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, 'Andersvænge', 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 β -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 gluco-cerebrosides in brain may be transformed by means of specific enzymes to galacto-cerebrosides. This ceramide, as well as galacto-gluco-ceramide, 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 14½ 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. Pneumoencephalography 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.

Received April 7, 1966.

* These investigations have been supported by Grant No. NB 05216-02 Public Service Grant, National Institutes of Health, Education and Welfare, Bethesda, U.S.A., and in part from the Research Funds for Mental Retardation, Copenhagen, Denmark.

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 $17 \times 17 \times 7$ cm.; the surface and the cut sections were yellow. The spleen (290 g.) was also enlarged measuring $14 \times 8 \times 6$ 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 $5\frac{1}{4}$ 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 $25 \times 15 \times 5$ cm., the spleen $9 \times 6 \times 3$ 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,

1965; Clausen, 1966). 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 (Schultze, Schmidtberger, 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 μ l. 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 μ l. methyl esters in a Perkin-Elmer flame-ionization gas-chromatograph F-11-(Clausen, 1966, in preparation) (column dimensions 2 m. $\times$ 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 + aqueous NH₃ (25%) (70 + 30 + 5) as an ascending

system. The pattern was developed with resorcinol-HCl-reagent for glycolipids as described by Svennerholm (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
Acid Mucopolysaccharide Excretion in Urine in Normal Subjects and in Case 2

Age (yr.)	Acid Mucopoly-saccharide Hexosamine (mg./24 hr.)	Acid Mucopoly-saccharide Glucuronic Acid (mg./24 hr.)
Normal subjects		
0-5	1.7 ($\pm$ 0.9)	2.1 ($\pm$ 0.5)
5-20	6.3 ($\pm$ 1.0)	4.9 ($\pm$ 0.5)
Over 20	3.5 ($\pm$ 0.9)	3.9 ($\pm$ 0.4)
Mean of all groups of age	3.9 ($\pm$ 0.5)	3.7 ($\pm$ 0.3)
Case 2	149.0	87.9

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
Case 2: Acid Mucopolysaccharides in Necropsy Specimens

	Fucose (μ g./mg. protein)	Hexosamine (μ g./mg. protein)	Uronic Acid (μ g./mg. protein)
Heart	1.57	25.00	13.20
Kidney	0.82	19.00	11.90
Normal kidney	0.14	0.16	0.14
Liver	3.82	99.50	73.10
Normal liver	0.29	0.83	0.38
Brain	1.17	12.00	6.82
Normal brain	0.79	0.53	0.15

FIG. 1.—Case 2. Paper electrophoresis of urine concentrated 100 times. The electrophoretic patterns are correlated to the markers of chondroitin sulphate and heparin. A fraction with the mobility of chondroitin sulphate is seen. (For experimental details, see the text.)

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 R_f value, 0.55, was found to be slower than that of normal galacto-cerebrosides (R_f 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 R_f value as

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

	Sf _{II} + Ps	Sf _I	Le	Pe	Ce
Kidney cortex from patient	13.4 ± 3.6		23.5 ± 3.4	10.4 ± 7.3	52.8 ± 9.2*
Kidney cortex from normal subject	19.3 ± 1.3		27.2 ± 1.2	16.1 ± 2.5	37.2 ± 3.2†
Brain, white matter from patient	14.5 ± 1.7	3.6 ± 0.3	16.9 ± 2.4	49.1 ± 2.1	15.9 ± 3.2
Brain, white matter from normal subject	12.5 ± 0.5	9.9 ± 0.1	14.7 ± 0.7	24.9 ± 0.6	38.6 ± 0.9
Liver from patient	13.3 ± 7.1		37.0 ± 7.3	18.1 ± 10.6	41.5 ± 9.1
Liver from normal subject	20.2 ± 2.5		33.0 ± 2.5	22.8 ± 3.7	22.3 ± 3.2

* This fraction has a retention factor R_f 0.55. † This fraction has a retention factor R_f 0.77.

Sf_{II} (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_I (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.

TABLE IV

Chemical Data of Glycolipid Fraction (R_f value 0.55) Isolated From Necropsy Specimens in Two Cases of Hurler's Syndrome

Case No.	Hexose/Hexosamine Ratio	Paper Chromatography		
		Glucose	Lactose	Galactosamine*
1	3.4/1	+	Traces	+
2	3.0/1	+	Traces	+

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

lactose was found. The hexose/hexosamine ratio was found to 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 R_f value of 0.55, slower than that of normal galacto-cerebrosides (R_f 0.77). In addition, the spleen revealed a high content of this component (Table VI).

The fatty acid composition of the abnormal

TABLE V

Case 1: Acid Mucopolysaccharides in Necropsy Specimens

	Fucose ($\mu\text{g./mg. protein}$)	Hexosamine ($\mu\text{g./mg. protein}$)	Uronic Acid ($\mu\text{g./mg. protein}$)
Brain	0.67	7.11	4.96
Normal brain	0.79	0.53	0.15

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 GM₁,₂ and ₃, GD_{1a}, 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 cerebroside fraction).

Discussion

The two cases of gargoylism were clinically typical of this disorder, with thick lips, prominent eyebrows and flat 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

TABLE VI

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

	Sf _{II} + Ps	Sf _I	Le	Pe	Ce
Kidney cortex from patient	14.9 ± 3.6		20.0 ± 3.4	11.2 ± 7.3	53.9 ± 9.2
Kidney cortex from normal subject	19.3 ± 1.3		27.2 ± 1.2	16.1 ± 2.5	37.2 ± 3.2
Spleen from patient	17.6 ± 7.3		25.4 ± 6.8	22.8 ± 3.1	34.2 ± 10.9
Spleen from normal subject	23.6 ± 2.6		31.3 ± 2.4	22.0 ± 1.1	22.7 ± 3.8
Brain, white matter from patient	20.8 ± 1.7	6.3 ± 0.3	16.0 ± 2.4	50.7 ± 2.1	6.3 ± 3.2
Brain, white matter from normal subject	12.5 ± 0.5	9.9 ± 0.1	14.7 ± 0.7	24.9 ± 0.6	38.6 ± 0.9

For key see Table III.

TABLE VII

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

Case No.	Fatty Acids															
	C-14	C-15	C-16	C-16:1	C*	C-17	C-18	C-18:1	C-18:2	C-18:3	C-20	C-20:1	C-20:2	C-20:3	C-20:4	C-22:1
1	3.8	1.9	14.1	3.5	0.5	1.3	16.0	20.7	6.0	0.9	0.7	1.3	1.4	2.3	20.0	1.3
2	0.9	0.8	17.1	3.8	1.1	1.1	27.0	38.5	0.6	0.4		2.9	1.5		1.4	6.3
																1.3

* Unidentified.

FIG. 2.—Case 1. Thin-layer chromatogram of glycolipids (gangliosides) from cortex of the brain correlated with that of normal cortex (adult). The polar lipids are developed with resorcinol (Svennerholm, 1963). The fraction named GM refers to the abnormal gangliosides of Case 1. (The chromatograms were developed for 30 minutes with chloroform + methanol + 10% aqueous NH_3 (60 + 35 + 8).) GM refers to mono-sialo-ganglioside fractions, GD to di-sialo-ganglioside, and GT to tri-sialo-gangliosides (for technical details, see text).

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

FIG. 3.—Thin-layer chromatograms of gangliosides extracted from the grey matter of the brain from Cases 1 and 2. GD and GM refer to the different gangliosides. (See Svennerholm (1963) and Fig. 2; the chromatograms were developed for 1½ hours.)

Mono- and di-sialo-gangliosides are found. No tri-sialo-gangliosides can be seen.

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. Because 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; Svennerholm 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 galactosamine 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 galactosamine (Schultze *et al.*, 1958), it seems reasonable to explain the findings in gargoylism by a lack of a β -galactosaminidase-like enzyme, leading to accumulation 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 instance 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 communication, 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 histiocytosis 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 compromised 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 gangliosides 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 accumulation 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.

REFERENCES

- Asboe-Hansen, G., and Clausen, J. (1964). Mastocytosis (urticaria pigmentosa) with urinary excretion of hyaluronic acid and chondroitin sulfuric acid. Changes induced by polymyxin B. *Amer. J. Med.*, **36**, 144.
- Austin, J., McAfee, D., Armstrong, D., O'Rourke, M., Shearer, L., and Bachhawat, B. (1964). Abnormal sulphatase activities in two human diseases (metachromatic leucodystrophy and gargoylism). *Biochem. J.*, **93**, 15c.
- Benda, C. E. (1952). *Developmental Disorders of Mentation and Cerebral Palsies*. Grune & Stratton, New York.
- Bishton, R. L., Norman, R. M., and Tingey, A. (1956). The pathology and chemistry of a case of gargoylism. *J. clin. Path.*, **9**, 305.
- Booth, D. A., Goodwin, H., and Cumings, J. N. (1966). Abnormal gangliosides in Tay-Sachs disease, Niemann-Pick's disease and gargoylism. *J. Lipid Res.*, **7**, 337.
- Brady, R. O., Kanfer, J., and Shapiro, D. (1965a). The metabolism of glucocerebrosides. I. Purification and properties of a glucocerebrosidase-cleaving enzyme from spleen tissue. *J. biol. Chem.*, **240**, 39.
- , —, and — (1965b). The metabolism of glucocerebrosides. II. Evidence of an enzymatic deficiency in Gaucher's disease. *Biochem. biophys. Res. Commun.*, **18**, 221.
- Brante, G. (1952). Gargoylism. A mucopolysaccharidosis. *Scand. J. clin. Lab. Invest.*, **4**, 43.
- Christensen Lou, H. O., Clausen, J., and Biering, F. (1965). Phospholipids and glycolipids of tumours in the central nervous system. *J. Neurochem.*, **12**, 619.
- Clausen, J. (1966). Polar lipids and structural proteins of human brain. Properties and pathological changes. In *A NATO Advanced Study Institute on the Molecular Basis of some Aspects of Mental Activity*, ed. O. Walaas. Academic Press, London. In the press.
- , and Asboe-Hansen, G. (1966). Urinary acid mucopolysaccharidosis in mastocytosis. A new technique for quantitative estimation. *Clin. chim. Acta*, **13**, 475.
- , Christensen Lou, H. O., and Andersen, H. (1965). Phospholipid and glycolipid patterns of infant and foetal brain. *J. Neurochem.*, **12**, 599.
- , Dyggve, H. V., and Melchior, J. C. (1963). Mucopolysaccharidosis. *Arch. Dis. Childh.*, **38**, 364.
- , and Rosenkist, P. (1962). Isolation of acid mucopolysaccharides of human brain. *J. Neurochem.*, **9**, 393.
- Craig, J. M., Clarke, J. T., and Banker, B. Q. (1959). A metabolic neurovisceral disorder with the accumulation of an unidentified substance. A variant of Hurler's syndrome. *A.M.A. J. Dis. Child.*, **98**, 577.
- de Duve, C. (1963). The lysosome concept. In *Ciba Foundation Symposium on Lysosomes*, ed. A. V. S. de Reuck and M. P. Cameron, p. 1. Little Brown, Boston, Massachusetts; Churchill, London.
- Dyggve, H. V., Melchior, J. C., and Clausen, J. (1962). Morquio-Ullrich's disease. *Arch. Dis. Childh.*, **37**, 525.
- Foster, T. S., and Pearce, R. H. (1961). Zone electrophoresis of acid mucopolysaccharides. *Canad. J. Biochem.*, **39**, 1771.
- Gaucher, P. C. E. (1882). De l'épithélioma primitif de la rate: hypertrophie idiopathique de la rate sans leucémie. Thesis, Paris.
- Gibian, H. (1959). *Mucopolysaccharide und Mucopolysaccharidasen*. Franz Deuticke, Vienna.
- Hagberg, B., Sourander, P., and Svennerholm, L. (1962). Sulfatide lipidosis in childhood. *A.M.A. J. Dis. Child.*, **104**, 644.
- Jatzkewitz, H. (1960). Cerebron-und Kerasin-schwefelsäureester als Speichersubstanzen bei der Leukodystrophie Typ Scholz (metachromatische Form der diffusen Sklerose). *Hoppe-Seylers Z. physiol. Chem.*, **320**, 134.
- Jervis, G. A. (1950). Gargoylism (lipocondrodystrophy). *Arch. Neurol. Psychiat. (Chic.)*, **63**, 681.
- Klenk, E., and Gielen, W. (1961). Über die Gehirnganglioside. *Hoppe-Seylers Z. physiol. Chem.*, **323**, 126.
- , and — (1963). Über ein zweites hexosaminhaltiges Gangliosid aus Menschengehirn. *ibid.*, **330**, 218.
- , Liedtke, U., and Gielen, W. (1963). Das Gangliosid des Gehirns bei der infantilen amaurotischen Idiotie vom Typ Tay-Sachs. *ibid.*, **334**, 186.
- Kuhn, R. (1961). Brain gangliosides. *Angew. Chem.*, **72**, 186.
- Landing, B. H., Silverman, F. N., Craig, J. M., Jacoby, M. D., Lahey, M. E., and Chadwick, D. L. (1964). Familial neurovisceral lipidosis. *A.M.A. J. Dis. Child.*, **108**, 503.
- Mehl, E., and Jatzkewitz, H. (1963). Über ein Cerebrosid-schwefelsäure-ester spaltendes Enzym aus Schweineniere. *Hoppe-Seylers Z. physiol. Chem.*, **331**, 292.
- , and — (1964). Eine Cerebrosidsulfatase aus Schweineniere. *ibid.*, **339**, 260.
- Melchior, J. C., Clausen, J., and Dyggve, H. V. (1965). The mucopolysaccharidoses. *Clin. Pediat. (Philad.)*, **4**, 468.
- Norman, R. M., Urich, H., Tingey, A. H., and Goodbody, R. A. (1959). Tay-Sachs' disease with visceral involvement and its relationship to Niemann-Pick's disease. *J. Path. Bact.*, **78**, 409.
- O'Brien, J. S., and Rouser, G. (1964). The fatty acid composition of brain sphingolipids: sphingomyelin, ceramide, cerebroside and cerebroside sulphate. *J. Lipid Res.*, **5**, 339.
- , and Sampson, E. L. (1965). Lipid composition of the normal human brain: gray matter, white matter and myelin. *ibid.*, **6**, 537.
- , Stern, M. B., Landing, B. H., O'Brien, J. K., and Donnell, G. N. (1965). Generalized gangliosidosis. *Amer. J. Dis. Child.*, **109**, 338.
- Philippart, M., and Menkes, J. (1964). Isolation and characterization of the main splenic glycolipids in Gaucher's disease. Evidence for the site of metabolic block. *Biochem. biophys. Res. Commun.*, **15**, 551.
- Rathbone, L. (1965). The effect of diet on the fatty acid compositions of serum, brain, brain mitochondria and myelin in the rat. *Biochem. J.*, **97**, 620.
- Schultze, H. E., Schmidtberger, R., and Haupt, H. (1958). Untersuchungen über die gebundenen Kohlenhydrate in isolierten Plasmaproteiden. *Biochem. Z.*, **329**, 490.
- Shanklin, W. M., and Salam, M. (1963). A comparison of the histochemistry of the cerebral cortex from siblings with gargoylism and Tay-Sachs' disease. *Acta neuroveg. (Wien)*, **25**, 297.
- Sourander, P., and Svennerholm, L. (1962). Sulphatide lipidosis in the adult with the clinical picture of progressive organic dementia with epileptic seizures. *Acta neuropath. (Berl.)*, **1**, 384.
- Steiness, I. (1961). Acid mucopolysaccharides in urine in gargoylism. *Pediatrics*, **27**, 112.
- Svennerholm, L. (1963). Chromatographic separation of human brain gangliosides. *J. Neurochem.*, **10**, 613.
- (1964). The gangliosides. *J. Lipid Res.*, **5**, 145.
- (1966). Lipid changes in mental and neurological disorders. In *A NATO Advanced Study Institute on The Molecular Basis of Some Aspects of Mental Activity*, ed. O. Walaas. Academic Press, London. In the press.
- , and Raal, A. (1961). Composition of brain gangliosides. *Biochem. biophys. Acta (Amst.)*, **53**, 422.
- Taghavy, A., Salsman, K., and Ledeen, R. (1964). An abnormal ganglioside pattern from a gargoyle brain. *Fed. Proc.*, **23**, 128.
- Walker, P. G. (1961). The enzymatic degradation of mucopolysaccharides. *Biochem. Soc. Sympos.*, **20**, 109.
- Wolfe, L. S., and Lowden, J. A. (1964). Studies on brain gangliosides. I. The isolation and composition of a trisialoganglioside. *Canad. J. Biochem.*, **42**, 1041.
- Zellweger, H., Ponseti, I. V., Pedrini, V., Stamler, F. S., and von Noorden, G. K. (1961). Morquio-Ullrich's disease. *J. Pediat.*, **59**, 549.