Chemical Studies in Gargoylism*

JØRGEN CLAUSEN, HOLGER V. DYGGVE, JOHANNES C. MELCHIOR, AND HANS OLAV CHRISTENSEN LOU

From the Neurochemical Institute, Danish Multiple Sclerosis Society, the University Clinic of Paediatrics, Rigshospitalet, Copenhagen, and the State Institution for Mentally Retarded 'Andersvaenge', Slagelse, Denmark

Recent studies of the chemical structure of glycolipids (Klenk and Gielen, 1961, 1963; Svennerholm and Raal, 1961; Kuhn, 1961; Wolfe and Lowden, 1964) have contributed to our knowledge of the metabolism of different glycolipids. Thus, a low concentration of \( \beta \)-glucosidase in human brain and other organs will give rise to a blocked degradation of gangliosides, with accumulation of glucocerebrosides, the disease associated with this enzymatic defect having previously been named Gaucher's disease (Gaucher, 1882; Philippart and Menkes, 1964; Brady, Kanfer, and Shapiro, 1965a,b; Svennerholm, 1966).

Again, the glucocerebrosides in brain may be transformed by means of specific enzymes to galacto-cerebrosides. This ceramide, as well as galacto-glucoceramide, may be sulphated by means of active sulphate (Svennerholm, 1966), to give rise to sulphatides which in their turn are degraded by the action of sulphatases (Mehl and Jatzkewitz, 1963, 1964), so that absence of the enzyme sulphatase, as occurs in metachromatic leucodystrophy, results in accumulation of the sulphatides (Jatzkewitz, 1960; Sourander and Svennerholm, 1962; Hagberg, Sourander and Svennerholm, 1962; Austin, McAfee, Armstrong, O'Rourke, Shearer, and Bachhawat, 1964).

Finally, the initial splitting of \( N \)-acetyl-neuraminic acid (NANA) may be impaired, as is the case in Tay-Sachs' disease (Klenk, Liedtke, and Gielen, 1963), when mainly mono-sialo-gangliosides accumulate.

Accumulation of carbohydrate derivatives occurs not only in the lipidoses but also in the mucopolysaccharidoses (Melchior, Clausen, and Dyggve, 1965). Thus, in Hurler's syndrome (gargoylism) there are accumulations of chondroitin sulphate B and heparitin sulphate (Brante, 1952; Bishton, Norman, and Tingey, 1956), and in Morquio-Ullrich's disease chondroitin sulphate B and keratosulphate and/or hyaluronic acid-like substances are stored and excreted in increased amounts (Zellweger, Ponseti, Pedrini, Stamler, and von Noorden, 1961; Clausen, Dyggve, and Melchior, 1963).

Because of the similarity between acid mucopolysaccharides (AMP) and polysaccharide moieties of glycolipids, pathological changes in the synthesis or breakdown of one or more of the carbohydrate constituents may cause changes in the other. Following on our previous studies (Dyggve, Melchior, and Clausen, 1962; Clausen et al., 1963), this communication gives some data concerning these two groups of carbohydrate-rich substances in two cases of Hurler's syndrome. These data suggest a metabolic defect common to the katabolism of both AMP and glycolipids in this disease.

Case Reports

Case 1. A boy, born September 1949, died aged 14\( \frac{1}{2} \) years. His case history has been published in detail by Clausen et al. (1963). There are no known similar diseases in the family. Following a normal infancy he deteriorated gradually into a typical case of gargoylism. He died from pneumonia and heart disease. Repeated slit-lamp examinations revealed no corneal opacities. No abnormal inclusions were found in his lymphocytes. EEGs on several occasions were moderately abnormal, with an increased amount of slow activity. Pneumencephalography at the age of 5 years showed a symmetrical dilatation of the lateral ventricles, with an Evans ratio of 0.35. Radiological examination showed thickening of the skull bones and typical changes of the spine and the hands. The spinal fluid protein was 44 mg./100 ml., with no cells.

 Necropsy (Dr. P. Christoffersen). The boy's appearance was typical of gargoylism with coarse facial features and extremities and a short neck. The cranium was enlarged with thickened bones measuring maximally 15 mm.

The heart was diffusely enlarged, weighing 240 g.
The myocardium was hypertrophic, the thickness of the left ventricle being 14 mm. The aortic and mitral valves were thickened but without vegetations. The coronary vessels were normal. Distally, in the aorta small yellow sub-intimal plaques were found. The liver (1540 g.) was enlarged and measured 17 × 17 × 7 cm.; the surface and the cut sections were yellow. The spleen (290 g.) was also enlarged measuring 14 × 8 × 6 cm. The lungs showed bronchopneumonia.

Histologically, there was ballooning of the liver cells, with eccentrically placed nuclei. There was a strong positive reaction to Sudan-staining, but only a slight reaction to PAS. In the myocardium there were infiltrations of lymphocytes, with oedema, and in the aorta there was also some lymphocyte infiltration. The skin showed oedema of the corium where some PAS-positive substance was seen. A lymph node revealed ballooned cells with PAS-positive substance.

The brain (Dr. Erna Christensen) showed some atrophy, with an increased amount of spinal fluid; the weight after removal of the right occipital lobe and after fixation was 920 g. The gyri were small and the sulci enlarged. The basal arteries were normal. On frontal section the ventricular system, including the 3rd ventricle, was seen to be much dilated. The white matter appeared underdeveloped and showed a great number of lacunae of up to 3 mm. Histologically, there were severe changes throughout the whole central nervous system. The layers of the cortex were poorly marked and the ganglion cells were ballooned, with a strong PAS-positive reaction and deposits of sudanophilic substance intracellularly. The lacunae contained coagulated fluid and were surrounded by perivascular proliferation of connective tissue, and macrophages. The myelination of the white matter was normal. The ependyma was lacking in several areas and the remaining showed some granular ependymitis. Throughout the brain the Purkinje cells were swollen and contained sudanophilic and PAS-positive material; these changes were less pronounced in the distal parts of the medulla and in the spinal cord.

No metachromasia was found by staining with toluidine blue and cresyl violet. The Bielschowsky staining for axis cylinders showed swollen neurites. The leptomeninges were thickened over large parts of the brain, with oedema and moderate amounts of lymphocytes, and macrophages containing PAS-positive and sudanophilic substances. Histological diagnosis was gargoylism.

Case 2. A boy, born December 1958, birthweight 3900 g. (for further details see Case 3 of Steiness, 1961), died when 51 years old. Two older sibs are healthy. Pregnancy and delivery were normal. At the age of 6 months the possibility of gargoylism was raised and the diagnosis was established at the age of 9 months, at which time an abnormal excretion of acid mucopolysaccharides was found. His mental and physical development was increasingly retarded, requiring admission to an institution for the mentally retarded where he died.

Necropsy (Dr. H. Wolthers). The facial appearance was typical of gargoylism with thick lips, a square cranial appearance, and a small body. The lungs showed an increased amount of mucopurulent secretion in the bronchi. The heart measured 7 × 7 cm., and the thickness of both ventricles was increased. The walls of the aorta were thickened and somewhat fibrotic, resembling the arteriosclerotic changes in older people. The liver measured 25 × 15 × 5 cm., the spleen 9 × 6 × 3 cm. The cranium showed thin bones. The subarachnoid fluid was increased. The vessels on the lower surface of the brain were normal. On section a fairly pronounced dilatation of the ventricular system was found, especially involving the lateral ventricles, and there was some swelling of the brain tissue. No histological examination was performed.

The diagnosis from the necropsy was gargoylism. During the illness urinary specimens drawn from 24-hour collections were used for qualitative and quantitative estimations of AMP. Necropsy specimens were taken 12 and 16 hours, respectively, after death, from the following organs: brain, liver, spleen, and kidney. These specimens were analysed for their content of polar lipids and AMP.

Methods

The AMPs present in urine were screened by paper electrophoresis of urine concentrated 100 times (Clausen and Rosenkast, 1962; Asboe-Hansen and Clausen, 1964; Dyggve et al., 1962; Clausen et al., 1963). The electrophoresis was performed in 0-2 M LiCl (pH 2-8) and the pattern was stained for AMPs with Alcian blue (Foster and Pearce, 1961). The total content of AMP in urine was estimated by content of hexosamine and uronic acid bound to AMP. AMP and some uromucoid were precipitated with cetyl-pyridium bromide (CPB), followed by an estimation of hexosamine, uronic acid, and fucose in the precipitate. Because of the known fucose/hexosamine ratio of uromucoid, the hexosamine of AMP can be estimated (Clausen and Asboe-Hansen, 1966). The content of AMP was thus expressed as mg. hexosamine and uronic acid excreted per 24 hours.

The AMP in the necropsy specimens was similarly determined by the total amount of hexosamine and uronic acid present per mg. extractable protein. However, the hexosamine value thus expressed also covers the CPB-precipitable gangliosides and glycoproteins of varying composition. The fucose/hexosamine ratio was therefore unknown, and the fucose content of the CPB precipitate (mg./mg. protein) was only used as an indicator for the total CPB-precipitable glycoprotein. The polar lipids of the necropsy specimens were extracted with chloroform : methanol (2 : 1) and separated into individual fractions by means of thin-layer chromatography (Clausen, Christensen Lou, and Andersen, 1965). The distribution of the sialic acid-free polar lipids was evaluated by photometric scanning of the chromatoplates. The patterns of polar lipids were developed quantitatively by spraying with ammonium-molybdate perchloric acid (Christensen Lou, Clausen, and Bierring,
The values obtained were correlated with those of normal necropsy specimens from adults.

The identification of the glycolipids was performed by infrared analysis of the fractions isolated from quantitative thin-layer chromatography (Clausen, 1966). The glycolipids were further characterized by their resistance to a 24-hour treatment (37°C) with 0.5 N KOH. The abnormal glycolipids visualized on thin-layer chromatography (see below) may be isolated by preparative chromatography (Christensen Lou et al., 1965). However, for analytical purposes, the abnormal glycolipid fraction found in liver, spleen, and kidney was isolated by column chromatography on silicic acid (O'Brien and Sampson, 1965). This revealed the abnormal glycolipid fraction to be eluted in pure state in Case 1 by chloroform + methanol (19 + 1) and in Case 2 by chloroform + methanol + H₂O (4 + 1 + 0.5% H₂O). The glycolipid fraction was hydrolysed with 4N HCl. The liberated carbohydrates were identified qualitatively by paper-chromatography, using 5% aqueous solutions of glucose, galactose, glucosamine, galactosamine, lactose, and maltose as standard markers (Schultz, Schmidt-berger, and Haupt, 1958). After 16 hours of developing, the papers were stained by spraying with anilinium phthalate (Schultze et al., 1958). Quantitatively the hexose/hexosamine ratio was determined by quantitative determination of hexose by the orcinol test and hexosamines by the Ehrlich's reaction (Clausen and Asboe-Hansen, 1966).

The fatty acid composition of the isolated glycolipid fraction (10 mg.) was evaluated by gas-chromatography of the methyl esters. The fatty acids of the glycolipid were saponified with 2 ml. 1 N KOH in 96% ethanol for 30 minutes as described by Rathbone (1965). The fatty acids were liberated by addition of 1.5 ml. 2 N HCl + 5 ml. H₂O. In a separating funnel the fatty acids were transferred to 10 ml. ethyl ether and washed three times with 10 ml. 0.1 N HCl. The ether phase was dried above CaCl₂ for 16 hours and concentrated to dryness below N₂. The fatty acids were afterwards methylated with 6 ml. super-dry methanol saturated with dry HCl gas. 250 μl. dry benzene was added as catalyst. Methylation was performed for 30 minutes at 80°C in sealed bottles. The methyl esters were extracted and washed as described above. Finally the esters were concentrated by evaporation of the ether below a stream of N₂. Gas-chromatography was performed on 0.2 μl. methyl esters in a Perkin-Elmer flame-ionization gas-chromatograph F-11-(Clausen, 1966, in preparation) (column dimensions 2 m. x 1 mm., stationary phase 8%, 1-4 butanediol succinate on Chromosorb W (mesh 80-100)).

Chromatograms were analysed by triangulation and the proportions of individual fatty acids were expressed as a percentage of the total. The fatty acids were identified by log plotting and by means of standard markers (Hormel Institute, Minnesota, U.S.A.). The sialic-rich glycolipids (gangliosides) were separated by means of thin-layer chromatography, using chloroform : methanol + aqeous NH₄ (25%) (70 + 30 + 5) as an ascending system. The pattern was developed with resorcinol-HCl reagent for glycolipids as described by Svenner-holm (1963). The gangliosides were developed with a violet colour, other glycolipids with a brownish colour.

**Results**

**Investigations of urine specimens.** In Case 2 paper-electrophoresis of urine concentrated 100 times revealed one fraction of AMP with the mobility of chondroitin sulphate (Fig. 1). Quantitative estimation of AMP in a 24-hour specimen of urine revealed a 20-fold increase in the amount of AMP-bound hexosamine and of AMP-bound uronic acid (Table I). In Case 1 also, as previously reported by Dyggve et al. (1962) and by Clausen et al. (1963), a fraction with the mobility of chondroitin sulphate was found, which, however, extended towards the heparin fraction. No quantitative estimation was performed in this case.

**TABLE I**

<table>
<thead>
<tr>
<th>Age (yr.)</th>
<th>Acid Mucopolysaccharide Excretion in Urine in Normal Subjects and in Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetyl Mucopolysaccharide Hexosamine (mg./24 hr.)</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>17 ± 1 (± 10)</td>
</tr>
<tr>
<td>0-5</td>
<td>6-3 (± 1-0)</td>
</tr>
<tr>
<td>5-20</td>
<td>3-0 (± 0-5)</td>
</tr>
<tr>
<td>Case 2</td>
<td>149-0</td>
</tr>
</tbody>
</table>

Figures in parentheses are standard deviations of the mean.

**Investigations of necropsy samples.** In Case 2 electrophoresis gave a fraction with the mobility of chondroitin sulphate in the heart, kidney, liver, and brain. Total estimation of fucose, hexosamine, and uronic acid in these organs revealed only a slight increase in the glycoproteins (fucose content), but a 20- to 230-fold increase in hexosamine, and an 85- to 455-fold increase in uronic acid (Table II).

**TABLE II**

<table>
<thead>
<tr>
<th>Case 2: Acid Mucopolysaccharides in Necropsy Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fucose (μg./mg. protein)</td>
</tr>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Kidney</td>
</tr>
<tr>
<td>Normal kidney</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Normal liver</td>
</tr>
<tr>
<td>Brain</td>
</tr>
<tr>
<td>Normal brain</td>
</tr>
</tbody>
</table>
Quantitative thin-layer chromatography revealed a relative predominance of a glycolipid fraction in liver and kidney cortex, but not in the other organs investigated (Table III).

The Rf value, 0·55, was found to be slower than that of normal galacto-cerebrosides (Rf 0·77). The glycolipid fraction was resistant to hydrolysis by 0·5 N KOH. In the brain necropsy specimen, an increase in the phosphatidyl-ethanolamine fraction was found, which may be explained by a partial overlapping of the abnormal glycolipid fraction and the ethanolamine-phosphatides, because this fraction in the brain chromatogram extended more towards the front of the developing fluid.

Paper chromatography of the abnormal fraction isolated from liver tissue revealed only glucose and galactosamine (five experiments), but no galactose. However, traces of a fraction with an Rf value as

**TABLE III**

*Case 2: Distribution of Phosphatides and Sialic Acid-free Glycolipids in Necropsy Specimens*

<table>
<thead>
<tr>
<th></th>
<th>Sf&lt;sub&gt;II&lt;/sub&gt; + Ps</th>
<th>Sf&lt;sub&gt;I&lt;/sub&gt;</th>
<th>Le</th>
<th>Pe</th>
<th>Ce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney cortex from patient</td>
<td>13·3 ± 3·6</td>
<td>23·5 ± 3·4</td>
<td>10·4 ± 7·3</td>
<td>52·8 ± 9·2*</td>
<td></td>
</tr>
<tr>
<td>Kidney cortex from normal subject</td>
<td>19·3 ± 1·3</td>
<td>27·2 ± 1·2</td>
<td>16·1 ± 2·5</td>
<td>37·2 ± 3·2†</td>
<td></td>
</tr>
<tr>
<td>Brain, white matter from patient</td>
<td>14·5 ± 1·7</td>
<td>3·6 ± 0·3</td>
<td>16·9 ± 2·4</td>
<td>15·9 ± 3·2</td>
<td></td>
</tr>
<tr>
<td>Brain, white matter from normal subject</td>
<td>12·5 ± 0·5</td>
<td>14·7 ± 0·7</td>
<td>24·9 ± 0·9</td>
<td>38·6 ± 0·9</td>
<td></td>
</tr>
<tr>
<td>Liver from patient</td>
<td>13·3 ± 7·1</td>
<td>37·0 ± 7·3</td>
<td>18·1 ± 10·6</td>
<td>41·5 ± 9·1</td>
<td></td>
</tr>
<tr>
<td>Liver from normal subject</td>
<td>20·2 ± 2·5</td>
<td>33·0 ± 2·5</td>
<td>22·8 ± 3·7</td>
<td>22·3 ± 3·2</td>
<td></td>
</tr>
</tbody>
</table>

* This fraction has a retention factor Rf 0·55. † This fraction has a retention factor Rf 0·77.

Sf<sub>II</sub> (C-18 sphingomyelins): polar lipid fractions demonstrated by means of infrared analysis to contain sphingomyelin (C-18). However, this fraction also covers the serine-phosphatide band (Ps).

Sf<sub>I</sub> (C-24 sphingomyelins): a fraction with a retention factor as C-24 sphingomyelins.

Le, a fraction with retention factor as lecithin.

Pe, a fraction with retention factor as ethanolamine-phosphatides.

Ce, a fraction with retention factors as cerebrosides and other glycolipids (traces of aldehyde-derivatives of split products of unsaturated fatty acids may adhere to these fractions (L. Svennerholm, 1965, personal communication)).

All fractions mentioned above have been isolated by infrared analysis and identified as described by Clausen (1966).

The values of the necropsy specimens are given ± SD. The normal values are based upon studies of 10 different macroscopically normal necropsy specimens, and are given ± SD of the means.
lactose was found. The hexose/hexosamine ratio was found to be 3.0 (W/W) (Table IV).

In Case 1 paper electrophoretic studies of the brain tissue revealed the presence of the AMP fraction found in urine. Quantitative estimations showed only a normal level of glycoproteins (fucose content), but a 13-fold increase in the hexosamine and a 33-fold increase in the uronic acid of the whole brain matter (Table V).

Quantitative thin-layer chromatography of liver extract revealed, as in Case 2, an increase of the glycolipids with Rf value of 0.55, slower than that of normal galacto-cerebrosides (Rf 0.77). In addition, the spleen revealed a high content of this component (Table VI).

The fatty acid composition of the abnormal glycolipids is seen in Table VII. Only traces of polyenoic acids of 22-C atoms were found and no C-24 derivatives could be detected. Staining of sialic components (gangliosides) in thin-layer chromatography revealed an increase in mono- and di-sialo-gangliosides (fraction GM₁₂ and GM₃, Svennerholm, 1963) in both Cases 1 and 2 (Fig. 2 and 3). No changes were found in other fractions, including the sulphatides which were normal (0.23 to 0.17 parts of the cerebrosid fraction).

**Discussion**

The two cases of gargoylism were clinically typical of this disorder, with thick lips, prominent eyebrows, and an elongated nose, and the large head and small body. Case 1 had a hearing loss and Case 2 had corneal opacities; both had hepatosplenomegaly. Their development was characteristic, with a fairly normal infancy and later deterioration, leading to severe psychomotor retardation. The mental retardation was associated with a slow development of the physical and mental abilities, and there were no abnormalities in the somatic growth. The myopia was absent.

**TABLE IV**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Hexose/Hexosamine Ratio</th>
<th>Paper Chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.4/1</td>
<td>Glucose: +</td>
</tr>
<tr>
<td>2</td>
<td>3.0/1</td>
<td>Lactose: +</td>
</tr>
</tbody>
</table>

* Other carbohydrates tested (galactose, maltose, glucosamine) were absent.

**TABLE V**

<table>
<thead>
<tr>
<th>Case 1: Acid Mucopolysaccharides in Necropsy Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
</tr>
<tr>
<td>Normal brain</td>
</tr>
</tbody>
</table>

**TABLE VI**

**Case 1: Distribution of Phosphatides and Sialid Acid-free Glycolipids in Necropsy Specimens**

<table>
<thead>
<tr>
<th></th>
<th>SfII + Ps</th>
<th>SfI</th>
<th>Le</th>
<th>Pe</th>
<th>Ce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney cortex from patient</td>
<td>14.9 ± 3.6</td>
<td>20.0 ± 3.4</td>
<td>11.2 ± 7.3</td>
<td>53.9 ± 9.2</td>
<td></td>
</tr>
<tr>
<td>Kidney cortex from normal subject</td>
<td>17.3 ± 3.1</td>
<td>27.2 ± 1.2</td>
<td>16.1 ± 2.5</td>
<td>37.2 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Spleen from patient</td>
<td>17.6 ± 7.3</td>
<td>25.4 ± 6.8</td>
<td>22.8 ± 3.1</td>
<td>34.2 ± 10.9</td>
<td></td>
</tr>
<tr>
<td>Spleen from normal subject</td>
<td>17.6 ± 7.3</td>
<td>23.6 ± 2.6</td>
<td>31.3 ± 2.4</td>
<td>22.7 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Brain, white matter from patient</td>
<td>20.0 ± 1.8</td>
<td>6.3 ± 0.3</td>
<td>16.0 ± 2.4</td>
<td>6.8 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Brain, white matter from normal subject</td>
<td>12.5 ± 0.5</td>
<td>9.9 ± 0.1</td>
<td>14.7 ± 0.7</td>
<td>6.3 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

For key see Table III.

**TABLE VII**

**Fatty Acid Composition (%) of Abnormal Glycolipid Isolated from Liver Tissue from Two Cases of Hurler’s Syndrome**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>C₁₀</th>
<th>C₁₂</th>
<th>C₁₄</th>
<th>C₁₆</th>
<th>C₁₈</th>
<th>C₂₀</th>
<th>C₂₂</th>
<th>C₂₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.8</td>
<td>1.9</td>
<td>1.1</td>
<td>7.0</td>
<td>3.2</td>
<td>2.2</td>
<td>2.0</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
<td>0.8</td>
<td>1.0</td>
<td>1.7</td>
<td>1.1</td>
<td>2.1</td>
<td>2.0</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* Unidentified.
idiocy. Radiologically there were typical bone changes, particularly of the spine.

Necropsy confirmed the diagnosis. In both cases there was hepatosplenomegaly, and abnormalities of the aorta, and naked-eye appearances of the brain were typical. In Case 1, where histological examination was performed, the findings were characteristic of a storage disease and the histochemical studies supported the presence of gangliosides. The latter finding is in agreement with those of Jervis (1950) and Benda (1952), who were the first to suggest a metabolic disorder. The deposits were proved to be a ganglioside, first by Brante (1952) and later by others (Norman, Urich, Tingey, and Goodbody, 1959; O'Brien, Stern, Landing, O'Brien, and Donnell, 1965).

The chemical studies revealed an accumulation of AMP in all organs investigated, while in addition the urine from Case 2 revealed a large increase in AMP. The paper electrophoretic studies of concentrated urine revealed a fraction with the mobility of chondroitin sulphate in both cases. Previous infrared studies revealed this fraction to be
identical with chondroitin sulphate B. Although
the presence of a small amount of heparitin sulphate
cannot be excluded (Bishton et al., 1956), the amount
of this component was too low to allow isolation by
alcohol precipitation before the infrared analysis
(Dyggve et al., 1962; Clausen et al., 1963). Our
results thus seem in agreement with those of Brante
(1952). The slightly increased fucose value may
indicate a slight increase in the level of glycoproteins
in one of these cases of gargoylism.

The quantitative thin-layer chromatography
revealed a predominance of the glycolipid fraction,
containing both galactosamine and glucose. Be-
cause the glycolipids are split products of the
degradation of gangliosides (see above), and because
mono- and di-sialo-gangliosides were found to be
increased in the brain matter of these two cases of
gargoylism, a common enzymatic defect in the
degradation of carbohydrate moieties of AMP,
glycolipids, and glycoproteins may explain the
findings.

Recent studies (Klenk and Gielen, 1961, 1963;
Kuhn, 1961; Wolfe and Lowden, 1964; Svenner-
holm and Raal, 1961; Svennerholm, 1964, 1966)
have shown the gangliosides to be composed of
lipamide-glucose-galactose-galactosamine-galactose-
(sialic acid)$_3$, with the probable attachment of one
sialic acid residue to the glucose molecule.

So, at the moment it cannot be decided whether
the glycolipid containing only glucose and galacto-
samine represents a new type of lipid, present
normally merely in traces, or whether it represents
an abnormal glycolipid only to be found in Hurler's
syndrome. However, it is of interest that Booth,
Goodwin, and Cumings (1966) have recently
described a nearly hexosamine-free sialic- and
hexose-containing glycolipid in necropsy specimens
from a case of Hurler's syndrome. The present
studies showed accumulations of both chondroitin
sulphate and of a glycolipid, both containing
galactosamine, and this may be explained on the
basis of a common metabolic defect in the following
way.

Chondroitin sulphate B (see survey by Gibian,
1959) and gangliosides may only be degraded to
liberate the galactosamine, when hexosaminidase
(β-galactosaminidase) is present (Walker, 1961).
Because the glycoproteins also may contain galacto-
samine (Schultze et al., 1958), it seems reasonable
to explain the findings in gargoylism by a lack of a
β-galactosaminidase-like enzyme, leading to accum-
ulation of glycolipids, chondroitin sulphate, and their
precursors. This may also explain, on the basis of
'stasis' in the pathway, the increased level of mono-
and di-sialo-gangliosides in the brain matter. This
explanation also seems in agreement with other
studies (to be published later), that in Farber's
disease a storage of both a glycolipid and AMP
occurs. Most degradative enzymes, as for in-
stance hexosaminidases, are localized to the
lysosomes intracellularly (de Duve, 1963). Because
both mucopolysaccharidoses and lipidoses may be
explained by lack of degradative enzymes and,
because, as demonstrated in the present communi-
cation, these diseases may be interrelated concerning
the chemical abnormalities, their pathogenesis may
be related to abnormalities in the lysosomes.

When compared with the fatty acid composition
of normal cerebroside (O'Brien and Rouser, 1964),
the present abnormal glycolipid seems to be lacking
higher polyenoic acids (C-24 derivatives) and only
the glycolipid from Case 1 contained minor amounts
of C-22 derivatives.

The present pathological findings are in agreement
with the histological studies of Craig, Clarke, and
Banker (1959), Norman et al. (1959), and Landing,
Silverman, Craig, Jacoby, Lahey, and Chadwick
(1964). These authors demonstrated foaming histio-
cytosis of viscera as found in Tay-Sachs' disease,
while in our two cases the increase in the glycolipids
was found in the spleen and liver, containing reticulo-
endothelial cells. The similarity of the findings in
Tay-Sachs' disease may be explained by the com-
promised degradation of the gangliosides. A family
with one child with gargoylism and another with
Tay-Sachs' disease has been described by Shanklin
and Salam (1963), and similarity between ganglio-
sides from a gargoyle brain and from a Tay-Sachs'
brain has been reported by Taghavy, Salsman, and
Ledeen (1964).

The finding of an increased sulphatase activity in
cases of gargoylism (Austin et al., 1964) may be due
to increased sulphation of galacto-AMP stored in
this disease.

Summary

Two cases of gargoylism were investigated
chemically. Necropsy specimens revealed accumu-
lation in liver, kidney, spleen, and brain of acid
mucopolysaccharides. In the liver and spleen, and
in the kidney cortex, there was an accumulation of
mono-, di-sialo-gangliosides and glycolipids.

These findings may be explained by a deficiency of
hexosaminidase (β-galactosaminidase) activity.

Our thanks are due to Dr. Annalise Dupont for
permission to publish one of the cases of gargoylism, and
to Drs. H. Wolthers, P. Christoffersen, and Erna
Christensen, for the necropsies.
Chemical Studies in Gargoylism

9

REFERENCES


Chemical studies in gargoylism.

J. Clausen, H. V. Dyggve, J. C. Melchior and H. O. Lou

Arch Dis Child 1967 42: 62-69
doi: 10.1136/adc.42.221.62

Notes