and 9). The pattern of the "empty" cell nuclei, observed under a light microscope, corresponded ultrastructurally to a chromatin deficit at the area of entire cell nucleus and to accumulation of tubular structures close to the nuclear envelope (Figs. 10 and 11). As a rule, the nuclear lesions were accompanied in the same cells by changes in RER system. The reticulum system was dilated and strongly osmophilic, particularly within its degranulated fragments. The membrane fragments frequently formed horseshoe-like structures (Figs. 12 and 13). Another modification of rough endoplasmic reticulum involved its folding and invaginations, corresponding to the patterns described in the literature as undulating membranes. In the lumen of the widened canals, long tubular structures were sometimes seen and, in other canals, a network of branching fibrils. The structures were not observed to pass through ER membranes to the cytoplasm. In our material the altered structure of mitochondria was noted in 10 out of 30 infected patients. In individual cases marked augmentation of the structures was detected, with shortened cristae and disappearance of Palade granules. Some mitochondria contained paracrystalline deposits (Figs. 5 and 6). Apart from the altered cell organella, the cytoplasm contained free, long tubular structure of a highly osmophilic character, similar to those seen within the cell nucleus. In every other patient, ultrastructural studies confirmed also presence in hepatocytes of numerous lipid vacuoles. Features of hepatocyte lysis could be observed, sometimes with evident outpouring of the cell content beyond limits of the cell. Disintegration of nuclear envelope was accompanied by passage of nuclear content to the cytoplasm [1, 12, 51].

In children with chronic type C hepatitis, the ultrastructural pattern did not differ from that detected in adults. Swollen, enlarged hepatocytes, exponents of hepatocyte lysis and of fatty degeneration were documented. Cell nuclei were altered with evidently loosened structure and margina-

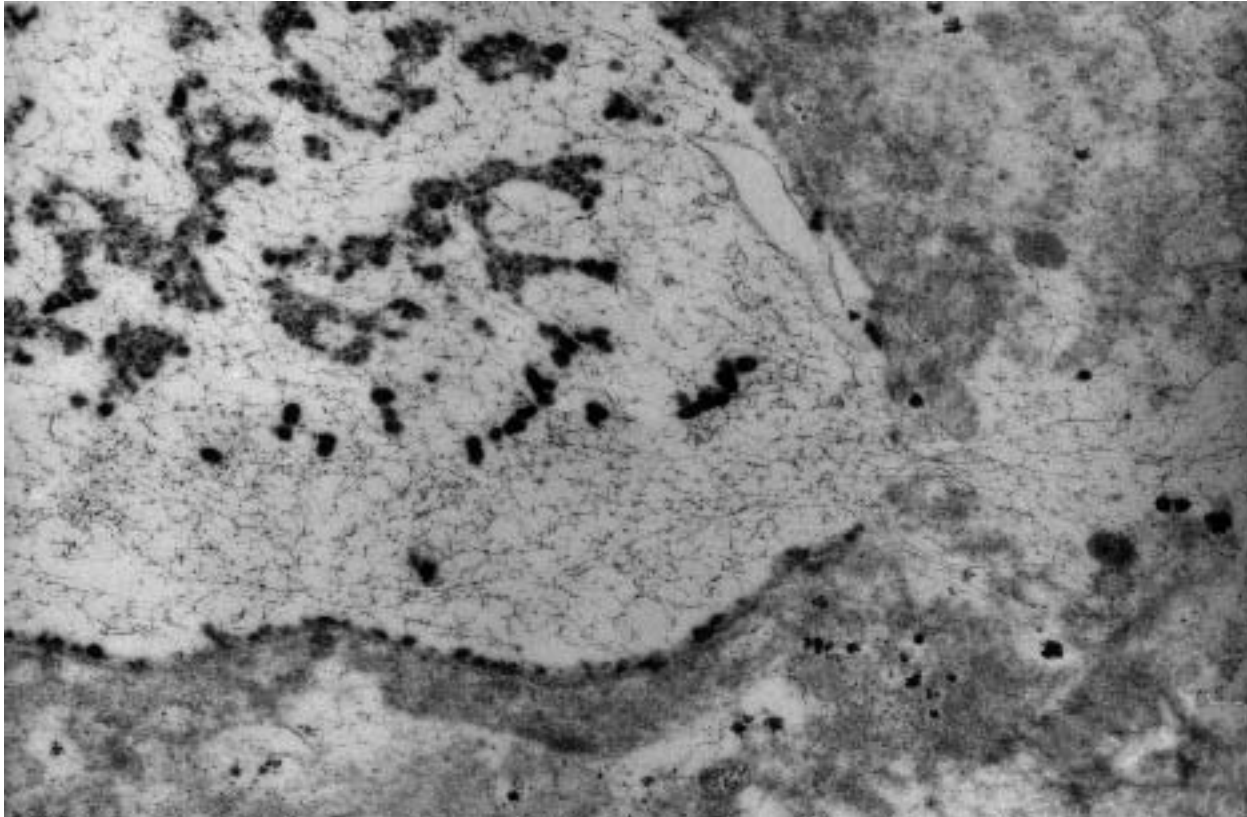


Fig. 7. Another hepatocyte of the region identical to that of Figs. 5 and 6. In the cell nucleus note delicate networks of osmophilic material, extending to the cytoplasm through the damaged nuclear envelope. Magn. 35000x.

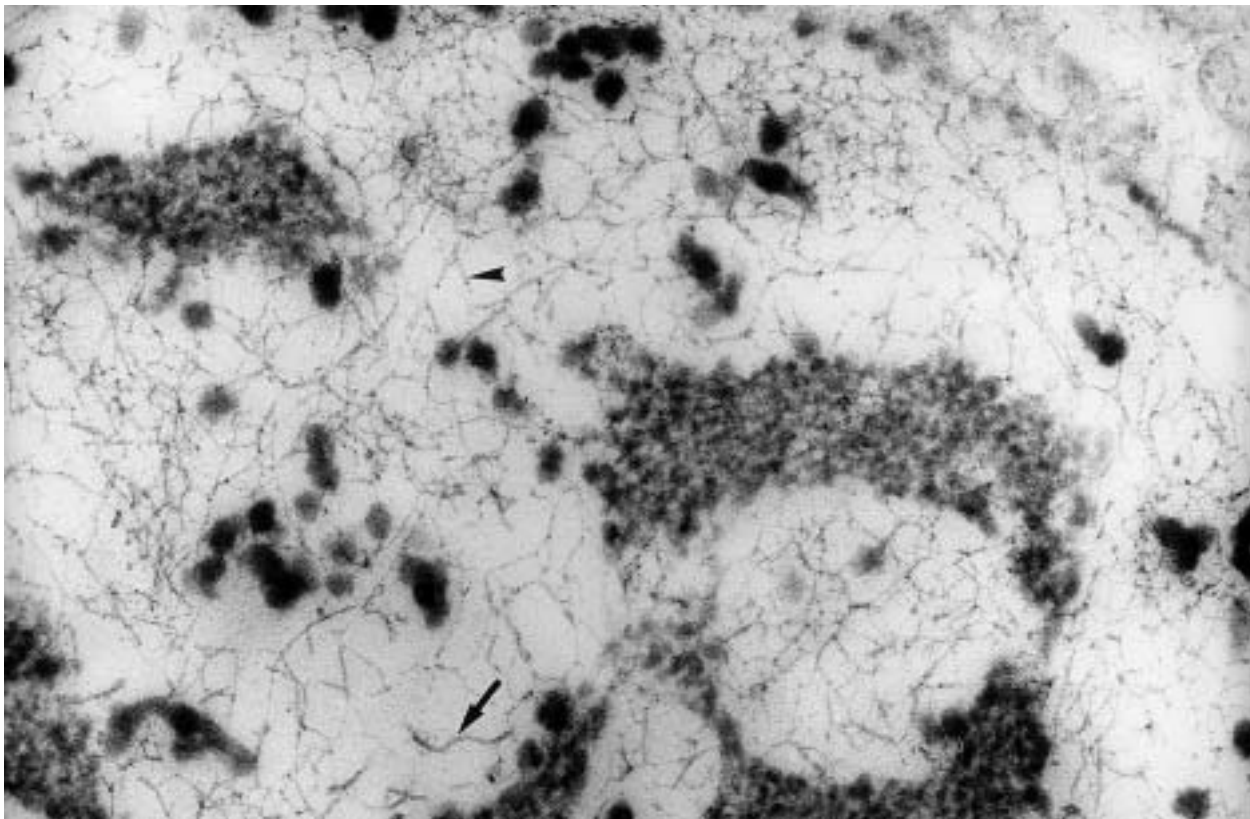


Fig. 8. Fragment of the cell nucleus illustrated in figure 7. Note different thickness of the network of nuclear fibrils: "single" threads (arrowhead) and "double" threads (arrow). Magn. 70000x.

TABLE 1

The most common cytoplasmic ultrastructural alterations in HCV infected cells

Electron microscope observations	Types of infected cells	Author
Dilated vesicular endoplasmic reticulum (ER)	Human hepatocytes TOFE cells	Adamek et al. [1]; Asti et al. [3]; Biczysko et al. [12]; Gastaldi et al. [37]; Kasprzak et al. [50]; Pawelek et al. [70] Serafino et al. [81]
Dilated, degranulated ER with double membranes of semicircular shape ("undulating")	Human hepatocytes Human and chimpanzee hepatocytes	Adamek et al. [1]; Biczysko et al. [12]; Kasprzak et al. [50]; Pawelek et al. [70] Schaff et al. [77]
Cytoplasmic vesicles with virus-like particles: of 50nm in diameter of 50 - 60nm in diameter of 40 - 45nm in diameter	Daudi cells and chimpanzee hepatocytes HepG2 cell line Human foetal hepatocytes TOFE cells Human hepatocytes	Shimizu et al. [83] Dash et al. [28] Iacovacci et al. [45] Serafino et al. [81] Bosman et al. [15]
Virus-like particles within ER cisternae: of 45nm in diameter of 20nm in diameter	HeLaG cells Human hepatocytes	Mizuno et al. [63] Bosman et al. [15] Adamek et al. [1]; Pawelek et al. [70]
Tubular structures: in cytoplasm in ER	TOFE cells Human hepatocytes Human and chimpanzee hepatocytes	Serafino et al. [81] Adamek et al. [1]; Ballercia et al. [5]; Biczysko et al. [12]; Faa et al. [33]; Kasprzak et al. [50] Shaff et al. [77]
Densely packed fibrils in ER	Human hepatocytes	Adamek et al. [1]; Ballercia et al. [5]; Biczysko et al. [12]; Faa et al. [33]; Kasprzak et al. [50]
Regular mitochondria with dense granules in the matrix	Human hepatocytes	Asti et al. [3]
Irregular mitochondria Irregular outlines of mitochondria with reduced number or absence of cristae	Human hepatocytes	Adamek et al. [1]; Asti et al. [3]; Kasprzak et al. [50]; Ballercia et al. [5]
Paracrystalline inclusions in mitochondria	Human hepatocytes Transgenic mice hepatocytes	Adamek et al. [1]; Asti et al. [3]; Kasprzak et al. [50] Moriya et al. [65, 66]
Increased number of lysosomes	Human hepatocytes TOFE cells	Adamek et al. [1]; Lefkowitz et al. [57]; Villari et al. [85] Serafino et al. [81]
Large lipid droplets	Human hepatocytes HepG2 and CHO cells Transgenic mice hepatocytes	Adamek et al. [1]; Kasprzak et al. [50]; Lefkowitz et al. [57]; Barba et al. [7] Moriya et al. [66]

tion of chromatin. Both in cell nuclei and in widened canals of endoplasmic reticulum tubular structures were found, of around 20nm in diameter, as well as virus-like particles of around 45nm in diameter [15]. The degranulated membranes of endoplasmic reticulum formed horseshoe-like structures [70].

Most of ultrastructural studies using immunocytochemical techniques and applying various models of investigations demonstrated a cytoplasmic localisation of HCV proteins [56, 83]. Envelope proteins E1 and E2 as well as the non-structural proteins NS2, NS3, NS5 were detected in rough endoplasmic

reticulum [13, 63, 76, 80], in the cytoplasm [13, 66] or in perinuclear area [44]. A subcellular localisation of the core protein (C protein) remains controversial but most of detection systems proved its presence also within the cytoplasm. The protein was observed to be bound to ER membranes [75] and lipid droplets in cytoplasm of the infected cells [7, 65]. Individual studies reported the nuclear [75] and mitochondrial expression of C protein [65, 66].

Our preliminary studies at the ultrastructural level demonstrated C and NS3 proteins in cytoplasm and in

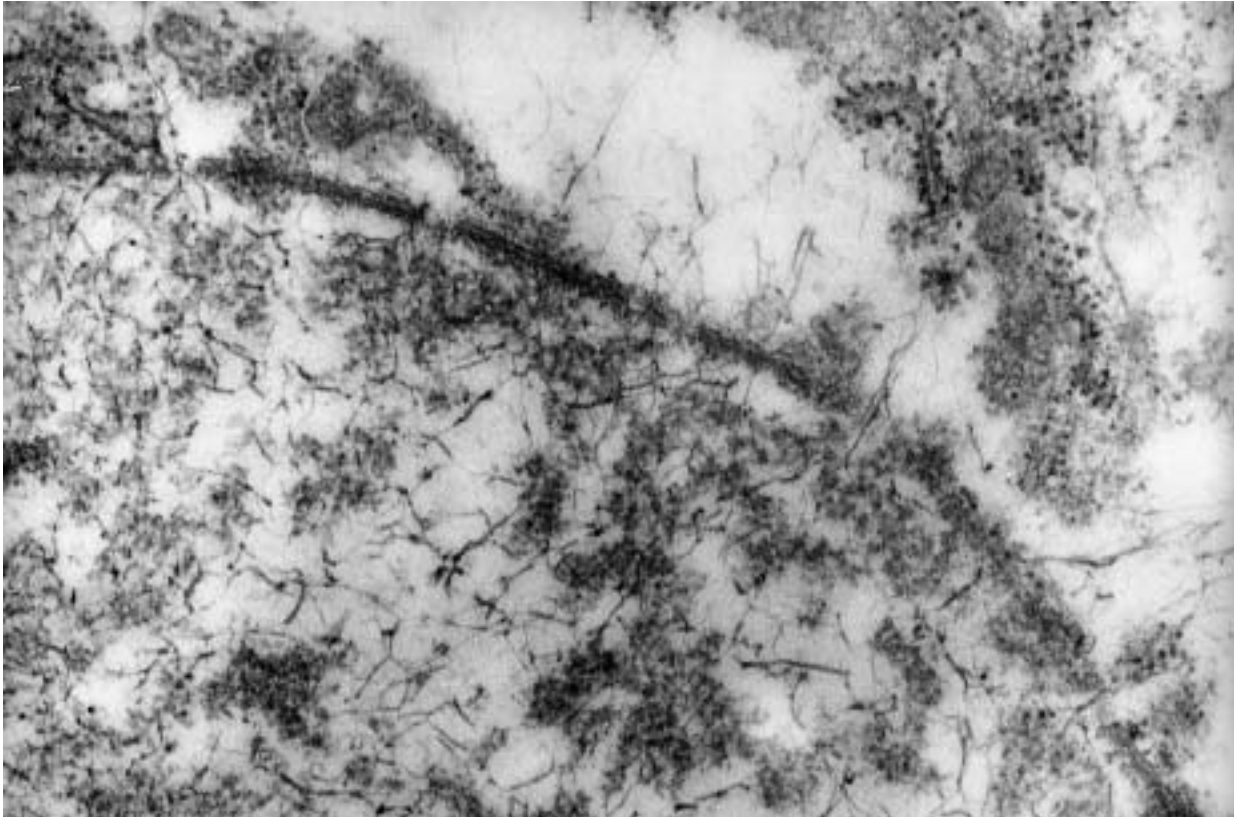


Fig. 9. Fragment of a hepatocyte of another patient with HCV infection. Cell nucleus filled with tubular structures, which form networks. The networks contains also thin threads. Tubular structures of a different thickness protrude through the nuclear envelope. Magn. 60000x.

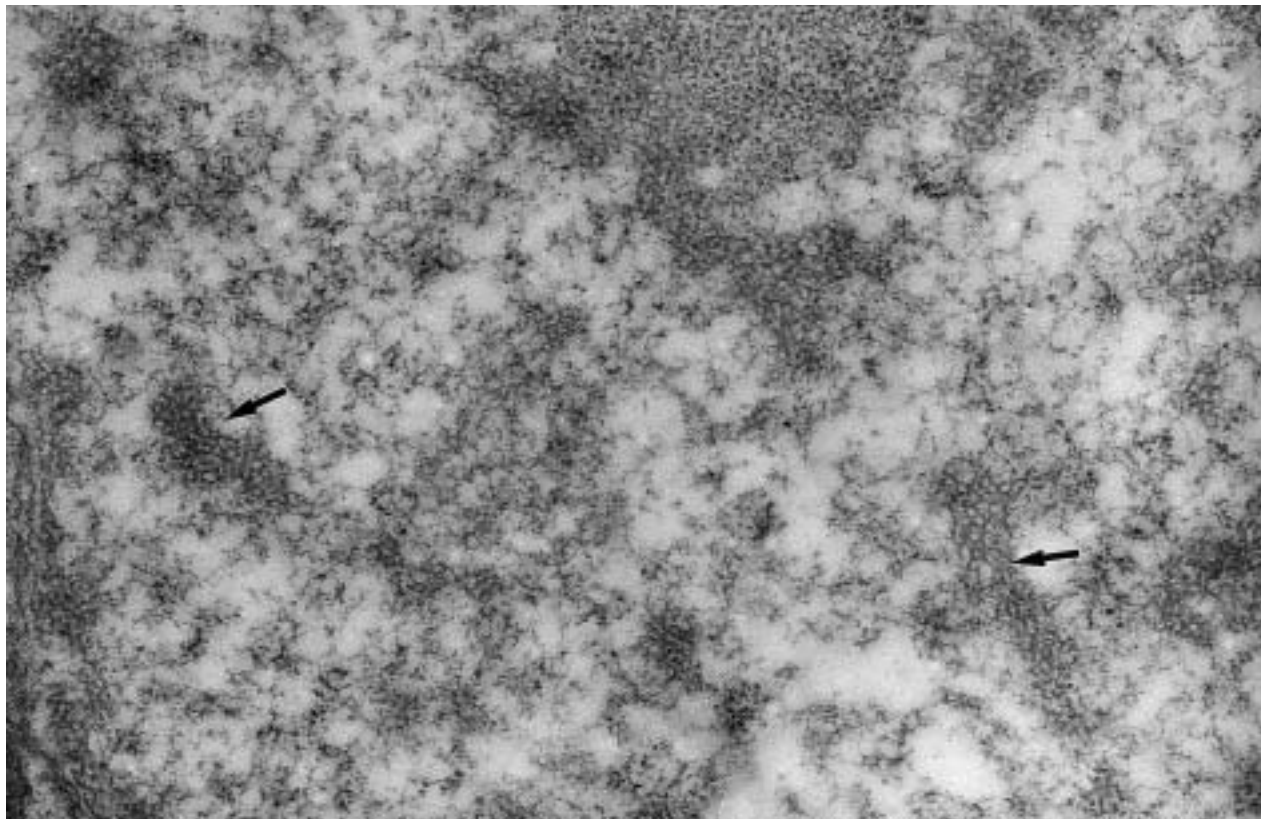


Fig. 10. Networks of tubular structures (arrows) in the hepatocyte nucleus of another HCV-infected patient. Note distinct density of the material in regions containing tubular structures. Magn. 42000x.

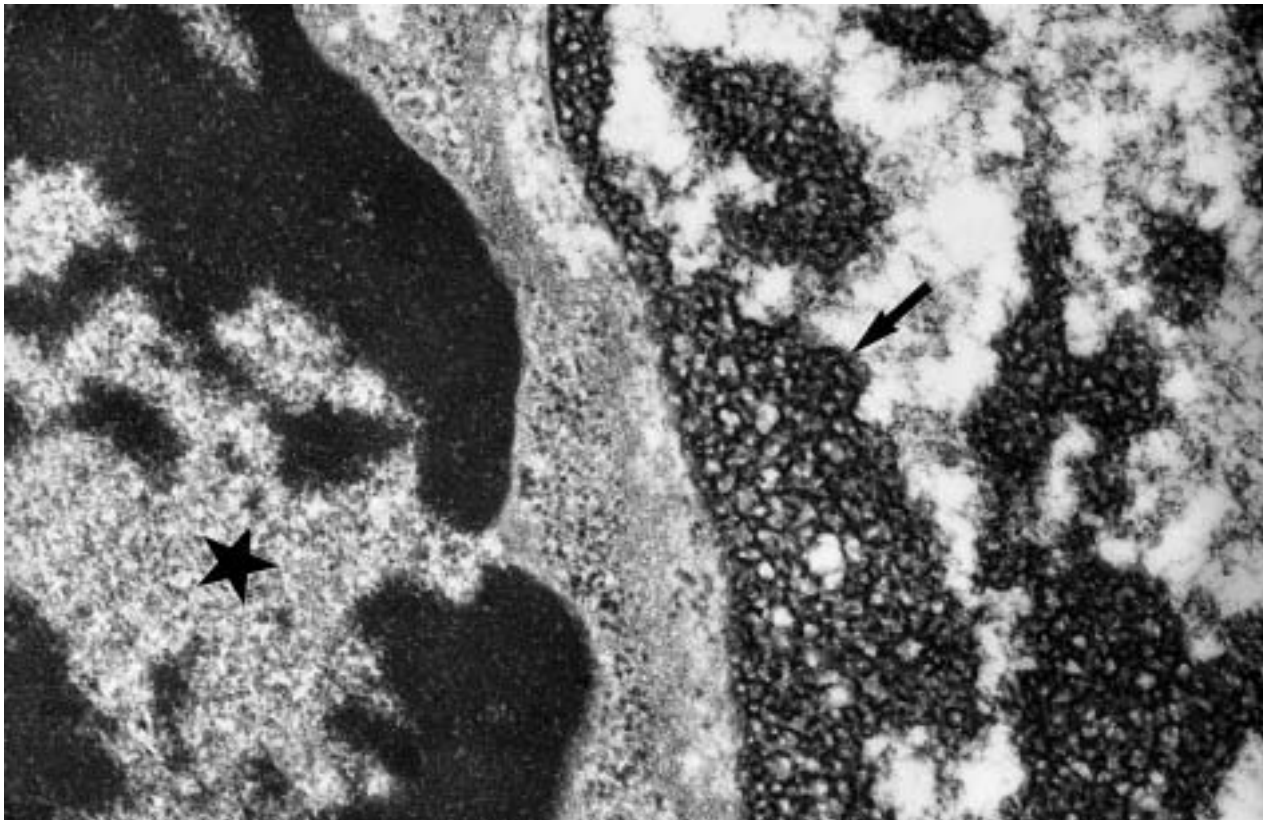


Fig. 11. Fragment of hepatocyte nucleus with numerous dense tubular structures (arrow), located also under nuclear envelope. In the vicinity the fragment of a lymphocyte (asterisk). Magn. 54000x.

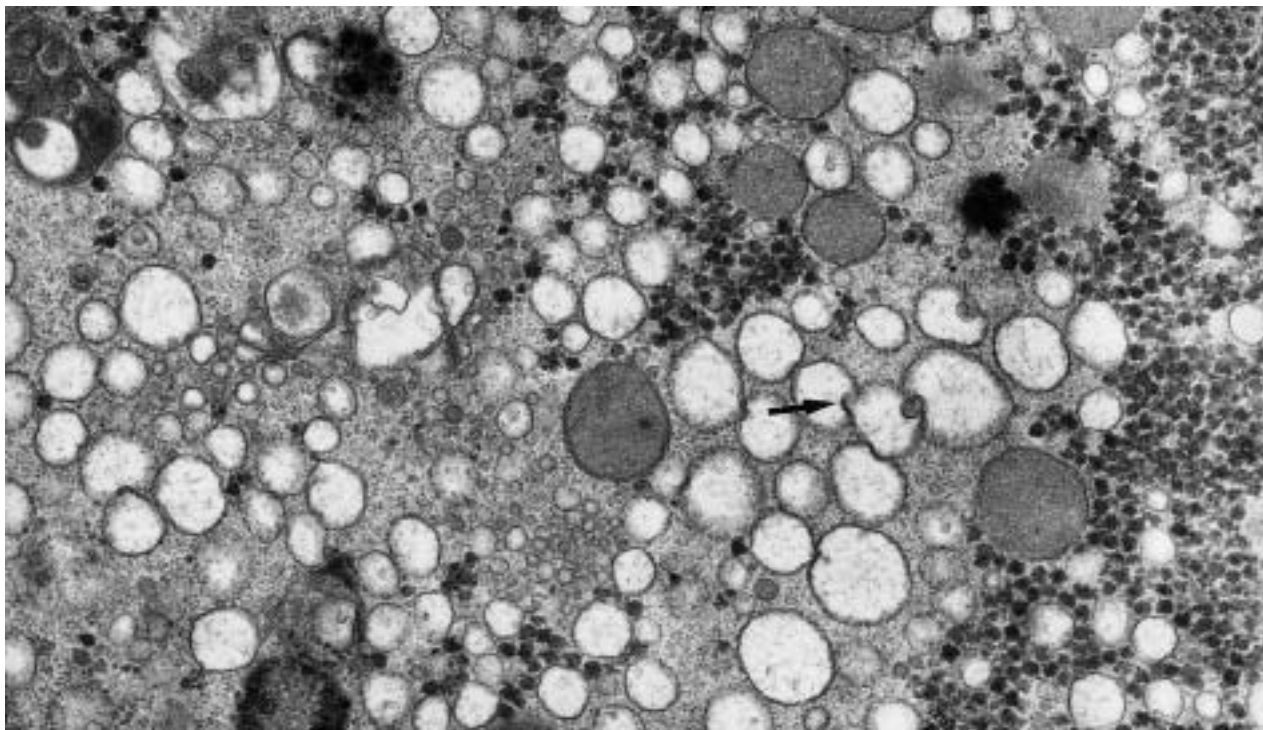


Fig. 12. Cytoplasm of a hepatocyte with numerous, partially or fully degranulated ER canals. Few partially degranulated canals form horseshoe-like structures (arrow). Magn. 25000x.

nuclei of HCV-infected cells. In the cytoplasm the proteins were observed to be connected with dilated endoplasmic reticulum canals (data not published).

The cellular and subcellular localisation of HCV antigenic proteins was consistent with localisation of HCV RNA disclosed using the molecular biology techniques (*in*

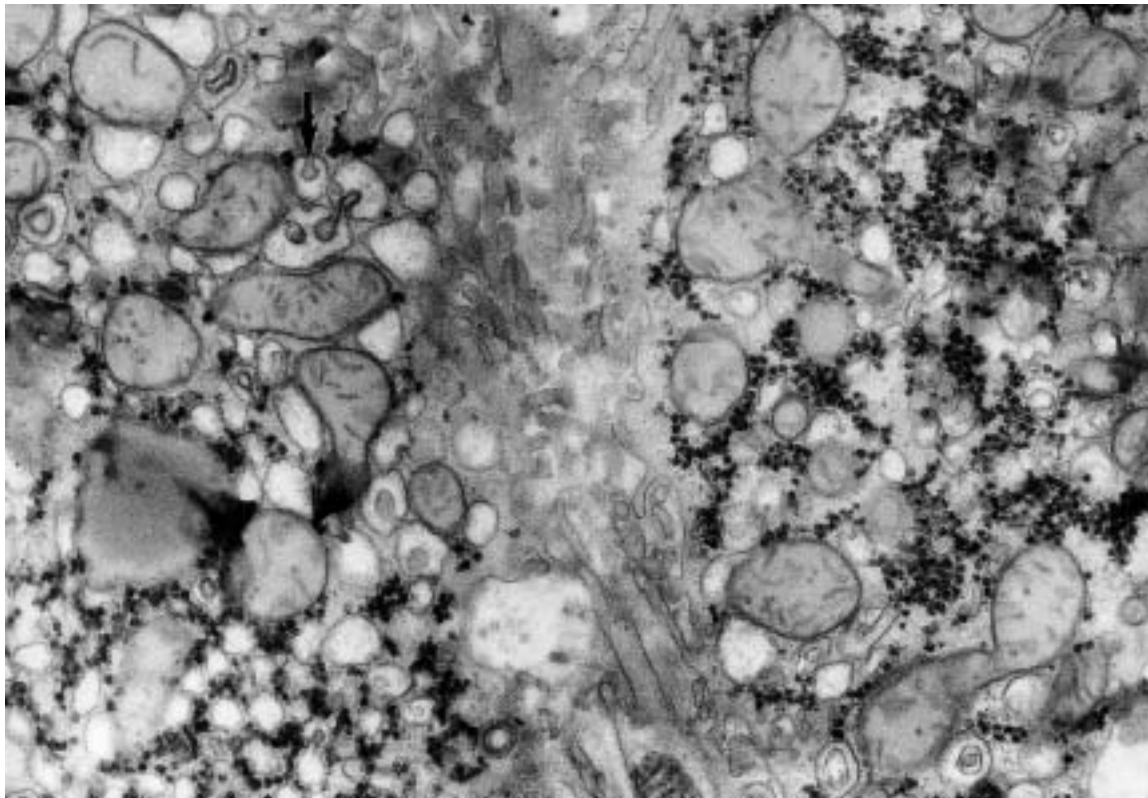


Fig. 13. Fragments of cytoplasm in two hepatocytes. The space between them is widened and filled with a fine granular material. In the cytoplasm of one of the cells numerous horseshoe shaped structures form in the region of ER canals, which are partially degranulated (arrow). Magn. 24500x.

situ hybridisation, PCR and its varieties). In studies on expression of HCV RNA, application of RT-PCR proved to be particularly valuable [15, 28, 39, 56]. The RT *in situ* PCR technique allowed for the rapid detection of hepatitis C cDNA in much more hepatocytes in a panlobular distribution in comparison with detection of the viral RNA using standard RNA-cDNA *in situ* hybridization at the light microscope level [69]. Application of the technique at the electron microscopy level permitted to detect plus and minus strands of HCV RNA in the cytoplasm of infected hepatocytes in children and adults [15]. This was consistent with light microscopy observations on cellular localisation of HCV RNA [37, 54, 67 - 69].

The results concerning ultrastructural lesions, immunocytochemical localisation of HCV proteins and hybridocytochemical detection of HCV RNA are listed in Table 2.

Concluding remarks

Most of the patients with chronic type C hepatitis demonstrate low activity of inflammatory lesions and low extent of fibrosis, which results in difficulties in distinguishing infection stages corresponding to those noted in chronic type B hepatitis and in employing the traditional classification of cases as chronic persistent hepatitis or chronic active hepatitis [8, 9, 64]. In the course of chronic type C hepatitis,

however, the necrotic-inflammatory lesions seem to result in stroma reconstruction and in early cirrhotic lesions more rapidly, particularly in young adults [41]. Cellular lesions in chronic viral hepatitis detected under light microscope are characteristic but they are not specific and cannot provide grounds for aetiological diagnosis [8, 64]. The extensive damage to nuclear chromatin ("empty" cell nuclei) represents the common trait of chronic type B and type C hepatitis, both in children and in adults [1, 12, 51]. Our immunocytochemical studies demonstrated the effective detection of both the core antigen (C protein) and one of the non-structural protein (NS3) in chronically infected HCV patients. In line with the currently modified classification of chronic hepatitis and with the potential of new antiviral therapy it seems very important to supplement routine staining techniques with studies at the molecular level, also at the ultrastructural level [50]. Interpretation of ultrastructural lesions in HCV infections remains to be a matter of dispute. Extensive differences have been noted in the observed lesions, particularly in those concerning cell nuclei [12, 16, 33, 57]. Application of immunocytochemical techniques and of hybridocytochemical techniques has demonstrated a markedly more frequent cytoplasmic localisation both of plus and minus strands of HCV RNA and of most viral proteins as compared to localisation of the elements in the cell nucleus [13, 50, 56,

TABLE 2
Characteristics of HCV

Virus type	HCV*
Family	<i>Flaviviridae</i>
Virion characteristics	Spheric particle of 50nm (36 - 65nm) in diameter with core (30nm) and envelope with extensions
Genom structure	Single stranded, linear RNA, of positive polarity, of around 10kb in length; one long open reading frame (ORF); yields 1 polyprotein, later structural proteins (C, E1 and E2) and 6 non-structural proteins (NS2, NS3, NS4a, NS4b, NS5a, NS5b)
Presence of viral components in cell nucleus	around 30nm spheric or tubular structures: - protein C - HCV RNA plus and minus strands (together with cytoplasmic localisation)
Presence of viral components in cell cytoplasm	- 45 - 60nm virus-like particles - 22 - 40nm-thick tubular structures - protein C (30nm particles), E2, NS3, NS4, NS5a, NS5b HCV RNA plus and minus strands
Applied models of studies	human hepatocytes; chimpanzee hepatocytes; transgenic mice hepatocytes; human B-cell line (Daudi cells); HepG2 cells; HeLaG cells, TOFE cells
Numbers of our microphotographies	5 - 13

*according to: Choo et al. [27]; Santolini et al. [75]; Mizuno et al. [63]; Gastaldi et al. [37]; Shimizu et al. [83]; Barba et al. [7]; Dash et al. [28]; Serafino et al. [81]; Bosman et al. [15]; Moriya et al. [65]; Kasprzak et al. [50]; Shi [82]

66]. Subcellular localisation of the HCV core protein requires further studies [7, 65].

Literature data and our experience indicate that application of direct visualisation of the virus supplemented by the use of molecular biology techniques allows to clarify more accurately the correlation between virus distribution on the one hand and morphological lesions of the liver, immune response of the host and clinical symptoms on the other hand in the natural history of the disease. A role of such studies in defining novel therapeutic aims and in construction of vaccines cannot be overestimated.

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