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Insulitis in Cytomegalovirus Infection in a Newborn Infant

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ABSTRACT

In an infant who died when less than one hour old, with clear morphological signs of cytomegalovirus infection in the liver, kidneys, lungs and pancreas, cell degeneration and necrosis were observed in the islets of Langerhans, with CMV cells and insulitis. Electronmicroscopically all three types of islet cells showed signs of involvement, but this was most distinct in the α_1 cells. No symptoms of diabetes had been observed.

INTRODUCTION

Pancreatitis with or without insulitis has been observed in various virus infections in man, e.g. mumps (1), rubella (2) and Coxsackie virus infection (3, 4). As clinical symptoms of diabetes have often been observed after mumps (5, 6, 7, 8, 9, 10, 11, 12) and in some cases after rubella (13, 14, 15) a relationship between virus infection, pancreatitis/insulitis and symptoms of diabetes has been considered probable. There seems, however, to be no convincing documentation of this complete triad in man, even though such evidence was sought as early as in 1899 by Harris (8) in the first report of diabetes following mumps. Experimental findings in animals, on the other hand, have clearly supported this relationship. Thus, diabetic symptoms have been induced in mice by encephalo-myocarditis virus (EMV), with damage to the islets of Langerhans (16, 17, 18, 19).

Symptoms of diabetes (20) and pancreatic changes (21) have also been demonstrated in spontaneous virus infection, foot-and-mouth disease, in four cows. Besides pancreatitis, extensive destruction of the pancreatic islets was observed in these cases.

In cytomegalovirus (CMV) infections inclusion bodies are said to occur fairly frequently in the pancreas. No electron microscopic observations of islet cell changes appear to have been reported previously. In view of the probable relationship, mentioned above, between virus infection, pancreatitis/insulitis and diabetic symptoms, it was considered that our observation of insular changes also in CMV infection warranted a report.

CASE REPORT

Clinical data (Nordvall)

The mother of the investigated child was 24 years old and primiparous. There were no complications in the first 5 months of pregnancy. In the seventh month hypertension and traces of protein in the urine were noted. Hydralazine (Apresolin®) was given in a dose of 12.5 mg × 3. Also in the seventh month a course of nitrofurantoin (Furadantin®) was given for a suspected infection of the urinary tract.

About 2 weeks before term the mother complained of dull abdominal pain and tenderness over the uterine region, and there was some sparse bleeding per vaginam. Twelve days before the estimated date of delivery she was admitted to hospital. On admission the foetal head was presenting but lay high and mobile in the pelvic inlet. The uterus was tense and painful. The foetal heart sounds were audible, at a rate of 136 beats per min. Partial separation of the placenta or hydramnios and threatening foetal asphyxia were suspected, and Caesarian section was therefore performed on the same day. When the uterus was opened a large quantity of clear, yellowish amniotic fluid poured out. The infant was smaller than is normal for the gestation time, showed signs of life, but did not cry or breathe spontaneously. The heart rate was below 40 beats per min. Despite attempts at resuscitation the infant died 40 min after the Caesarean section.

The infant displayed signs of multiple malformations, with short extremities, bilateral talipes equinovarus and a rather large skull with enlargement of the anterior fontanelle. The palate was cleft far back in the oral cavity and there was some protrusion of the eyeballs. Extensive petechial haemorrhages were seen. The mother showed a negative Wasserman reaction and also a negative complementbinding reaction for Listeria. The Sabin-Feldman dye test gave a value of 1/50 (borderline value).

Autopsy findings (Sundström)

The autopsy was performed within 5 days of death. The body had been placed in formalin within a few hours post mortem.

The child weighed 1 300 g. The crown-to-heel length was 33 cm. Multiple petechial haemorrhages were present in the

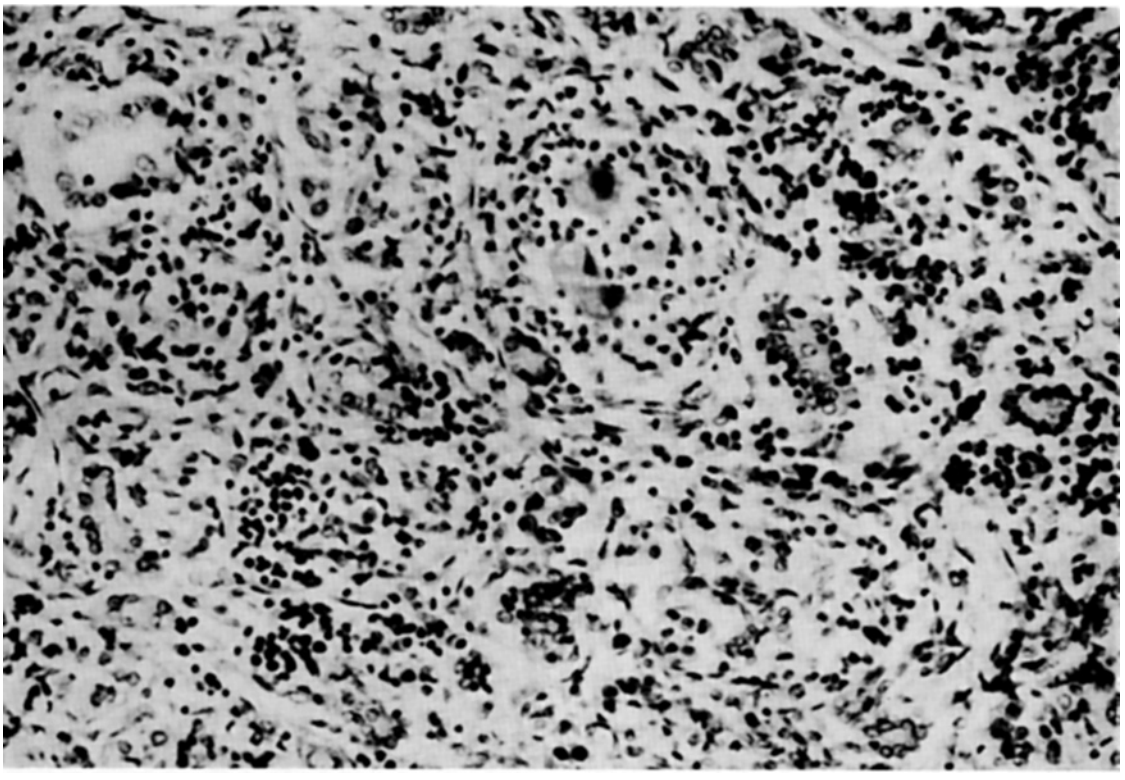

Fig. 1. A group of islets of Langerhans, some exocrine acini and small ducts, with interstitial lympholeucocytic infiltration, at various sites, including close to an islet with three CMV cells. ×130, Weig.–v. G.

skin. The heart (weight 10 g) was normal, with an open ductus arteriosus and foramen ovale. The lungs floated on water but had a rather solid appearance; minor petechial haemorrhages were found both superficially and in the lung parenchyma. The spleen was enlarged (weight 24.5 g) but had a normal gross appearance. Grossly, the kidneys were normal (combined weight 18 g). The left renal pelvis and ureter were somewhat dilated. The left ureter described a rather tortuous course. The liver, pancreas and intestine were normal. Ecchymosis was present in a few places in the meninges. The brain was normal. The placenta was of normal size and appearance. The umbilical cord was inserted near the placental margin.

Microscopically, cytomegalic inclusion bodies were seen in the lungs, liver, pancreas and kidneys. In the lungs minor haemorrhages and small infiltrates of lymphocytes and granulocytes were also noted. A variegated infiltration of inflammatory and haematopoietic cells was observed in the spleen.

Death was considered to have followed from multiple malformations and haemorrhagic diathesis due to intrauterine cytomegalovirus infection.

Examination of the pancreas (Hultquist)

Material that had been fixed in neutral formalin and embedded in paraffin was examined in the light microscope. The stains used were Weigert haematoxylin–van Gieson (Weig.–v. G.), haematoxylin-eosin, paraldehyde-fuchsin–Ponceau fuchsin for β granules, (22) and silver stains for α granules (23, 24).

Electron-microscopic examination was performed on formalin-fixed (form.) material that had been post-fixed in glutaraldehyde (glut.) and OsO_4 and embedded in Epon. After contrast-staining with uranyl acetate and lead citrate, suitable islets were selected from 1 μ-thick sections in a phase-contrast microscope and these were cut on an LKB Ultrotome and examined in a Zeiss EM9 electron microscope at 60 kV.

Light-microscopic examination

The cell changes typical for cytomegalovirus infection, with inclusion bodies, were obeserved in acinar cells, duct cells and islet cells. In the interstitial connective tissue there were inflammatory cell infiltrates (Fig. 1) with abundant lymphocytes and granulocytes, occasional plasma cells and histiocytes. In certain areas the infiltrates were profuse between acini, around ducts and around and within islets, and sparse in other places. Some areas were free from cell infiltrates.

The CMV cells were seen singly or in small groups of two to three in exocrine acini. The islets also showed single or groups of up to four to six CMV cells per islet (Fig. 2). Inflammatory cell infiltation was not always observed in the immediate vicinity of the CMV cells. In the light-microscopic

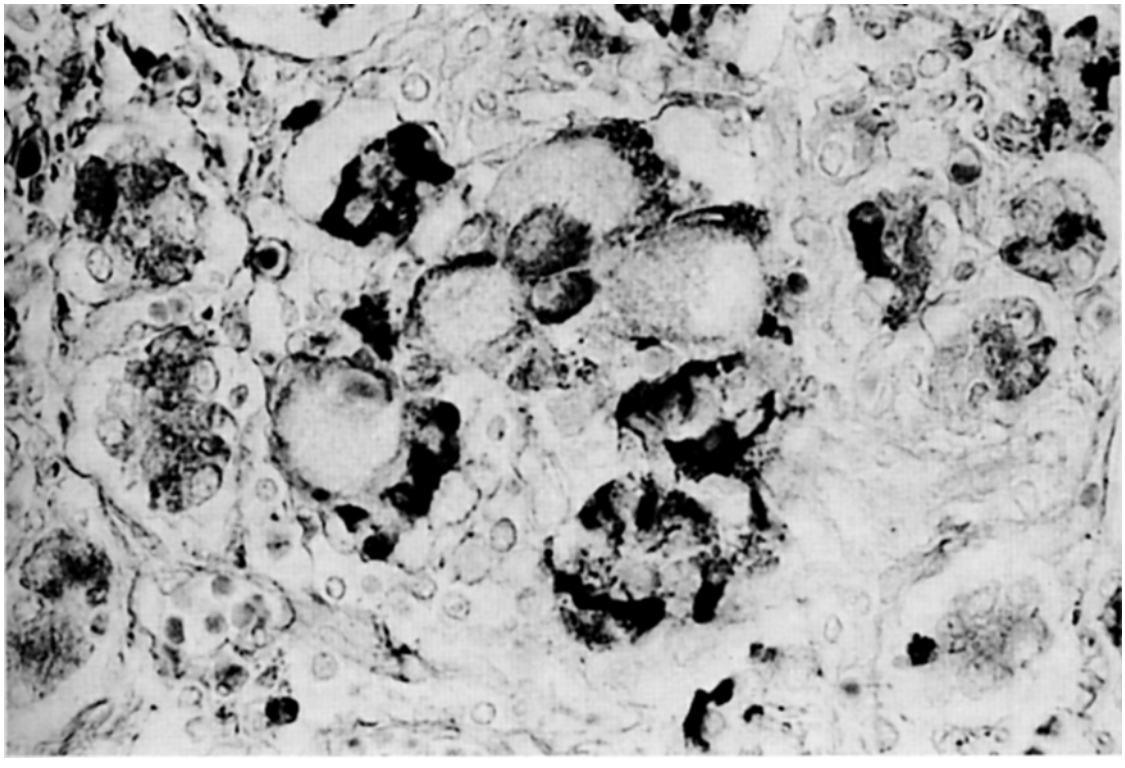

Fig. 2. An islet of Langerhans surrounded by some exocrine acini and occasional small islet fragments. In the large islet there are four CMV cells that occupy almost half of the islet area. Close to some CMV cells there is a crescent-shaped accumulation of silver granules. ×700, Grimelius' staining.

sections it was difficult, with the stains used for β, α_1 and α_2 granules, to ascertain with certainty which cell types were involved. Granules in single CMV cells were possibly of the β type. It was also difficult to demonstrate with certainty silver-positive granules in CMV cells. Some CMV cells were surrounded by a crescent-shaped accumulation of silver granules (Fig. 2), possibly suggesting that the silver-granulated cells had been compressed by the CMV cells. The CMV cells lay mainly in the peripheral parts of the islets and only a few were seen in the centres. The cells were chiefly localized to the mantleformed cell layer that surrounds the central β cell mass and where at this age the α cells are located.

Electron-microscopic examination

In spite of the fact that the examination was performed on autopsy material, fairly well preserved structures were observed, especially islet cell granules but also mitochondria, with quite distinct cristae.

Some regions of the islets displayed necrosis and degenerative changes especially in the endoplasmic reticulum, mitochondria and Golgi complex, and the nuclei were pyknotic. It was generally possible to recognize the type even of such cells. The CMV cells showed the characteristic structure, with intranuclear and intracytoplasmic virus accumulations and a honeycomb inclusion in the nucleus.

Within cells that were greatly changed and contained inclusion bodies, remnants of islet cell granules of the α_1 type were seen only exceptionally. In α and β cells with less severe changes groups of vesicles, usually lying within a vacuole, were seen in the cytoplasm and/or nucleus (Figs. 3, 4). Similar changes were found also close to virus accumulations in CMV cells (Fig. 5) and corresponded to those that have been interpreted as immature virus particles (25). In the cytoplasm of one α_1 cell a few mature virus particles were observed (Fig. 6). In islet cell nuclei, single nuclear bodies of the type that have been observed in human material (26) were seen, but often granules surrounded by a clear zone, i.e perichromatin granules (Fig. 3) that have been described in rats and mice (27) and resemble the dense foci that have been seen in parenchymal cell nuclei in mice infected with cytomegalvirus (25) and dense bodies surrounded by a halo in human renal cells in CMV infection (28).

DISCUSSION

In view of the finding of CMV cells the changes in this case can be said to be pathognomonic for cytomegalovirus infection. They were seen most clearly

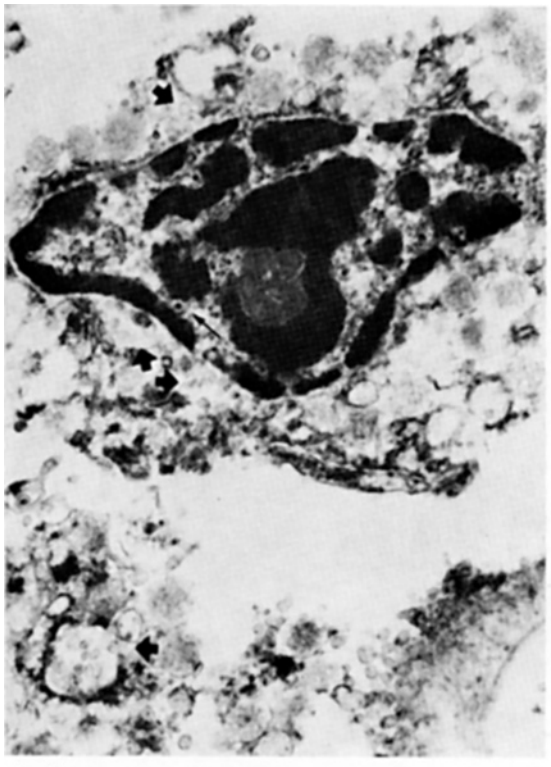

Fig. 3.

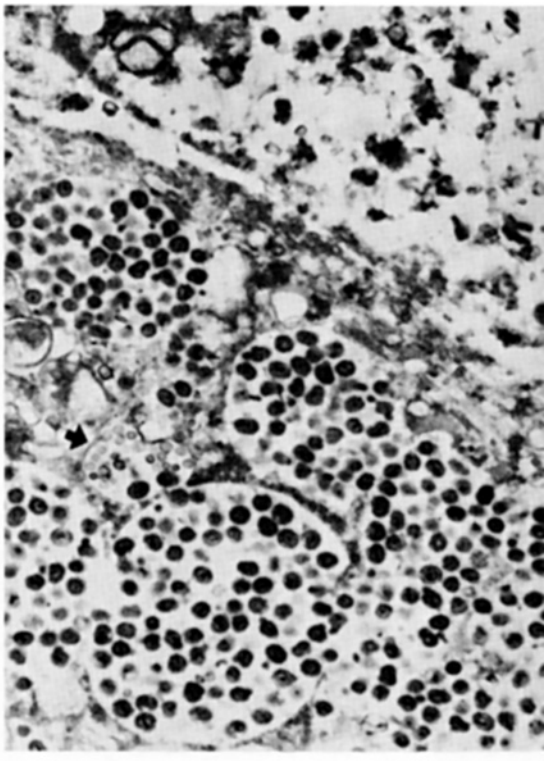

Fig. 5.

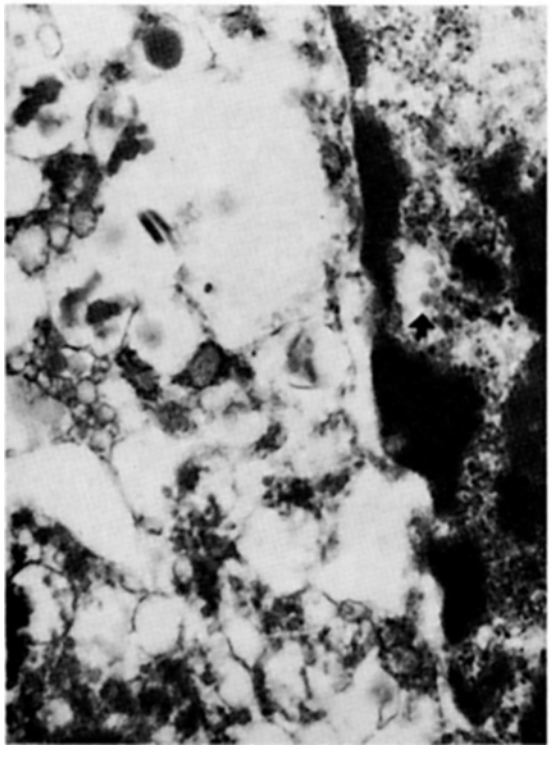

Fig. 4.

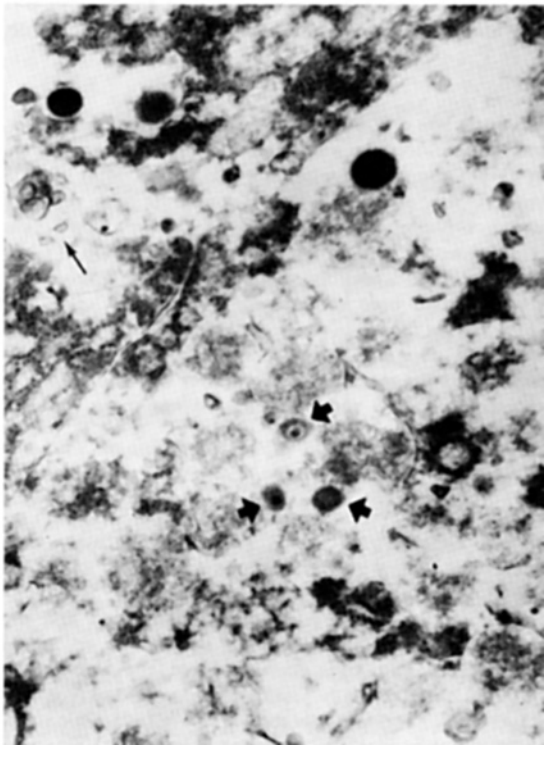

Fig. 6.

in the kidneys, liver and lungs and in the exocrine and endocrine parts of the pancreas.

Of especial interest is the question of what type of pancreatic islet cells were involved and the clinical importance of the changes observed. It seemed probable even from the findings at light microscopy that the CMV changes had also affected the endocrine cells of the islets. CMV inclusions were not identified with certainty in vascular endothelial or stromal cells. The advanced destruction of the CMV cell structure made it difficult to identify details, for identification of the cell type. In some CMV cells, however, structural details were found which closely corresponded to secretion granules in α_1 cells, but not to β or α_2 granules. In islet cells with less severe destruction there were few mature virus particles, and structural details with distinct or probable characteristics of immature virus particles were observed. Such findings applied most distinctly to α_1 cells, but also to β and α_2 cells.

Although their nature is not yet fully established, nuclear bodies have been associated with virus infection. They have most commonly been observed in the nuclei of cells infected with a number of DNA-containing viruses (29). Other origins of nuclear bodies have been discussed and the phenomenon has been claimed to be related to cellular hyperactivity in general (physiological or induced by hormones, drugs, viruses or tumours) (26) or to an altered or special metabolic state of the nucleus (30). It has been considered that the perichromatin granules might also be viruses or in some way related to viruses (25, 27, 28). Nuclear bodies and perichromatin granules were observed in the nuclei of some islet cells from our infant. The value of this finding as a support for the standpoint of virus infection in the cells is difficult to judge in view of the above-mentioned variation in the opinions on their type, pathogenesis and importance.

The findings in this investigation thus indicate that both β and α cells in the pancreatic islets were attacked by virus infection.

The question of the clinical importance of the changes is difficult to answer. Can the pancreatic alterations, especially the insulitis, possibly be sufficient to cause a disturbance of the islet function, giving clinical manifestations of diabetes? The islet cell changes included necrosis, and sometimes there was a corresponding inflammatory cell reaction of considerable strength in numerous islets. On the other hand many islets appeared, on light microscopy, to be relatively well preserved. Since the conditions for electron-microscopic examination were not ideal, it cannot be decided with certainty whether the damage on the ultrastructural level was general. The spectrum of pancreatitic-insulitic changes that according to the literature are seen in diabetes is so broad that there is good reason to assume that the findings in our infant lay within the limits of variation of these changes.

Since it is probable that the risk of functional disturbances in insulitis does not depend solely upon the degree of islet alteration but also on the presence or absence of a genetic factor for diabetes, no definite opinion on the functional importance of the islet changes can be expressed in our case on the basis of the morphological findings alone. Furthermore, because of the short survival time, no clinical symptoms of disturbances of the carbohydrate metabolism had been observed. This case would seem to be of interest in principle, however, as the potentially diabetogenic injurious effect of CMV infection on the pancreatic islets may conceivably be a cause of diabetes in non-fatal cases of intra-uterine CMV infection exhibiting diabetic symptoms.

Fig. 3. Remnants of cytoplasm of an α_1 cell with a partially pyknotic nucleus with some perichromatin granules (*long arrow*). The nucleus is surrounded by secretory granules. Adjacent to the nuclear membrane and elsewhere in the cytoplasm some groups of small vesicles (*short arrows*) with the same appearance as immature virus particles in CMV cells (Fig. 5) are seen. × 14 365, Form.-glut.-OsO_4 fix.

Fig. 4. Part of a β cell. A few secretory granules are seen, some with a rectangular granule core (*top left*). In the nucleus (*right*) there is a vacuole (*arrow*) with some vesicles resembling immature virus particles (Fig. 5). × 26 700. Form.–glut.–OsO_4 fix.

Fig. 5. Part of the cytoplasm of a CMV cell (*bottom*) with numerous, large vacuoles containing mature virus particles and a smaller vacuole containing immature virus vesicles (*arrow*). Part of a β cell (*top*) with no obvious particles seen. × 14 365. Form.–glut.–OsO_4 fix.

Fig. 6. Part of an α_1 cell (*bottom*) and α_2 cell (*top*) with partly indistinct boundaries. In the α_1 cell there are three mature virus particles (*short arrows*) and in the α_2 cell a few irregular vesicles which might be immature virus particles (*long arrow*). × 26 700. Form.–glut.–OsO_4 fix.

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