Invited Review

Ground-glass hepatocytes: light and electron microscopy. Characterization of the different types

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Summary. Morphological observations of paraffin-embedded histological sections stained with H&E led to the discovery of some cytoplasmic changes which occur in different conditions, although they look alike under the light microscope. These hepatocytic changes consisted basically of homogeneous areas which are weakly eosinophilic in H & E-stained sections. They are frequently referred to as «inclusion» bodies, even when they are not true inclusions. The hepatocytic changes observed in HBsAg carriers, in chronic alcoholic patients treated with cyanamide to discourage them from drinking alcohol, in Lafora's disease, and in glycogenosis type IV, look very similar in paraffin sections stained with H&E. Nevertheless, they can be differentiated using ancillary techniques. On electron microscopy they do not look alike. Of particular interest are the «inclusion» bodies induced by cyanamide, a predictable and reproductible lesion, which in man eventually leads to cirrhosis.

Other types of hepatocytic changes also giving a rather vague «ground-glass» appearance to the cytoplasm are those resulting from intracytoplasmic accumulation of proteins, particularly fibrinogen, and those observed in patients treated with different drugs.

Key words: Ground-glass hepatocytes, Liver-cell inclusions, HbsAg, Cyanamide, Lafora's disease, Glycogenosis type IV, Fibrinogen inclusions

Introduction

Morphological observations of paraffin-embedded histological sections stained with haematoxylin and eosin led to the discovery of some cytoplasmic changes which appear in different conditions, although they look alike under the light microscope. The exact interpretation of

these changes depends on the degree of expertise of the pathologist and particularly on the results of special stainings and ultrastructural studies. These hepatocytic changes consist basically of more or less large, homogeneous areas which are weakly eosinophilic in haematoxylin and eosin-stained sections.

When these areas are well-demarcated they are referred to as inclusion bodies, even when they are not true inclusions, since most of them are not membrane-bound, and correspond to transformed or altered cytoplasm or, being membrane-bound, contain a material produced by the cell itself.

Ground-glass hepatocytes in HBsAg carriers

The best known type of ground-glass hepatocytes is that observed in Australia antigen (HBsAg) carriers in relation to the hepatitis B viral infection (Fig. 1). Hadziyannis et al. (1973) described the presence of hepatocytes with ground-glass appearance in HBsAg-seropositive patients. These hepatocytes were associated with bright HBsAg-specific cytoplasmic immunofluorescence. These ground-glass cytoplasmic areas can be stained with orcein, aldehyde-fuchsin and aldehyde-thionin and give a strong reactivity when treated with antibodies raised against HBsAg (Hadziyannis et al., 1973). In addition, they give a PAS (periodic-acid-Schiff)-positive reaction.

On electron microscopy the ground-glass areas show hyperplasia of smooth endoplasmic reticulum which displaces other organelles to the cell periphery. Within the smooth endoplasmic reticulum profiles there are long filaments, 20-26 nm in diameter and of variable length up to 1 µm, which are similar to the HBsAg filaments found in the serum of these patients (Huang and Groh, 1973; Gerber et al., 1974; Winckler et al., 1976). This filamentous material corresponds to virus-induced coat protein synthesized by the endoplasmic reticulum of the infected liver cells (Huang and Groh, 1973). The ground-glass hepatocytes are found mainly in chronic hepatitis B
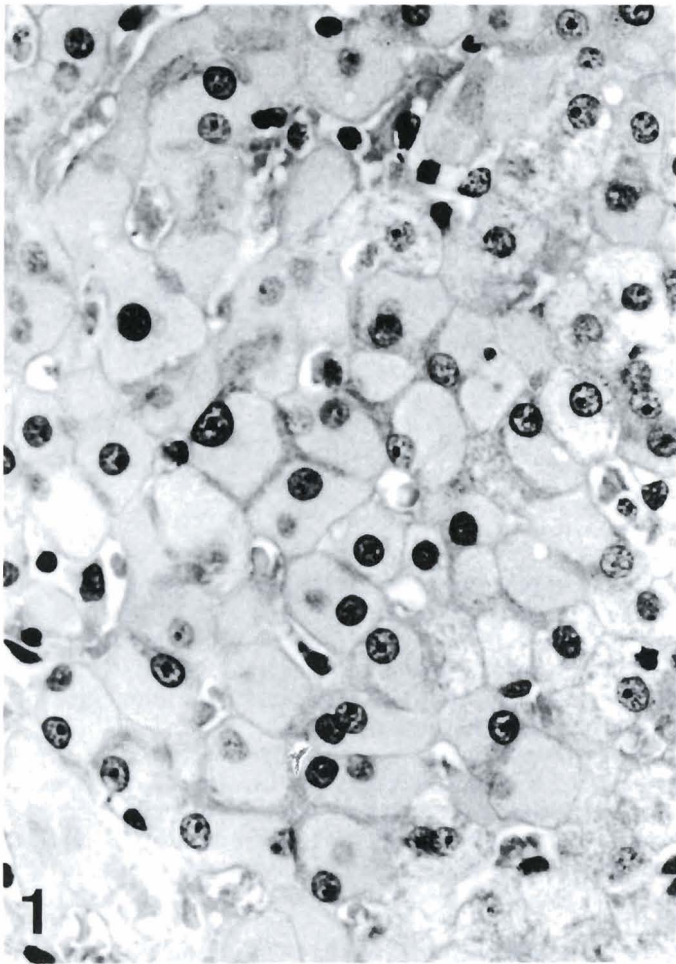
Fig. 1. Numerous ground-glass hepatocytes in a hepatitis B virus carrier. Needle biopsy. H & E, × 620

Fig. 2. Micrograph taken from a patient on cyanamide. Most of the hepatocytes bear «inclusion» bodies. H & E, × 620

and particularly in carriers without evidence of hepatitis. They are observed with a random distribution through the lobule.

Cyanamide-induced «inclusion» bodies

The liver cells bearing «inclusion» bodies induced by cyanamide in chronic alcoholic patients, treated to discourage them from drinking alcohol, have a close resemblance to the «ground-glass» hepatocytes found in the HBsAg carriers.

The «inclusion» bodies induced by cyanamide consist of distinctive round or kidney-shaped cytoplasmic areas, which are homogeneous or partly granular in haemato-xilin- and eosin-stained sections (Fig. 2). These bodies appear slightly eosinophilic and are very often surrounded by an artifactual halo. They frequently displace the nucleus to the cell periphery and often occupy most of the cell body sparing only a thin rim of normal looking cytoplasm, giving the cells a signet-ring appearance (Vázquez and Pardo-Mindán, 1979).

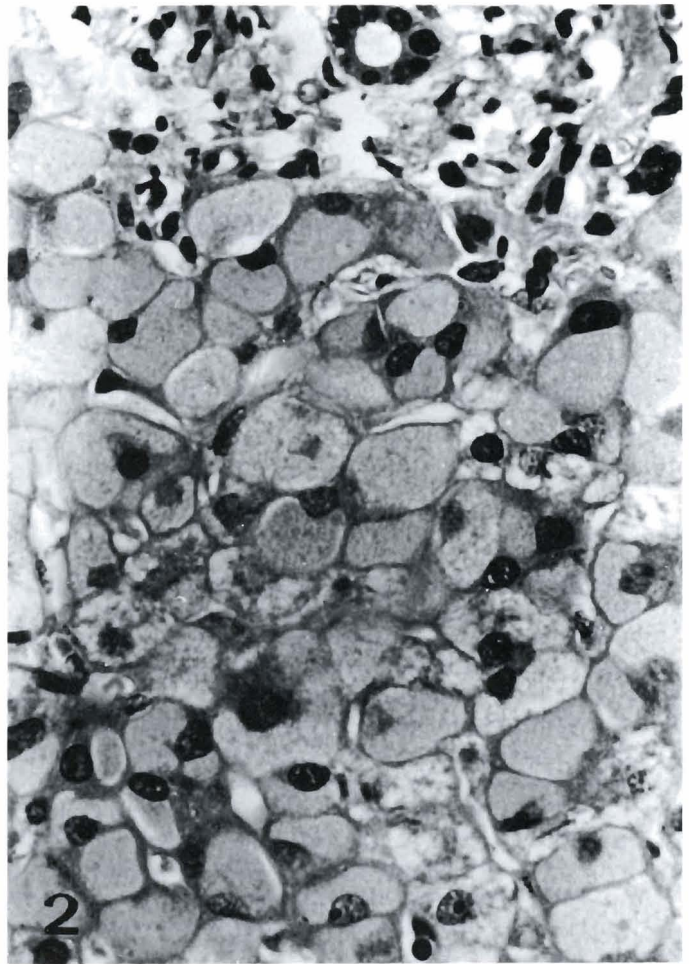In PAS-stained preparation the «inclusions» give a strongly positive reaction. This PAS-positive material is completely hydrolized by incubation with saliva, but the time required for its total digestion is often longer than that required for hydrolysis of glycogen in a normal liver «Inclusion» bodies stained also give a positive colloidal iron reaction, are deeply stained with methanamine silver, giving a light to moderate positive Prussian blue reaction. On the contrary, Shikata stain for HBsAg is negative.

In semi-thin sections of plastic-embedded material the «inclusion» bodies frequently show dense granules and fine vacuoles. In these semi-thin sections the artifactual halo around the «inclusions» is not seen.

On electron microscopy the «inclusions» contain a large amount of glycogen disposed in beta-granules, secondary lysosomes containing whorled lamellar structures, occasional lipid droplets, a filamentous matrix, and residues of degenerating organelles: rough and smooth endoplasmic reticulum and mitochondria (Fig. 3). Smooth endoplasmic reticulum is almost completely absent. Glycogen is the predominant component of «inclusions» in some patients, while debris resulting from organelle breakdown predominates in others.

Table 1. Differential diagnosis

Type	PAS stain	Shikata stain[a]	Acid fuchsin[b]	Immunocytochemistry	
				HBsAg	Fibrinogen
HBsAg	+	+	−	+	−
Cyanamide	+	−	−	−	−
Lafora's	+	−	−/+	−	−
Glucogenosis type IV	+	−	−	−	
Fibrinogen	−	−	−	−	+
Oncocytic reaction	−	−	+	−	−

[a] orcein;
[b] Cowdry stain for mitochondria, Masson's trichrome stain.
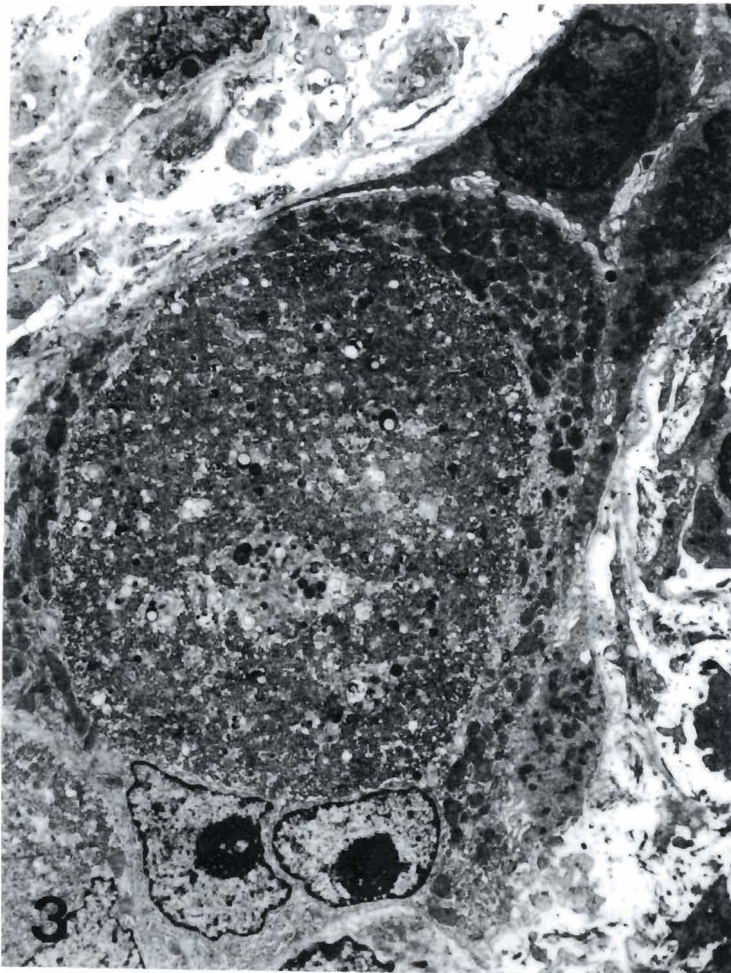

Fig. 3. Electron micrograph of a cholangiole made up of two biliary cells, upper right, and one binucleate liver cell, which bears an inclusion body containing abundant glycogen and degenerating organelles. × 3,000
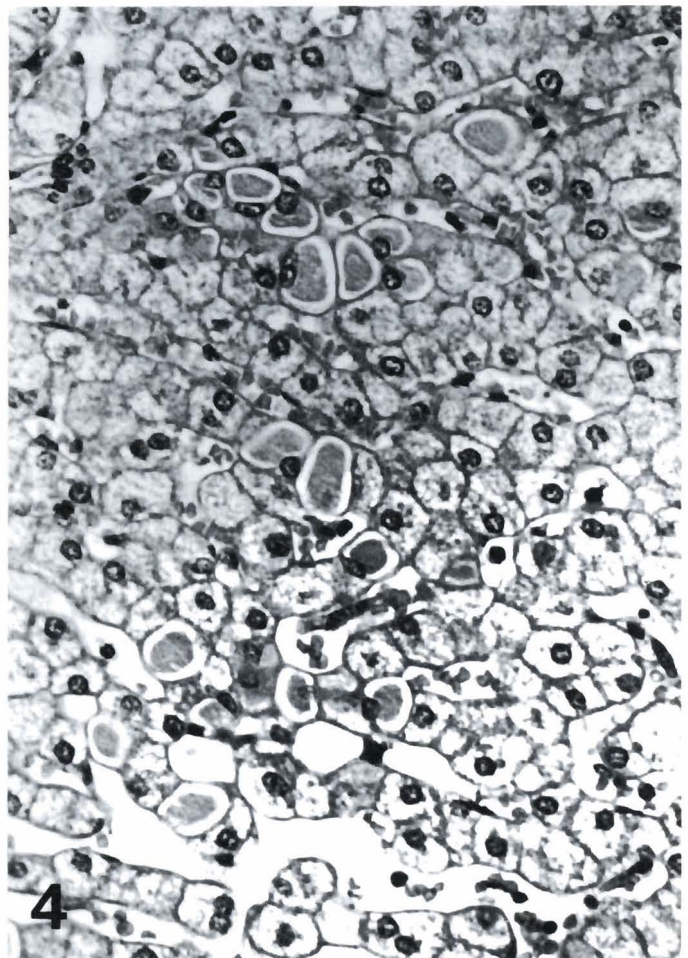
Fig. 4. Needle biopsy from a patient suffering from myoclonic epilepsy (Lafora's disease). Some hepatocytes contain «inclusion» bodies. Note the characteristic halo around the ground-glass areas. H & E, × 400
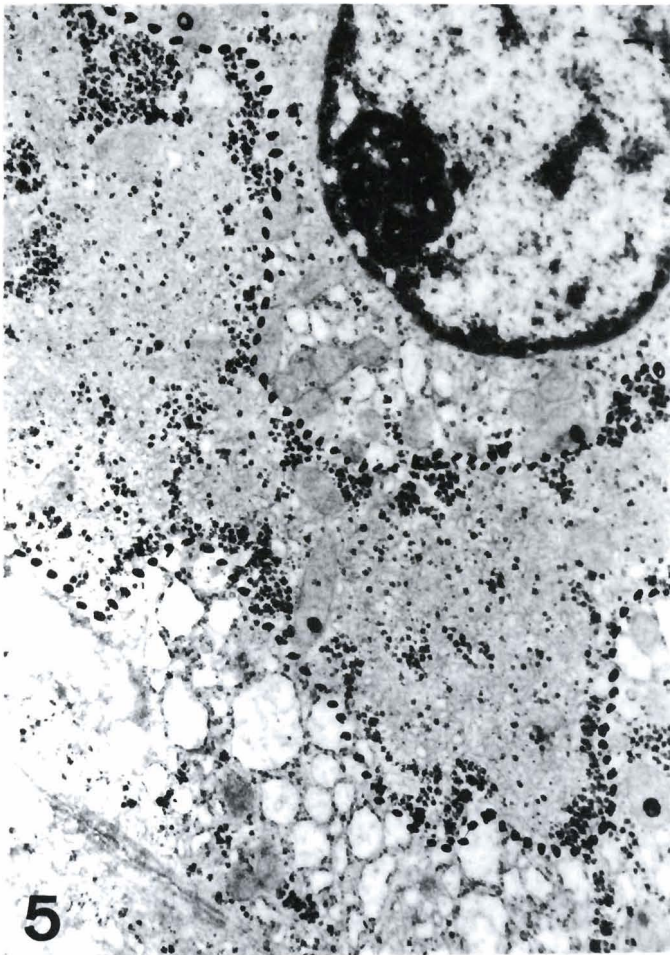
Fig. 5. Lafora's disease. Hepatocyte bearing inclusion body (dotted line). Note the abundant glycogen. Some mitochondria and smooth endoplasmic reticulum profiles are seen inside the inclusion. × 14,400

Fig. 6. Biopsy taken from a patient on antiepileptic drugs. The cytoplasm is clearer than normal because of the smooth endoplasmic retioulum increase. H & E, × 620

The peripheral rim of cytoplasm disposed around the «inclusion» contains numerous mitochondria and rough endoplasmic reticulum profiles, as usually seen in normal hepatocytes. Strikingly, this rim usually gives a PAS-negative reaction because it does not contain any glycogen.

The distribution of «inclusion» bodies through the lobule varies from case to case, according to the duration of treatment and the existence of pauses in the treatment. The lesion starts at the periphery of the lobule and progresses towards the centre. Therefore, in patients treated recently and for a short time only the periportal liver cells contain «inclusions», whereas in those treated for long periods and particularly having had some pauses in the treatment, the affected hepatocytes have a more random distribution throughout the lobule.

Patients treated with cyanamide develop a progressive fibrosis (Moreno et al., 1984). In cases with intense fibroblastic activation, the hepatocytic plates in the periportal area undergo a tubular transformation; «inclusion» bodies are also seen in the liver cells forming part in the lining of the cholangioles (Vázquez et al., 1983a).
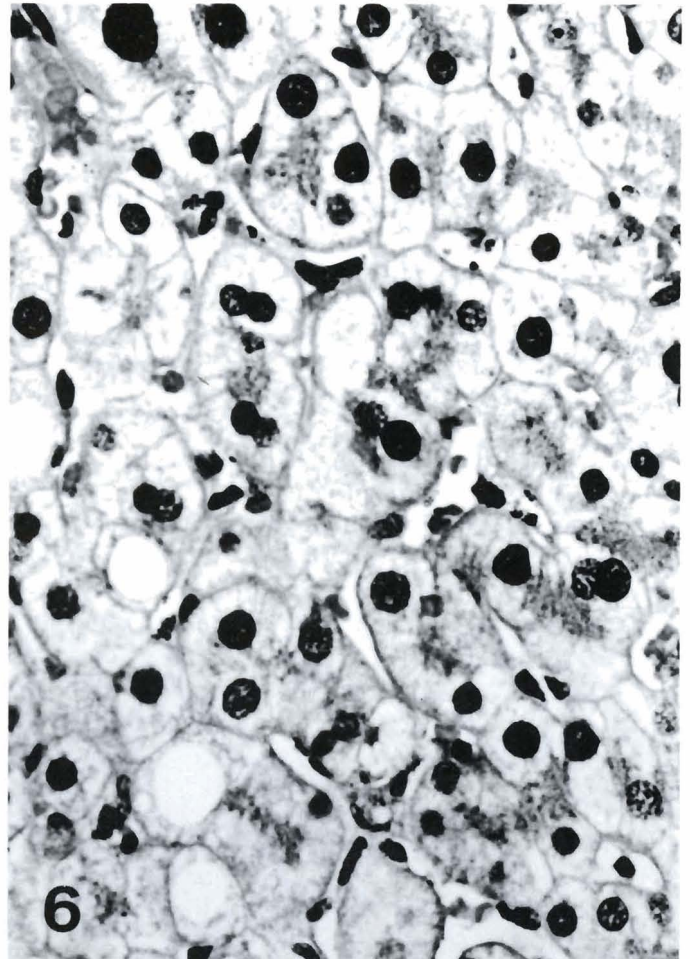
The lesion induced by cyanamide is predictable; that is, it appears in all patients treated with this drug, and is reproducible in the rat liver (Vázquez et al., 1983b; Guillén and Vázquez, 1984).

Two events are particularly important in the development of «inclusion» bodies induced by cyanamide: the storage of a rather abnormal glycogen and the breakdown of organelles. In normal liver cells glycogen is arranged in alfa-granules. On the contrary, in these «inclusions» it is disposed in beta-granules and in some filaments, giving a colloidal iron-positive reaction. The accumulation of such glycogen could be explained by the inhibition of certain hepatic enzymes by cyanamide. In addition, the almost complete disappearance of smooth endoplasmic reticulum is accompanied by the loss of its membrane-bound enzymes. The breakdown of smooth endoplasmic reticulum and mitochondrial membranes inside lysosomes may well result in the whorled lamellar, structures seen by the electron microscopy (Vázquez and Pardo-Mindán, 1979).

The cyanamide-induced «inclusions» eventually

Fig. 7. Electron micrograph taken from a hepatocyte showing smooth endoplasmic reticulum hyperplasia (adaptative change). × 14,400
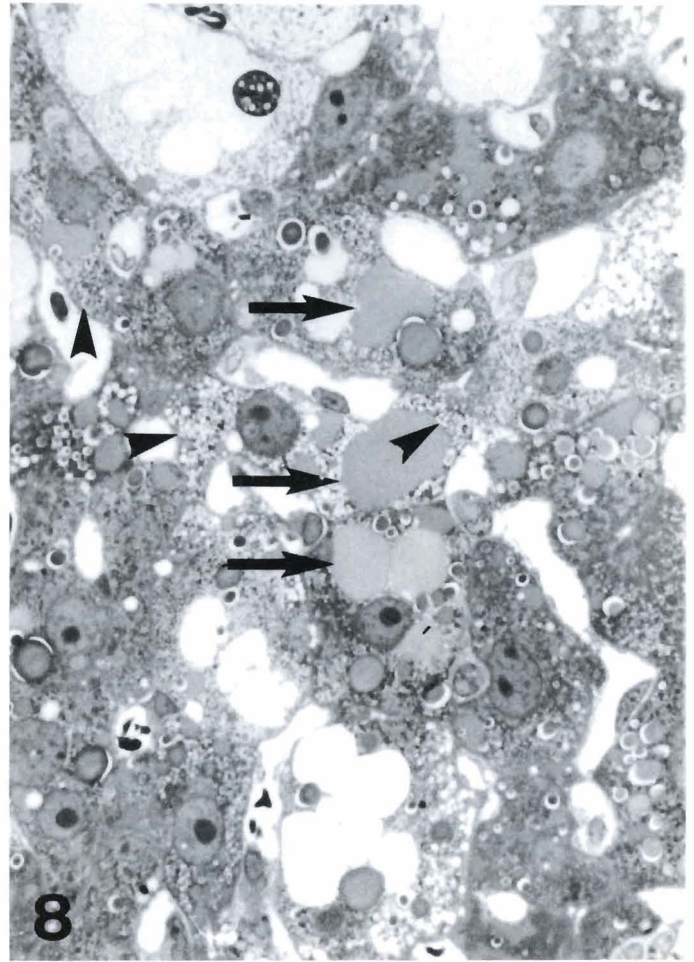

Fig. 8. Liver cells bearing inclusions containing fibrinogen (arrows). In addition, there are other degenerative changes: microvesicular fatty change, smooth endoplasmic reticulum hyperplasia and vesiculation (arrow heads). H & E, × 620

disappear after discontinuing the drug consumption, mainly through a process of removal of the inclusion-bearing liver cells; an event which has been studied in human beings (Thomsen and Reinicke, 1981; Vázquez et al., 1983b; Idoate, 1987) and in rats (Idoate, 1987). The disappearance of «inclusions» in human beings may be expected three years after withdrawal of the drug. In rats it takes place much sooner.

«Inclusion» bodies in Lafora's disease

«Inclusion» bodies described in the liver tissue in cases of myoclonic epilepsy (Austin and Sakai, 1976; Ramón y Cajal et al., 1974) are quite similar to the cyanamide-induced «inclusions» on observation by light microscopy. The affected hepatocytes are also periportal in location. Hepatic «inclusion» bodies in Lafora's disease are usually less well demarcated and are rarely numerous (Fig. 4). They are found in groups of hepatocytes close to the portal space, but never in all periportal hepatocytes as seen in cyanamide-treated

patients at the beginning of the treatment. They give a PAS-positive reaction, although weaker than that of cyanamide bodies, which also disappears after saliva digestion. On electron microscopy the hepatocytes consist of glycogen disposed in beta-granules and some mitochondria that look normal (Fig. 5).

Similar bodies may be found in other organs, particularly in the secretory portion of sweat glands, which makes an easy diagnosis available by means of a skin biopsy.

«Inclusion» bodies in glycogenosis type IV

Quite similar to the previously described bodies are the «inclusions» observed in type IV glycogenosis which is due to branching enzyme deficiency. They are also located predominantly in the acinar zone 1 (Schochet et al., 1970), and in haematoxylin- and eosin-stained sections are faintly eosinophilic or amphophilic and rather granular. As in cyanamide bodies, a clear artifactual halo often surrounds the inclusions. The

Fig. 9. Fibrinogen inclusion. There is a membrane around the homogoneous material. In addition, some lipid vesicles are seen. × 9,000
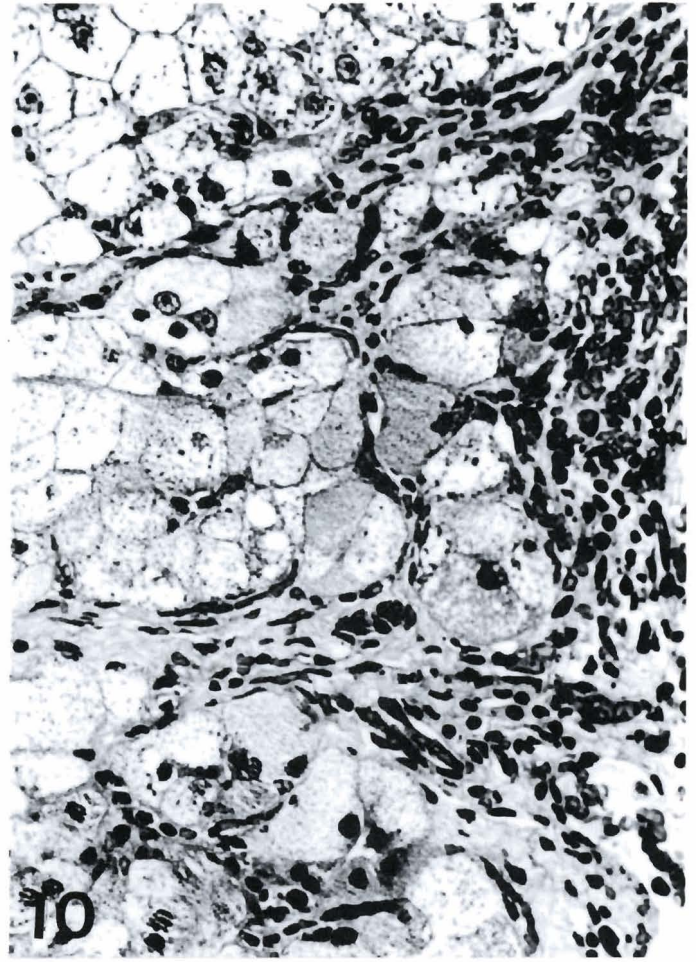

Fig. 10. Needle biopsy taken from a patient with cirrhosis. Some hepatocytes are strongly eosinophilic and granular due to the abundance of closely-packed mitochondria. H & E. × 620

abnormal glycogen which makes up the «inclusion» bodies stains with PAS and colloidal iron. It is only partially digested with diastase, but can be completely digested by pectinase (Reed et al., 1968; Schochet et al., 1970). The lesion is followed by a progressive fibrosis which eventually progresses to cirrhosis. On electron microscopy the inclusions are not membrane-bound and contain fibrillar material.

In addition to the liver «inclusions», basophilic deposits of polysaccharides may be found in muscle fibers, particularly from the tongue.

Although the type IV glycogen storage disease typically manifests itself in infants, with death occurring between the second and fourth year of life (Greene et al., 1988), cases occurring in children and even in adults have been reported (Ferguson et al., 1983; Greene et al., 1987).

Hepatocytes with ground-glass appearance induced by other drugs

Many drugs produce liver cell changes which vaguely resemble the ground-glass hepatocytes (Fig. 6). In this way, they may be considered the so-called adaptative changes observed after long-term treatment with anticonvulsant drugs (Jezequel et al., 1984; Pamperl et al., 1984), phenobarbital or phenytoin (Klinge and Bannash, 1988) rifampicin (Jezequel, 1971; Scheuer, 1974) chlorpromazine (Popper, 1973) and in patients receiving more than one drug (Winkler, 1976). In the cases in which an ultrastructural study was carried out a pronounced increase of smooth endoplasmic reticulum was observed (Fig. 7).

Ground-glass hepatocytes bearing inclusions of different types, induced by two or more drugs may be found in the same patient (Alonso-Martí et al., 1990).

Fibrinogen «inclusions»

Other types of «inclusions» also giving a rather vague «ground-glass» appearance to the cytoplasm are those resulting from intracytoplasmic accumulation of proteins (Pfeifer and Klinge, 1974; Porte et al., 1977; NG et al., 1989), particularly fibrinogen whose deposit was

described both in non-neoplastic hepatocytes (Callea et al., 1986; NG et al., 1989) and in carcinoma cells (Stromeyer et al., 1980). On haematoxilin- and eosin-stained sections the fibrinogen inclusions are round or kidney-shaped, homogeneous and weakly eosinophilic. They give negative reactions with orcein, PAS and silver methenamine. In PAS staining, a rim of glycogen is sometimes observed in the ring of cytoplasm around the inclusions. Immunocytochemically they react for fibrinogen very strongly, giving a granular appearance. The inclusions vary in size and more than one of them may be seen in the same hepatocyte (Fig. 8).

Ultrastructurally, the fibrinogen inclusions described by Porte et al. (1977) and Callea et al. (1986) in non-neoplastic hepatocytes are bound by a membrane which bears ribosomes, considered then as being dilated cisternal of the rough endoplasmic reticulum. The content of this dilated cisternae is an amorphous, granular or slightly fibrillar, moderately electron-dense material. We also observed a membrane around the fibrinogen inclusions in non-neoplastic hepatocytes (Fig. 9). Stromeyer et al. (1980) described a similar alteration in carcinoma cells in three cases of fibrolamellar hepatocarcinoma, but they described the fibrinogen deposits as non-membrane-bound. Nevertheless, the material they studied was previously fixed in formalin and although the ultrastructure was fairly well preserved, the cytoplasmic membranes do not show up well; they appear fragmented. Craig et al. (1980) and Goodman et al. (1985) also described the presence of eosinophilic, hyaline, cytoplasmic PAS-negative globules in cases of lamellar hepatocarcinoma; they did not carry out ultrastructural study.

The intracellular retention of fibrinogen may be the result of a molecular defect which interferes with its transportation towards the smooth endoplasmic reticulum (Callea et al., 1986).

Oncocytic change of hepatocytes

Among other changes in the cytoplasm of liver cells resembling the ground-glass hepatocytes, there is also the oncocytic change sometimes observed in patients having cirrhosis. The cytoplasm has a granular appearance and stains intensively with eosin and acid fuchsin.

Ultrastructurally, large amounts of mitochondria are seen. The hepatocytes with oncocytic reaction are arranged in small groups, very often close to connective tissue septa (Fig. 10).

Differential diagnosis of ground-glass hepatocyte types

The hepatocytic changes observed in HBsAg carriers, in patients on cyanamide, in Lafora's disease and in gly-cogenesis type IV look very similar in paraffin sections stained with hematoxylin and eosin. Nevertheless, they can be differentiated using ancillary techniques (Table 1). On electron microscopy they look unalike.

References

Alonso-Martí C., Moreno A., Barat A., Solera J.C. and Oliva H. (1990). Co-existence of hepatocyte ground-glass inclusions from several causes. Histopathology 16, 304-307.

Austin J. and Sakai M. (1976). Disorders of glycogen and related macromolecules in the nervous system. In: Handbook of Clinical Neurology. Vol. 27. North-Holland. Amsterdam. pp 169-219.

Callea F., de Vos R., Togni R., Taardanico R., Vanstapel M.J. and Desmet V.J. (1986). Fibrinogen inclusions in liver cells: a new type of ground-glass hepatocyte. Immune light and electron microscopic characterization. Histopathology 10, 65-73.

Craig J.R., Peters R.L. and Edmondson H.A. (1980). Fibrolamellar carcinoma of the liver. A tumor of adolescent and young adults with distinctive clinico-pathologic features. Cancer 46, 372-379.

Ferguson I.T., Mahon M. and Cumming W.J.K. (1983). An adult case of Andersen's disease. Type IV glycogenosis. J. Neurol. Sci. 60, 337-351.

Gerber M.A., Hadziyannis S., Vissoulis C., Schaffner F., Paranetto F. and Popper H. (1974). Electron microscopy and immuno-electronmicroscopy of cytoplasmic hepatitis B antigen in hepatocytes. Am. J. Pathol. 75, 489-502.

Goodman Z.D., Ishak K.G., Langloss J.M., Sesterhenn I.A. and Rabin L. (1985). Combined hepatocellular-cholangiocarcinoma. A histologic and immunohistochemical study. Cancer 55, 124-135.

Greene G.M., Weldon D.C., Ferrans V.J., Cheathman J.P., McComb R.D., Brown B.I., Gumbiner C.H., Vanderhoof J.A., Itkin P.G. and McManus B.M. (1987). Juvenile polysacchari-dosis with cardioskeletal myopathy. Arch. Pathol. Lab. Med. 111, 977-982.

Greene H.L., Brown B.I., McClenathan D.T., Agostini R.M. Jr. and Taylor S.R. (1988). A new variant of type IV glycogenosis: deficiency of branching enzyme activity without apparent progressive liver disease. Hepatology 8, 302-306.

Guillén F.J. and Vázquez J.J. (1984). Cyanamide-induced liver cell injury. Experimental study in the rat. Lab. Invest. 50, 385-393.

Hadziyannis S., Gerber M.A., Vissoulis C. and Popper H. (1973). Cytoplasmic hepatitis B antigen in «ground-glass» hepatocytes of carriers. Arch. Pathol. 96, 327-330.

Huang S-N. and Groth V. (1973). Immunoagglutination electron microscopic study on virus-like particles and Australia antigen in liver tissue. Lab. Invest. 29, 353-366.

Idoate M.A. (1987). Mecanismo de regresión de los cuerpos de inclusión inducidos por la cianamida en el hígado. Estudio en el hombre y en la rata. Tesis Doctoral. Facultad de Medicina, Universidad de Navarra.

Jezequel A.M., Orlandi F. and Tenconi L.T. (1971). Changes of the smooth endoplasmic reticulum induced by rifampicin in human and guinea-pig hepatocytes. Gut 12, 984-987.

Jezequel A.M., Librari M.L., Mosca P., Novelli G., Lorenzini I. and Orlandi F. (1984). Changes induced in human liver by long-term anticonvulsant therapy. Functional and ultrastructural data. Liver 4, 307-317.

Klinge O. and Bannash P. (1958). Zur Vermehrung des glatten endoplasmatischen Reticulum in Hepatocyten menschlicher Leberpunktate. Verh. Dtsch. Ges. Pathol. 52, 568-571.

Moreno A., Vázquez J.J., Ruiz del Arbol L., Guillén F.J. and Colina F. (1984). Structural hepatic changes associated with cyanamide treatment: cholangiolar proliferation, fibrosis and cirrhosis. Liver 4, 15-21.

NG I.O.L., Lai E.C.S. and Wu P.C. (1989). Endoplasmic storage disease of liver: characterization of intracytoplasmic hyaline inclusions. Histopathology 15, 473-481.

Pamperl H., Gradner W., Fridrich L., Pointner H. and Denk H. (1984). Influence of long-term anticonvulsant treatment on liver ultrastructure in man. Liver 4, 294-300.

Pfeifer U. and Klinge O. (1974). Intracisternal hyaline in hepatocytes of human liver biopsies. Virchows Arch. (B). 16, 141-155.

Popper H. (1973). Drug induced liver injury. In: The Liver. Gall E.A. and Mostofi F.K. (eds). Williams and Wilkins. Baltimore. Maryland. pp 182-198.

Porte A., Batzenschlager A., Stoeckel M.E., Bockel R., Girard M. and Doffoël M. (1977). Storage of proteins in the rough endoplasmic reticulum of human hepatocytes in a patient with normal blood protein, on oral contraceptives. Virchows Arch. Path. anat. Histol. 375, 241-248.

Ramón y Cajal S., Blanes A., Martínez A., Saénz E. and Gutiérrez M. (1974). Lafora's disease. An ultrastructural and histochemical study. Acta Neuropathol. (Berl.) 30, 189-196.

Reed G.B., Dixon J.E.P., Neustein H.B., Donnel G.N. and Landing B.H. (1968). Type IV glycogenesis. Lab. Invest. 19, 546-557.

Scheuer P.J., Summerfield J.A., Lal S. and Sherlock S. (1974). Rifampicin hepatitis. A clinical and histological study. Lancet 1, 421-425.

Schochet S.S. Jr., McCormick W.F. and Zellweger H. (1970). Type IV glycogenosis (amylopectinosis). Light and electron microscopic observations. Arch. Pathol. 90, 354-364.

Stromeyer F.W., Ishak K.G. and Gerber M.E. (1980). Ground-glass cells in hepatocellular carcinoma. Am. J. Clin. Pathol. 74, 254-258.

Thomsen P. and Reinicke V. (1981). Ground-glass inclusions in liver-cells in an alcoholic treated with cyanamide (Dipsan). Liver 1, 67-73.

Vázquez J.J. and Pardo-Mindán J. (1979). Liver cell injury (bodies similar to Lafora's) in alcoholics treated with disulfiram (Antabuse). Histopathology 3, 377-384.

Vázquez J.J., Díaz de Otazu R., Guillén F.J., Zozaya J. and Pardo F.J. (1983). Hepatitis induced by drugs used as alcohol aversion therapy. Diagn. Histopathol. 6, 29-37.

Vázquez J.J., Guillén F.J., Zozaya J. and Lahoz M. (1983). Cyanamide-induced liver injury. A predictable lesion. Liver 3, 225-230.

Winckler K., Junge U. and Creutzfeldt W. (1976). Ground-glass hepatocytes in unselected liver biopsies. Ultrastructure and relationship to hepatitis B surface antigen. Scand. J. Gastroenterol. 11, 167-170.