Reye Syndrome: Light and Electron Microscopic Studies of 7 Cases.

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Histopathological and electron microscopical studies were made on 7 cases of Reye syndrome. Histologically brain edema with neuronal degeneration and fatty change in the liver were constant findings, and fatty change of kidneys, heart and pancreas were variably observed. Electron microscopy revealed characteristic mitochondrial abnormalities in the hepatocytes, proximal convoluted tubular cells of kidneys, pancreatic acinar cells and nerve cells, in which the mitochondria were distended with expansion of matrix space and the matrix substance was converted to granular or flocculent material. Lipid droplets of small size were also observed in the various cells. In the hepatocytes abnormal shaped microbodies were noted and the rough endoplasmic reticulum was dilated. Virus-like particles were not found.

It is suggested that Reye syndrome is not a rare disease-entity in Korea since the cases of Reye syndrome comprised 3.2 per cent of all pediatric autopsies during the same period. The significance and the specificity of the mitochondrial changes in Reye syndrome are discussed.

Encephalopathy and fatty degeneration of the viscera were reported as early as 1929 (Brain et al.), but it is only in the past 15 years that the clinical and pathological features have been clearly defined (Reye et al., 1968), and the concurrence of encephalopathy and fatty degeneration of the viscera has been called “Reye syndrome”.

Clinically, Reye syndrome is characterized by the sudden onset of vomiting, altered consciousness and seizures in a previously healthy child associated with elevated SGOT and often, refractory hypoglycemia and hyperammonemia (Nelson, 1975). The outcome is often fatal within a few days (Bourgeois et al., 1971). The diagnosis has usually rested on histologic findings obtained at autopsy: Edema, neuronal degeneration, and absence of inflammation are found in the brain, whereas in the liver and, to lesser extent, the kidneys and myocardium contain fine cytoplasmic lipid deposits (Dvorackova et al., 1966; Bourgeois et al., 1971).

In spite of great numbers of studies about this syndrome, considerable problems still exist regarding many aspects of the disorder. The etiology remains unknown and the pathogenesis is poorly understood.

Several reports about electron microscopic...
study of Reye syndrome has been presented in the literature (Morales et al., 1969; Thaler et al., 1970; Partin et al., 1971; Chang et al., 1973; Partin et al., 1975; Tang et al., 1975), but there is still much controversy in regard to the ultrastructural changes of the hepatocytes in this disease entity. Many of the investigators have emphasized mitochondrial changes of the hepatocytes as a characteristic ultrastructural feature of Reye syndrome (Morales et al., 1969; Partin et al., 1971; Chang et al., 1973; Partin et al., 1975; Tang et al., 1975); other workers have paid attention to the increased number of microbodies (Thaler et al., 1970) and to the abnormal shape of microbodies in the hepatocytes (Svoboda the Reddy, 1975); and still other workers have insisted on the presence of virus-like particles within the hepatocytes. In the brain several ultrastructural changes, including mitochondrial abnormalities and intranuclear inclusions, have also been noted (Partin et al., 1975).

In view of uncertainty of the etiology and significance of ultrastructural abnormalities in Reye syndrome, we are reporting light and electron microscopic findings on 7 cases of Reye syndrome.

MATERIALS AND METHODS

Seven cases of pathologically proven Reye syndrome were selected from the autopsy file of the Department of Pathology, Yonsei University College of Medicine during the period from 1971 to 1977. Review of the clinical chart, autopsy protocol and microscopic slides was made in every case. In all cases the formalin-fixed tissues were available for the histochemistry to detect the presence of lipid; frozen section was made from the liver, kidneys, heart and brain and was stained with Oil Red O.

Processings for the electron microscopy had been made at autopsy in four cases out of seven. Tissue blocks, measuring about 1 cubic millimeter, from the liver, kidneys, brain, pancreas and adrenals had been fixed in 4 per cent glutaraldehyde, double-fixed by 1 per cent osmium tetroxide, dehydrated by graded alcohol and embedded within Epon-812. The appropriate foci for the electron microscopy were selected by examining the sections of 1 micron-thickness. Ultra-thin sections were made by a glass knife, stained by lead hydroxide and uranyl acetate, and examined by the Electron Microscope, Hitachi HU-11-E.

RESULTS

A. Clinical Manifestations

Of the seven pathologically confirmed cases of Reye syndrome, five were male babies and two were female, and the ages ranged from four months to seven years with the average of 2 years.

All seven cases were transferred to the hospital with suddenly developed neurological manifestations, such as vomiting, dyspnea, convulsions and altered consciousness. Febrile episodes and nonspecific URI-like symptoms were also noted shortly before the neurologic symptoms in some cases. All cases studied went on a rapidly downhill course and died in a very short period of time; the interval between the onset of symptoms and the death was less than three days with two days average (Table 1).

B. Autopsy Findings

The cases studied here were selected from 294 cases of postmortem examination; Reye syndrome represented 2.4 per cent of all autopsied cases and 3.2 per cent of all pediatric autopsies during the years 1971 to 1977.
a. Gross Findings (Table 1):

In every case the liver was mildly to moderately enlarged and appeared yellowish or light brown in hue with a greasy and fatty appearance. Besides the change in the liver, the brain of all seven cases revealed the same findings of interest; the gyri were flattened and widened due to edema of the brain. Four cases had definite tonsillar herniation of the brain and two cases had questionable tonsillar hernia.

Other organs, such as kidneys, heart and other viscera were not remarkable, with some exceptions; a case had pulmonary edema and another showed pneumonitis but these changes were thought not to be related to the main disease.

b. Light Microscopic Findings:

In all cases examined the most striking findings were always presented in the liver and in the brain (Table 2).

Histologically the lobular architecture of the liver was relatively well preserved with some degree of sinusoidal congestion. The hepatocytes throughout the entire lobule had fine vacuolar droplets in their cytoplasm, which did not impinge or displace the nuclei. The size of the vacuoles was not larger than that of the nuclei (Fig. 1). This vacuolar change was generally diffuse throughout the entire lobule with a little tendency to be more severe in the periphery of the lobule. In one case (Case 3), in addition to the vacuolar change, there was a

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Autopsy No.</th>
<th>Sex</th>
<th>Age</th>
<th>Clinical Manifestations (Duration)</th>
<th>Gross Findings at Autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A-71-3</td>
<td>M</td>
<td>2 yrs.</td>
<td>Convulsions, coma, dyspnea (2 days)</td>
<td>Fatty liver, brain herniation, pulm. edema</td>
</tr>
<tr>
<td>2</td>
<td>A-74-28</td>
<td>M</td>
<td>2 yrs.</td>
<td>Convulsions, dyspnea, unconsciousness (1 &amp; ½ days)</td>
<td>Fatty liver, brain herniation, pneumonitis</td>
</tr>
<tr>
<td>3</td>
<td>A-75-44</td>
<td>M</td>
<td>6 mos.</td>
<td>Vomiting, dyspnea, coma, fever (3 days)</td>
<td>Fatty liver, ?brain herniation</td>
</tr>
<tr>
<td>4</td>
<td>A-76-17</td>
<td>M</td>
<td>4 mos.</td>
<td>7 days' URI and diarrhea, semicoma, dyspnea (3 days)</td>
<td>Fatty liver, tonsillar herniation</td>
</tr>
<tr>
<td>5</td>
<td>A-77-23</td>
<td>M</td>
<td>7 yrs.</td>
<td>Convulsions, dyspnea, coma (1 and ½ days)</td>
<td>Fatty liver, uncal and tentorial herniation</td>
</tr>
<tr>
<td>6</td>
<td>A-77-28</td>
<td>F</td>
<td>18 mos.</td>
<td>3 days' URI, vomiting, dyspnea (2 days)</td>
<td>Fatty liver, brain edema</td>
</tr>
<tr>
<td>7</td>
<td>A-77-29</td>
<td>F</td>
<td>6 mos.</td>
<td>Vomiting, convulsions, dyspnea, coma (1 day)</td>
<td>Fatty liver, ?brain herniation</td>
</tr>
</tbody>
</table>
moderate degree of eosinophilic degeneration of hepatocytes, mainly in the periphery of the lobule; the eosinophilic bodies were in the hepatic cords or in the sinusoidal spaces. Otherwise the histology of the liver was not remarkable, i.e., there was no discernible necrosis or definite inflammatory reactions.

The sections from the brain disclosed moderate to severe edema of the brain with anoxic neuronal changes (Fig. 2). The edema of the brain was evidenced by great enlargement of the perivascular and perineuronal clear spaces in the cortex, basal ganglia, hippocampus, and brain stem. No inflammatory cells were present. There were anoxic neuronal changes consisting of shrunken, homogeneous, eosinophilic cytoplasm, and pyknotic nuclei. The cerebral cortex was a region of severe involvement by neuronal degeneration, and in some brains the degeneration was laminar, affecting the third and fifth layers. Sommer’s sector and basal ganglia were also affected. In addition, central

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
<th>Brain</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diffuse fine vacuolation. No infl. or necrosis.</td>
<td>NR</td>
<td>NR</td>
<td>Edema, anoxic changes.</td>
<td>NR</td>
</tr>
<tr>
<td>2</td>
<td>Massive fine vacuolation, diffuse. No infl. or necrosis.</td>
<td>Basal vacuolation in prox conv</td>
<td>NR</td>
<td>Edema, anoxic changes, central chromatolysis.</td>
<td>Focal vacuol. in pancre. acini. aspiration pneumonia.</td>
</tr>
<tr>
<td>4</td>
<td>Diffuse fine vacuolation. Sclerosis of CV.</td>
<td>NR</td>
<td>NR</td>
<td>Edema, anoxic changes.</td>
<td>Interstitial pneumonitis, mild.</td>
</tr>
<tr>
<td>6</td>
<td>Diffuse fine vacuolation. Sl. infiltr. by polys in sinusoid.</td>
<td>NR</td>
<td>NR</td>
<td>?Edema, anoxic changes.</td>
<td>NR</td>
</tr>
<tr>
<td>7</td>
<td>Vacular degeneration, diffuse. No infl. or necrosis.</td>
<td>Granular material in Bowmann’s capsule</td>
<td>NR</td>
<td>Edema, ?anoxic changes.</td>
<td>NR</td>
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</table>

NR : Not remarkable.
chromatolysis was also noted on two occasions.

There were no constant histologic abnormalities in the kidneys, except for one case (Case 6), in which a universal basal fine vacuolation was identified in the epithelial cells of the proximal convoluted tubules (Fig. 5).

The myocardium in all cases was not remarkable histologically, except some degree of congestion and edema in one case (Case 5) which seemed not to be specifically related to the main disorder.

Several other pathologic changes were noted from the various organs, i.e., aspiration pneumonia, interstitial pneumonia, and generalized lymphoid hyperplasia were also found. Interestingly, the case with vacuolar change in the renal convoluted tubules disclosed also foci of fine vacuolar degeneration in the exocrine portion of the pancreas but the vacuoles were not so large as to distort the outer shape of the pancreatic acinar cells (Case 6, Fig. 4). Generalized lymphoid hyperplasia was noted on two occasions (Cases 3 and 5), in which follicular lymphoid hyperplasia with many plump and vacuolated macrophages in the sinusoids was prominent in various lymphoid tissues throughout the body.

The distribution of the lipid in various organs was determined by Oil Red O stain. In every case the highest positivy was noted in the liver, in which most of the hepatocytes and some of the sinusoidal lining cells were stained diffusely throughout the entire lobule with a little tendency to be more marked in the periphery. The Oil Red O positive droplets (lipid droplets) were relatively of small size and did not fuse to form large lipid globules (Fig. 5). Demonstrable lipid droplets were found in the kidneys too, in which the lipid droplets were present mainly in the epithelium of proximal convoluted tubules by trace to mild degree in 6 out of 7 cases (Fig. 6). The glomeruli and collecting ducts were devoid of lipid droplets. The myocardium from the ventricular septum demonstrated the mildest degree of lipid-content with patch distribution in 3 out of 7 cases; the myocardium of the remaining 4 cases was negative for Oil Red O stain. All 5 brain tissues available for lipid-stain were negative for the lipid.

c. Electron Microscopic Findings:

An electron microscopic study was available on the liver, kidneys, and brain in four cases (Cases 3, 5, 6 and 7), and on the pancreas and adrenals in one case (Case 5).

In the hepatocytes, several ultrastructural changes in the subcellular organelles were observed. Numerous lipid-vacuoles of varying sizes were present in the cytoplasm in all cases and even within the nucleus in one case. Mitochondrial changes were of interest; most of the mitochondria were distended with expansion of the matrix space. The mitochondrial cristae were destroyed slightly and intramitochondrial dense granules were always absent. The outer mitochondrial membrane was not ruptured. The matrix substance was converted to granular or flocculent material in almost all mitochondria (Fig. 8). Besides the appearance of lipid-vacuoles and mitochondrial abnormalities, change in the configuration of microbodies (peroxisomes) was found as well.

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Table 3. Results of Oil Red O stain in Reye Syndrome

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<thead>
<tr>
<th>Case No.</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+++</td>
<td>+</td>
<td>±</td>
<td>-</td>
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<td>2</td>
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<td>3</td>
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<td>7</td>
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as an increase in the number of microbodies. Most of the microbodies in the hepatocytes had a low electron-dense matrix and had an unusual noncrystalline centrally located core (Fig. 7). Other organellar changes occurred in the liver parenchyma parallel with the above findings. The rough endoplasmic reticulum was dilated in most cases. In most of cases glycogen seemed to be reduced. The nucleus, Golgi membrane, smooth endoplasmic reticulum and other organelles had no great changes.

The appearance of intracytoplasmic lipid vacuoles and the changes in mitochondria, identical to those seen in the hepatocytes, were also observed in the epithelial cells of the proximal convoluted tubules of the kidneys (Fig. 9 and 10). However there were no such changes in cells other than proximal convoluted tubular cells in the kidneys.

Most of nerve cells from basal ganglia were shrunken and their cytoplasm was moderately electron-dense. Within the electron-dense cytoplasm, scattered swollen mitochondria with flocculent material were found and small droplets of lipid were occasionally present (Fig. 11).

The acinar cells of the pancreas of one case also demonstrated the mitochondrial swelling and flocculent material within the mitochondria which were observed in the hepatocytes, renal tubular cells and nerve cells (Fig. 12), but adrenocortical cells did not show any abnormalities in the mitochondria.

In all cases examined ultrastructurally, there were no virus-like particles in the cytoplasm or within the nucleus of the parenchymal cells of the liver, kidneys or brain.

**DISCUSSION**

After Reye, Morgan, and Baral focused attention on the concurrence of acute encephalopathy and fatty visceral infiltration in certain children with mild upper respiratory tract infections (1963), the pathogenesis of Reye syndrome has been the subject of interesting study (Evans et al., 1970; Morales et al., 1971; Mowat, 1973; Brown and Ishak, 1976). However the etiology or pathogenesis is not understood and current therapy is entirely empirical (Thaler, 1975).

Information gathered from several related areas of investigation suggests the possibility that Reye syndrome may be precipitated in susceptible individuals during exposure to a variety of viruses, toxins, and drugs (Mowat, 1973). Reye syndrome has been linked to infection with influenza B and chickenpox viruses, ingestion of toxins such as hypoglycin A, aflatoxin, and salicylates, and exposure to pesticides or chemical fertilizers. However, it is apparent that only a minute proportion of children coming in contact with these pathogens develops Reye syndrome, suggesting that a susceptible subpopulation may be involved (Thaler, 1975). Recently, Thaler (1975 and 1976) proposed a certain defect in the intramitochondrial portion of urea cycle enzymes as an important predisposing condition. He reported that combined as an important predisposing condition. He reported that combined deficiency of ornithine transcarbamylase and carbamyl phosphate synthetase might occur in most cases of Reye syndrome, whereas isolated ornithine transcarbamylase deficiency due to inherent errors in enzyme structure appeared to be the underlying abnormality in other patients.

Considering that Reye syndrome comprised 2.4 per cent of the total cases of autopsy and 3.2 per cent of pediatric autopsies performed during the same period of this study, it could be said that Reye syndrome is a relatively fre-
quent condition in Korea. The macroscopic findings at autopsy were the same as those previously described in the literature, including edema with or without herniation of the brain, neuronal degeneration, fatty liver of fine droplet nature, fatty change of proximal convoluted tubules of the kidneys, and sometimes fatty change in myocardium or pancreas.

Ultrastructurally the following findings were observed in the present study. Besides the appearance of lipid vacuoles, interesting changes in the mitochondria were noted in the hepatocytes, epithelium of the renal proximal convoluted tubules, nerve cells of basal ganglia and in the pancreatic acinar cells. The mitochondria were swollen and contained electron-dense flocculent material but did not rupture. Microbodies were increased in number in the cytoplasm of the hepatocytes and some of the microbodies were of abnormal shape, i.e., they had centrally located non-crystalline cores.

Morales et al. (1969) were the first to report the ultrastructural changes in Reye syndrome and they said that mitochondrial changes and dilatation of the endoplasmic reticulum were noted in the liver cells. Many investigators described mitochondrial changes similar to our findings in the liver, nerve cells and in the heart, and Partin et al. (1971) reported that the mitochondrial changes were reversible when the patient survived. The floccular degeneration of the mitochondria was strongly suggested as a characteristic of Reye syndrome by Chang et al. (1973) who had made electron microscopic observations upon an autopsy case. However Partin et al. (1975) criticized the ultrastructural alterations from autopsy material because they reflected the sum of severe injury due to the disease process and the effects of post-mortem changes. However, mitochondrial changes such as those seen in the present study have been reported from biopsy materials (Tang et al., 1975) as well as from autopsy materials (Chang et al., 1973). With regard to the mitochondrial damage, Thaler (1976) proposed that an inherited or acquired defect in ammonia metabolism located in mitochondria might play a major role in the pathogenesis of fatty liver, hyperammonemia and encephalopathy in Reye syndrome, and we agree with the idea that mitochondrial damage is profoundly related to the pathogenesis of Reye syndrome.

Some of the investigators also described changes in the number and shape of microbodies in Reye syndrome but the importance of these changes in the microbodies has not been clarified. Microbodies (peroxisomes) contain enzymes involved in the metabolism of hydrogen peroxide (DeDuve and Baudhuin, 1966). This organelle proliferates when the hypolipemic agent ethyl-a-chlorophenoxyisobutyrate is administrated to rats (Svoboda et al., 1969). However, the role of peroxisome in the lipid metabolism must await further elucidation.

Other investigators disclosed that some virus-like particles and intranuclear inclusions (Partin et al., 1975) were observed ultrastructurally. Tang et al. (1975) confirmed the presence of herpes-like virus and myxovirus/paramyxovirus in Reye syndrome by electron microscopy combined with isolation and serologic tests. However we were not able to find any inclusions or virus-like particles by electron microscopy.

Our studies confirmed previously reported ultrastructural changes in Reye syndrome. However, the specificity of the findings and their significance in the pathogenesis of Reye syndrome remain to be determined by future studies.
REFERENCES


Brain WR, Hunter H, Turnbull HM: Acute meningo-encephalomyelitis of childhood. Lancet 1: 221, 1929


Morales AR, Bourgeois CH, Chulacharit E: Pathology of the heart in Reye's syndrome (encephalopathy and fatty degeneration of the viscera). Amer J Cardiol 27: 314, 1971


Fig. 1. Histologic picture of the liver in Case 5. The hepatocytes reveal diffuse fine intracytoplasmic vacuolation. H-E stain, 400x

Fig. 2. Histologic picture of the brain in Case 6. Perineuronal and perivascular clear space is greatly widened due to edema, and neuronal degeneration is evident. H-E stain, 400x

Fig. 3. Photomicrograph of histologic section of the kidney in Case 2. Subnuclear clear vacuolation is regularly present in convoluted tubular epithelium. H-E stain, 400x

Fig. 4. Photomicrograph of histologic section of the pancreas in Case 2. The pancreatic acinar cells contain vauolar droplets within their cytoplasm. H-E stain, 400x

Fig. 5. Photomicrograph of Oil Red O stain of the liver in Case 3. All of the hepatocytes show diffuse positivity by small droplet-type. Oil Red O stain, 100x

Fig. 6. Photomicrograph of the kidney stained for fat in Case 6. The tubular epithelium disclosed mild degree of positivity. Oil Red O stain, 400x
Fig. 7-8. Electron micrographs of the hepatocyte in Case 3 (Fig. 7) and Case 5 (Fig. 8). Mitochondria (M) are swollen and contain flocculent material (arrows). An abnormally shaped peroxisome (P) with noncrystalline central core is noted. Original magnification, 22,500x.

Fig. 9-10. Electron micrographs of the proximal convoluted tubular epithelium of the kidney in Case 6 (Fig. 9) and Case 7 (Fig. 10). The nucleus is not remarkable. In the cytoplasm there are increased lipid vacuoles (L) and swollen mitochondria with flocculent material (arrows) like those seen in the hepatocytes. Original magnifications, 22,500 and 30,000x, respectively.
Fig. 11. Electron microscopic picture of a nerve cell (Case 5). The cytoplasm is shrunken and electron-dense. Lipid material (L) is present and abnormal mitochondria (arrows) are seen in the cytoplasm. Original magnification, 30,000x

Fig. 12. Electron microscopic picture of the pancreatic acinar cell (Case 5). Swollen and flocculent material-containing mitochondria (M and arrows) are also seen in the cytoplasm. Original magnification, 10,700x