Virchows Arch [Pathol Anat] (1984) 405:119–129

Virchows Archiv A

Electron microscopic examination of congenital cytomegalovirus hepatitis

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Summary. The electron microscopic features of cytomegalovirus hepatitis in the liver biopsy of a three-week-old infant were studied.

The liver cells did not contain virus, but severe alterations similar to virus hepatitis were observed. In the bile duct cells, nuclear and cytoplasmic virus inclusions were demonstrated. In the nuclear inclusions virus particles of various degrees of maturity were embedded in dense granular material. The cytoplasm of the infected cells contained vacuoles with mature viruses. The Golgi zone seemed to play an important role in vacuole formation. In another type of infected cell, viruses were lying free in the cytoplasm and passed into the lumen of the bile ducts. It is concluded that viruses are eliminimated by the bile. Based on this electron microscopical observations, the examination of duodenal fluid is recommended as a new diagnostic procedure for demonstrating viruses.

Key words: Cytomegalovirus hepatitis – electron microscopy – bile duct cells – liver biopsy.

Cytomegalovirus infections show an increasing incidence world-wide. There are a large number of symptomless hosts, but the manifest disease is also becoming more frequent, mostly in newborn infants and in immunosuppressed adults (Hamazaki 1983; Lennartz and Piesbergen 1983; Medearis 1964; Nankervis and Kumar 1978). In fetal life, infection occurs diaplacentally (Benirschke et al. 1974; Collaborative study, 1970; Finegold and Karpenter 1982). Intrauterine infection can pass off without any symptoms, may produce a grave septic clinical picture or, after a symptom-free period, severe delayed complications may appear (as e.g. blindness, deafness and mental retardation) (Berenberg and Nankervis 1970; Hanshaw et al. 1976; McCracken et al. 1969).

At present there is still no cure for the disease, however, encouraging attempts have been made at preventing it or curing it by vaccination (Balfour 1983; Elek and Stern 1974; Sarov and Abady 1975; Stinski et al. 1979). A familiarity with, and an exact diagnosis of, the disease is important not only because of the increasing number of case but because the infection may be transmitted by mother's milk, blood transfusion and by saliva (Nankervis and Kumar 1978).

One of the major symptoms of congenital cytomegalovirus disease is hepatitis associated with hepatomegaly and jaundice (Krech et al. 1971; Hamazaki 1983). Light microscopically, it resembles the hepatitis caused by hepatitis viruses (Seifert and Oehme 1957). An exact diagnosis can be made on the basis of the pathogonomic inclusions (Krech et al. 1971; Medearis 1964).

We present a cytomegalovirus hepatitis diagnosed in the liver biopsy of a three-week-old infant. The disease caused the symptoms of a perinatal sepsis. The hepatitis associated with severe histological changes was cured within two months. On follow-up examination, at the age of one-and-a-half years, the child was found to have bilaterally impaired hearing. In the present study the electron microscopic characteristics of the infected bile duct cells are reported for the first time in the literature. The electron microscopic study of cytomegalovirushepatitis has so far been reported only by Donnellan et al. (1966) and by Wills (1972), however, they did not detect virally infected cells.

Case report

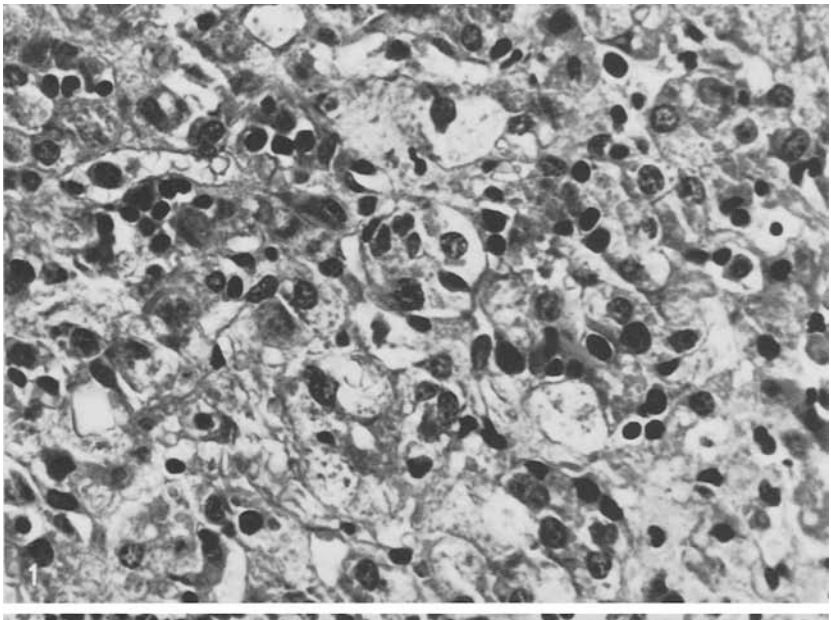
The patient, a pre-term male infant with a birthweight of 2450 g was admitted to the 3rd Department of Medicine of the Apáthy Paediatric Hospital at the age of 24 h. His skin was icteric, there were petechiae and suffusions all over his body. He had marked hepatosplenomegaly. His thrombocytopenia was controlled by repeated administration of thrombocyte suspensions, his grave acidosis by that of 4.2% sodium bicarbonate. During the first three weeks, due to acute circulatory failure, resuscitation was performed on several occasions. The assumed diagnosis was perinatal sepsis.

Bacteriological inoculation was made from all his body orifices and discharges, repeatedly with negative results. The virological examinations excluded the possibility of hepatitis.

At the age of three weeks, owing to persisting jaundice and continuous hepatosplenomegaly, needle biopsy of the liver was performed. The result of histology revealed a severe form of viral hepatitis associated with cholestasis and with persisting extramedullary haematopoiesis (Fig. 1). The electron microscopic examination showed virus particles of a size corresponding to the herpes virus group in the lumen of the bile ductules. Then serial sections of paraffin blocks were made revealing inclusions that contained the characteristic cytomegalovirus in the duct cells (Fig. 2). Diagnosis: cytomegalovirus hepatitis.

Method of electron microscopic examinations

1 mm^3 pieces of liver tissue were fixed in 1 per cent Palade buffered osmium tetroxide, then dehydrated in a graded series of ethanol and embedded in Araldite. The sections were prepared by Reichert's ultramicrotome and photographed by the JEM 100 CX electron microscope. Serial semi-thin sections were made of each block and the infected duct cells were selected from them.



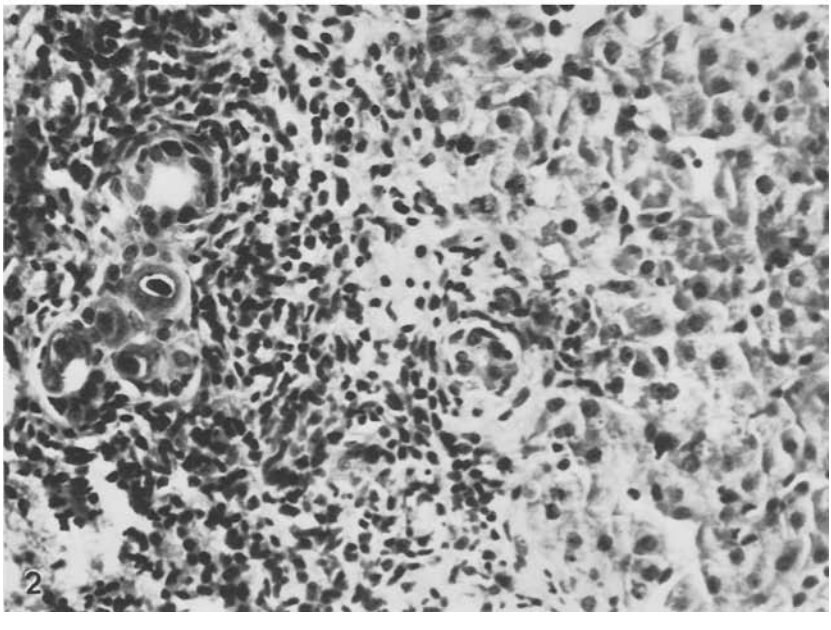
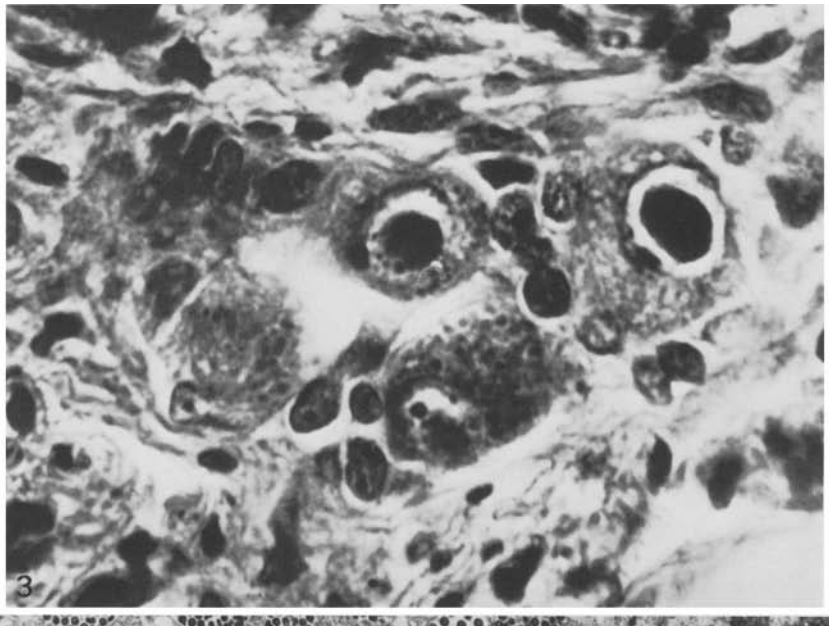


Fig. 1. The light microscopic picture shows the hydropic degeneration and ballooning of hepatic cells, dark-staining Kupffer cells and haemopoietic foci. H and E. × 140

Fig. 2. On the left, cytomegalovirus inclusions of bile duct cells are present, around them, there is a chronic inflammatory infiltration. H and E. × 160



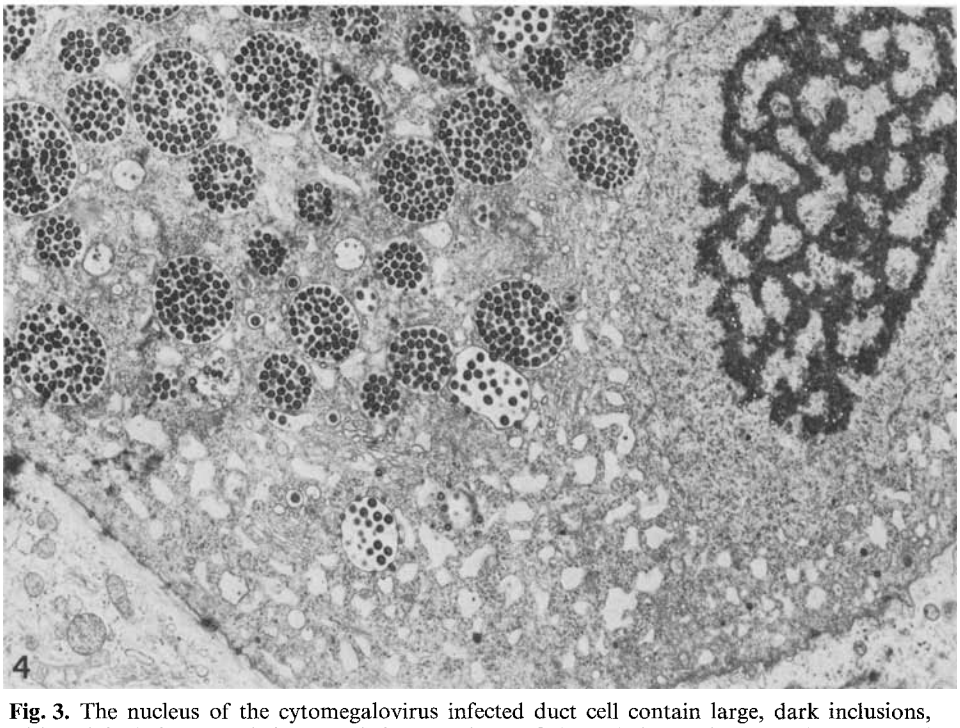


Fig. 3. The nucleus of the cytomegalovirus infected duct cell contain large, dark inclusions, with a light halo around them, and the cytoplasm of the cells is roughly granulated. H and E. $\times 240$

Fig. 4. The electron microsopic picture shows an epithelial cell of the bile duct infected by cytomegalovirus. On the right, the nucleus contains a network of inclusion. In the cytoplasm (on the left) virus-containing vacuoles are present. $\times 8{,}250$

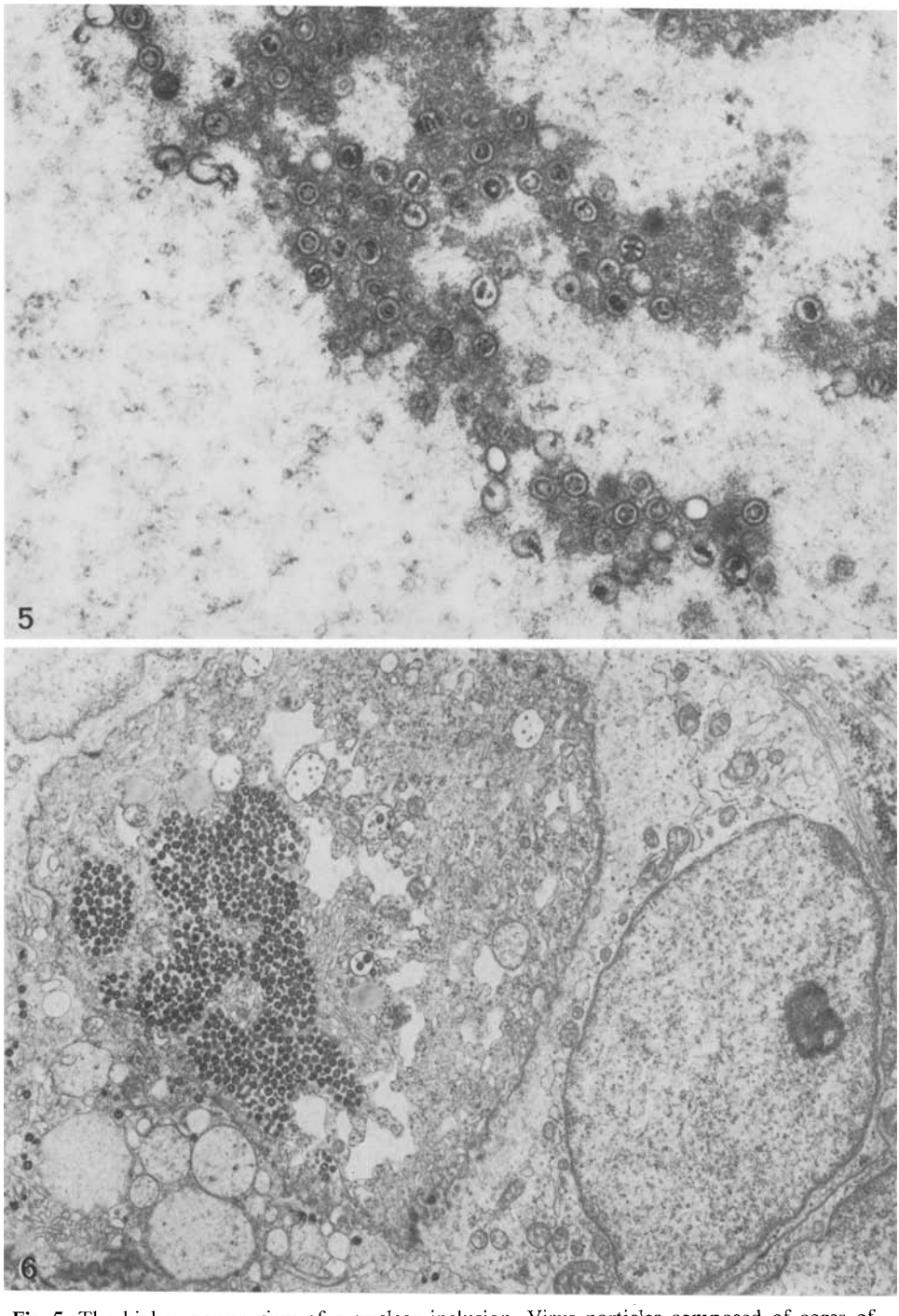


Fig. 5. The higher power view of a nuclear inclusion. Virus particles composed of cores of varying density and of nucleocapsids are embedded in a dense granular substance. × 82,500

Fig. 6. Details of three duct cells. In the cell, on the right, there is no virus infection, the cell in the middle shows virus inclusions near the Golgi zone and dilated endoplasmic reticulum. In the cell on the left, the viruses are lying free in the cytoplasm. × 12,500

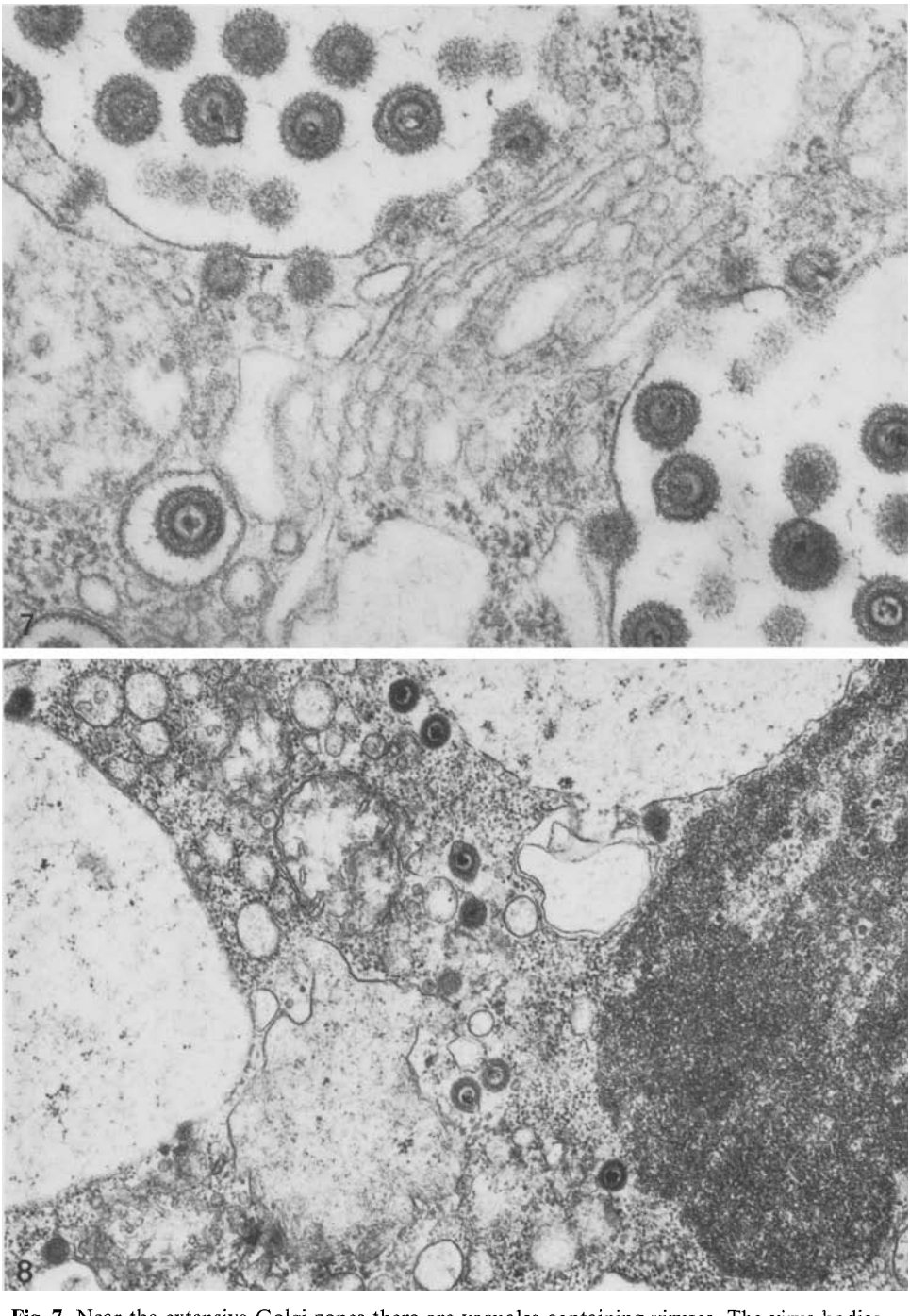
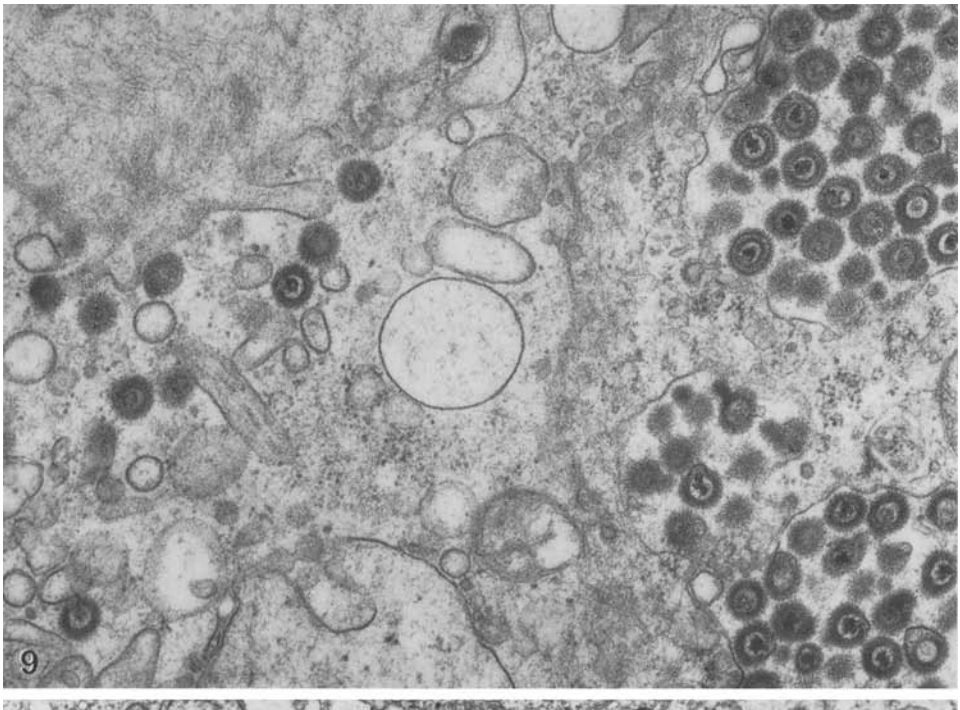


Fig. 7. Near the extensive Golgi zones there are vacuoles containing viruses. The virus bodies can be seen in the process of leaving or entering the vesicles. × 40,000

Fig. 8. In the duct cell, there is a dilatation of the endoplasmic reticulum, the mitochondria are destructed and there are free lying virus particles in the cytoplasm. × 40,000



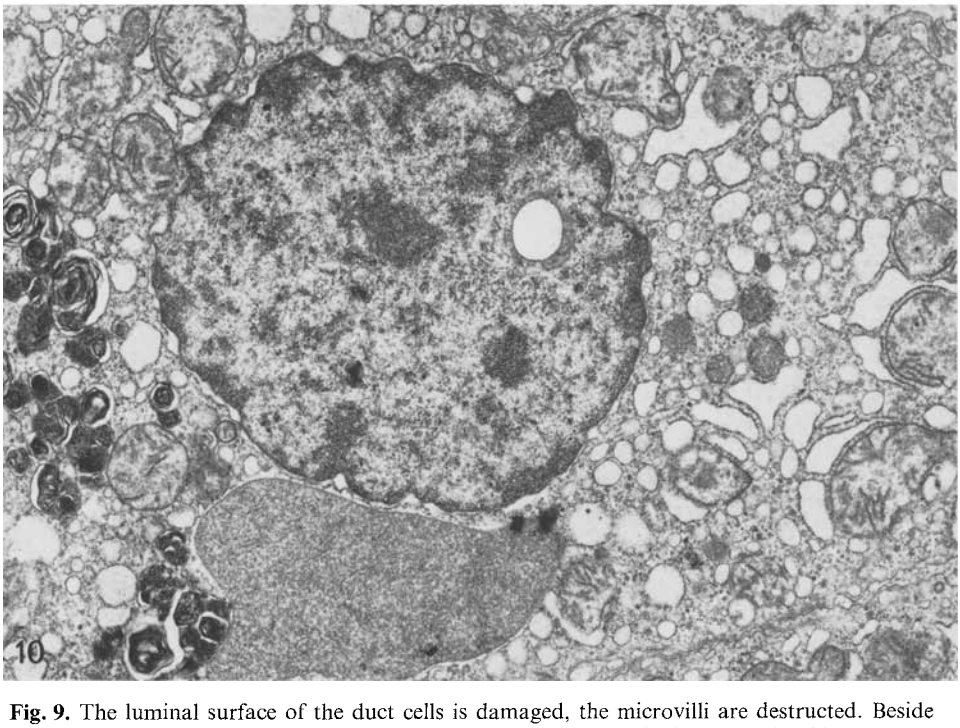


Fig. 9. The luminal surface of the duct cells is damaged, the microvilli are destructed. Beside the cytoplasmic parts there are several mature virus particles in the lumen. × 40,000

Fig. 10. Detail of a liver cell. In the cytoplasm the rough endoplasmic reticulum is distended, the mitochondria are destructed and extensive bile deposits are present. × 12,500

Results

The light microscopic pictures showed a clear halo around the dark inclusions in the nucleus of the infected bile duct cells ("owl's eye" inclusion). The course granulation in the cytoplasm of the cells indicated the cytoplasmic inclusions (Fig. 3).

In electron microscopic examinations, the infected bile duct cells could be easily recognized. They preserved their connection and were attached to the adjacent noninfected cells (Fig. 4). The cells were larger than the surrounding duct cells but they did not obstruct the lumen.

The nucleus retained its oval shape, but, in general, no nucleolus could be seen. The amount of nuclear chromatin had become sparser containing fine, evenly distributed granules. Marginal clumping had disappeared. The nuclear membrane could hardly be recognized. The nucleus contained inclusions occupying about two-thirds of the nucleus. In the inclusions there was a network of dense, granular material in which virus particles of various degrees of maturity were embedded (Fig. 5). These had ring-like capsids of a diameter of 80 to 100 nm and cores of varying shape and density. The cores ranged between 50 and 70 nm in diameter. In some places, fine filaments could be observed around the viruses.

In the cytoplasm, the viruses appeared in two forms. In most of the cells (type I) they were situated in vacuoles surrounded by a single membrane. Within them the viruses possessed a double membrane measuring 120 to 180 nm in diameter (Fig. 6). These cells contained extensive Golgi zones, the vacuoles with one or two viruses had become detached from the Golgi apparatus. The vacuoles fused giving rise to extensive cytoplasmic inclusions. The close link between the Golgi zones and the virus inclusions was striking (Fig. 7).

In the other form of infected cells (type II), the viruses were lying free in the cytoplasm (Fig. 8). Here, no extensive Golgi zones were present. The endoplasmic reticulum was dilated. On the luminal surface, sequestration of parts of the cytoplasm could be observed (Fig. 9).

In the lumen of the bile ductules and ducts free lying mature viruses could be demonstrated. These originated from the infected type II duct cells. The viruses with the separated cytoplasm parts passed into the lumen. Virus particles were also present in the lumen of the greater bile ducts, allowing the conclusion to be drawn that these are eliminated in the bile.

There was no viral infection of the hepatic cells. In the cytoplasm there was a dilatation of the endoplasmic reticulum. The mitochondria were damaged and extensive bile deposits were formed (Fig. 10). The bile canaliculi were normal and contained no bile cylinders. The vascular cell membrane was seriously damaged. In the Disse's space detached cytoplasmic parts could be observed. Phagocyte activity in the Kupffer cells was marked.

Discussion

Electron microscopic study of human cytomegalovirus is has been made on human fibroblast culture (Krech et al. 1971; Ruebner et al. 1965;

Ruebner et al. 1966), and on human diploid amnion-cell culture (Vonka et al. 1976). Only a few researchers have succeeded in demonstrating human cells infected with cytomegalovirus electron microscopically. Donnellan et al. (1966) studied the electron microscopic characteristics of the infected tubular epithelial cells of the kidney, and Martin and Kurtz (1966) in the alveolar epithelial cells of the lung.

An important factor in cytomegalovirus research is that the cytomegalovirus infections of the mouse and the guinea pig seem to have an almost similar course to human infections. The size of viruses has become known as well as the mechanism of replication (Smith and Harven 1973) and the electron microscopic changes in infected cells have been described (Fong and Brigati 1982; Fong and Hsiung 1980; Fong et al. 1983; Ruebner et al. 1966; Smith and Harven 1974). Special attention is devoted to the biochemical examination of viral nucleocapsids and the dense material surrounding the viruses, since their antigen components are assumed to determine the hosts response (Sarov and Abdy 1975; Stinski et al. 1979). The experiments revealed that the infection proceeds in a different way in various tissues (Fong et al. 1983).

Since electron microscopic descriptions of cytomegalovirus-infected bile duct cells cannot be found in the literature, the results of our investigations were compared with those of animal experiments. We found that the infected bile duct cells are similar to the duct cells of the salivary glands of guinea pigs infected with cytomegalovirus (Fong and Hsiung 1980).

We observed that the viruses are surrounded by a dense granular substance in nuclei. The fibrillar substance described in animal experiments was absent. In the cytoplasm, mature viruses could be seen in large vacuoles, surrounded by a single membrane. The Golgi vesicles took part in enveloping the viruses. The close link between the cytoplasmic vacuoles and the Golgi apparatus has been observed by several authors (Donnellan et al. 1966; Ruebner et al. 1965; Smith and Harven 1973 and 1974). In guinea pig cytomegalovirus infection, virus particles containing vacuoles were found only in the duct cells of the salivary glands, they were not observed in the liver, spleen and lungs (Fong et al. 1983).

In other type of the infected duct cells the viruses were lying free in the cytoplasm. Severe destruction of the organelles was seen in these cells. On the luminal surface, sequestration of the cytoplasmic parts were observed. The viruses together with the cytoplasmic processes passed into the lumen of the ductules. Viruses were also present in greater bile ducts where the epithelial cells had not been infected. This suggested that the viruses are eliminated by the bile. Our observation calls attention to the fact that viruses can pass into the duodenal fluid and can be demonstrated here.

In the liver cells, severe changes occurred, similar to those seen in viral hepatitis (Lapis and Schaff 1979). Viruses were absent from the liver cells.

Our conclusions are as follows: 1. In cytomegalovirus hepatitis liver biopsy is not only a reliable diagnostic procedure but offers a good possibility for the detailed study of the infection.

2. Electron microscopic changes in the liver cells correspond to those caused by hepatitis viruses.

3. In the nucleus of the infected bile duct cells, viruses are surrounded by a dense granular substance.

4. In the cytoplasm of the infected duct cells, viruses are present in two forms. Mostly, they are in vacuoles, enveloped by single membrane. The Golgi apparatus plays an important role in forming virus containing vacuoles.

5. In some cells, viruses are found lying free in the cytoplasm and entering the lumen of the bile ducts. The examination of duodenal fluid for demonstrating viruses is recommended as a new diagnostic procedure.

6. In cytomegalovirus infections in guinea pigs, the duct cells of the salivary glands showed similar electron microscopic changes to the human bile duct cells described in this study. The statements of Fong et al. (1983), that guinea pig cytomegalovirus infection is a reliable model for studying the cytopathological implications of human infection, are supported.

Acknowledgement. We thank Dr. V.F. Lukács (Apáthy Pediatric Hospital, Budapest, Hungary) for providing us with the clinical data.

References

Balfour HH (1983) Cytomegalovirus disease: Can it be prevented? Ann Int Med 98:544–546

Benirschke K, Mendoza GR, Bazeley PL (1974) Placental and fetal manifestations of cytomegalovirus infection. Virchows Arch (Cell Pathol) 16:121–139

Berenberg W, Nankervis G (1970) Long-term follow-up of cytomegalia inclusion disease of infancy. Pediatrics 46:403–410

Collaborative Study (1970) Cytomegalovirus infection in the North West of England. A report on a two-year study. Arch Dis Child 45:513–522

Donnellan WL, Cantra-Umporn S, Kidd JM (1966) The cytomegalic inclusion cell: An electron microscopic study. Arch Pathol 82:336–348

Elek SD, Stern H (1974) Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero. Lancet I:1–5

Finegold M, Carpenter R (1982) Obliterative cholangitis due to cytomegalovirus: a possible precursor of paucity of intrahepatic bile ducts. Hum Pathol 13:662–665

Fong CKY, Brigati D (1982) Ultrastructural localization of viral antigen in nuclear inclusions of cytomegalovirus infected Guinea pig cells. Arch Virol 74:125–133

Fong CKY, Hsiung GD (1980) Ultrastructural development and persistence of guinea pig cytomegalovirus in duct cells of Guinea pig submaxillary gland. Arch Virol 64:97–108

Fong CKY, Lucia H, Bia FJ, Hsiung GD (1983) Histopathologic and ultrastructural studies of disseminated cytomegalovirus infection in strain 2 Guinea pigs. Lab Invest 49:183–194

Hamazaki M (1983) Histological study of congenital and acquired cytomegalovirus infection. Acta Pathol Jpn 33:89–96

Hanshaw JB, Scheiner AP, Moxley AW, Gaev L, Abel V, Scheiner B (1976) School failure and deafness after "silent" congenital cytomegalovirus infection. New Eng J Med 295:468–470

Krech U, Jung M, Jung F (1971) Cytomegalovirus infections of man. Karger, Basel, pp 6–17

Lapis K, Schaff Zs (1979) Acute viral Hepatitis. In: Johannessen JV (ed) Electron microscopy in human medicine, vol 8. McGraw-Hill INC, New York, pp 124–136

Lennartz H, Piesbergen H (1983) Clinical and diagnostic aspects of cytomegalovirus infection in the adult. Dtsch Med Wochenschr 37:1403–1406

Martin AM, Kurtz SM (1966) Cytomegalic inclusion disease. An electron microscopic histochemical study of the virus at necropsy. Arch Pathol 82:27–34

McCracken GH, Shinefield HR, Cobb K, Rausen AR, Dische MR, Eichenwals HR (1969) Congenital cytomegalic inclusion disease. Am J Dis Child 117:522–538

Medearis DN (1964) Observations concerning human cytomegalovirus infection and disease. Bull Johns Hopkins Hosp 114:181–211

Nankervis GA, Kumar ML (1978) Diseases produced by cytomegaloviruses. Med Clin North Am 62:1021–1035

Ruebner BH, Hirano T, Slusser R, Osborn J, Medearis DN (1966) Cytomegalovirus infection. Viral ultrastructure with particular reference to the relationship of lysosomes to cytoplasmic inclusions. Am J Pathol 48:971–990

Ruebner BH, Hirano T, Slusser RJ, Medearis DN (1965) Human cytomegalovirus infection. Electron microscopic and histochemical changes in cultures of human fibroblasts. Am J Pathol 46:477–496

Sarov I, Abady I (1975) The morphogenesis of human cytomegalovirus. Isolation and polypeptide characterization of cytomegalovirions and dense bodies. Virology 66:464–473

Seifert G, Oehme J (1957) Pathologie und Klinik der Cytomegalie. VEB Georg Thieme, Leipzig

Smith JD, De Harven E (1973) Herpes Simplex Virus and Human Cytomegalovirus replication in WI-38 cells. J Virol 12:919–930

Smith JD, De Harven E (1974) Herpes Simplex Virus and Human Cytomegalovirus Replication in WI-38 Cells. II. An ultrastructural study of viral penetration. J Virol 14:945–955

Stern H (1972) Cytomegalovirus and EB virus infections of the liver. Br Med Bull 28:180–185

Stinski MF, Mocarski ES, Thomsen DR, Urbanowski ML (1979) Membrane glycoproteins and atigens induced by human cytomegalovirus. J Gen Virol 43:119–129

Vonka V, Anisimová E, Macek M (1976) Replication of cytomegalovirus in human epitheloid diploid cell line. Arch Virol 52:283–296

Wills EJ (1972) Electron microscopy of the liver in infectious mononucleosis hepatitis and cytomegalovirus hepatitis. Am J Dis Child 123:301–303

Accepted August 1, 1984