point and solubility. Also, they claim that mixtures of optical isomers can be converted to the racemate if the KBr disk technique is applied (see also Right [7]). Therefore, the optical isomers can 'find' each other inspite of the great quantity of KBr, consistent with our result on the 'dry' D-glucose-urea mixture. Brockmann and Musso state that probably hydrogen bonding is responsible for the effects they found. The same explanation may be valid here, but further experiments are undoubtedly necessary to verify this interpretation. Therefore, although KBr disk spectra are of great value to the analyst they must be used with some reservation in view of the present incomplete experimental coverage of the field and the lack of adequate theoretical insight.

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On Sialic Acid in Brain Tissue

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Gangliosides were isolated from brains by exhausted extraction with methanol-chloroform (2:1, v/v). After evaporation of the solvent *in vacuo* and drying the residue with ethanol the lipids were redissolved in waterfree solvent of the same composition. An insoluble rest remained, and it was considerably larger when the tissue was extracted directly with methanol-chloroform without previous drying with acetone. It was composed of low molecular substances, such as amino acids, purines and salts but also substances of high molecular weight. The low molecular substances were removed by repeated dialysis against distilled water. The total amount of carbohydrates (hexose, hexosamine and sialic acid) was high (20–50 % in different samples) in the dialysed material. After hydrolysis several amino acids were also identified with paper chromatography. If strandin prepared according to procedure C of Folch, Arsove and Meath [1] was redissolved in methanol-chloroform (2:1) a substance containing hexosamine and sialic acid remained undissolved.

Only a few similar observations have been found in the literature. Klenk and Lauenstein [2] isolated a substance from methanol-chloroform extract of erythrocyte stroma which was only soluble in hot pyridine and contained a high amount of hexosamine. Rosenberg, Howe and Chargaff [3] have recently identified several amino acids after hydrolysis of strandin prepared by procedure C of Folch *et al.*

The observations described indicate that amino sugar and sialic acid are not only occurring in the lipopolysaccharides (gangliosides) but also in other substances in brain.

A quantitative estimation of sialic acid with Bial's reagent [4] in brain tissue, from which the lipids had been extracted, demonstrated that the amount of 'protein-bound' sialic acid was higher than that of lipid-bound. The amount of sialic acid was higher in old brains than in young ones and about twice as high in grey as compared with white matter. In adult brain the content was about 0.6 %, calculated on lipid-free brain tissue. N-Acetyl-sialic acid has now been isolated from lipid-free brain tissue and its X-ray diagram compared with that of sialic acid from gangliosides and serum proteins.

Analytical methods. Nitrogen was determined by the Kjeldahl micromethod. Hexose was determined with an orcinol-sulphuric acid method [5] and calculated as galactose. Hexosamine was assayed with a modified Elson and Morgan method [6], and glucosamine was separated by the procedure outlined by Gardell [7]. The determination of sialic acid was performed with Bial's reagent and calculated as N-acetyl sialic acid (mol.wt. 309) [4]. The X-ray powder diagrams were taken with a Guinier camera using copper $K\alpha$-radiation.

Materials. Brains from old people were freed from membranes and blood vessels. Grey matter was dissected free from the main part of white matter and water removed by two successive treatments with 4 vol. of acetone at 0°. The dried brain powder was extracted with

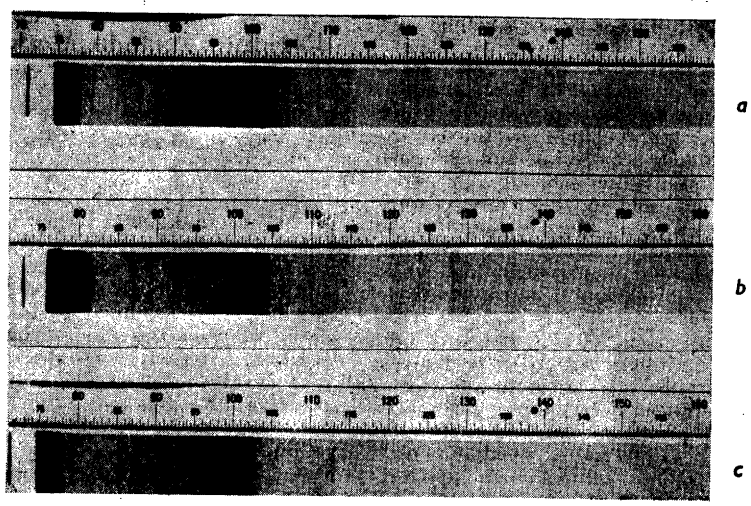

Fig. 1. X-ray powder diagram of N-acetyl-sialic acid isolated from (a) serum, (b) gangliosides and (c) lipid-free brain tissue.

methanol-chloroform (2:1, v/v) in a modified Soxhlet apparatus during 24 hours.

Gangliosides were prepared from the lipid extract by chromatography on cellulose columns [8]. Analysis of the potassium salt of gangliosides gave the following composition: nitrogen 2.70 %, chondrosamine (base) 6.45 %, hexose 27.5 %, N-acetyl-sialic acid 25.2 %.

Lipid-free substance. Portions of 100 g extracted tissue were suspended in about 3 l of 0.01 N sulphuric acid at 0° and shaken for at least 4 hrs. The liquid was filtered off and the treatment of the tissue was repeated until pH of the filtrate did not exceed 2.5. The substance was then hydrolysed as described below. *Analysis* (figures calculated on dry weight). Hexosamine 0.7 % (ratio glucosamine: galactosamine = 3:1), N-acetyl-sialic acid 0.9 %.

Serum. Pooled human serum was added four volumes of ethanol and brought to boiling. The precipitate was then treated as described for lipid-free brain substance. *Analysis* (calc. on dry weight). Hexose 1.8 %, hexosamine 1.3 % (glucosamine:galactosamine = 12:1), N-acetyl-sialic acid 1.0 %.

Isolation of N-acetyl-sialic acid. The procedure used [9] for isolation of N-acetyl-sialic acid has in the main been the same as that described by Zilliken, Braun and György [10] for the isolation of gynaminic acid from human milk.

The yield of crystalline acid was 40—50 % calculated from the quantitative estimation of N-acetyl-sialic acid with Bial's reagent on the starting material.

The isolated acids were tested by partition chromatography on Whatman paper No. 1. The solvents used were *n*-butanol-acetic acid-water (4:1:5), *sec*-butanol-acetic acid-water (4:1:5) and *sec*-butanol-acetone-acetic acid-water (3:3:1.5:2.5). After drying of the chromatograms the sialic acids were detected by spraying with [10]: orcinol-trichloroacetic acid [11]. All the crystallized acids ran as only one spot and had the same R_F-values. The X-ray diffraction patterns of the acids were identical (Fig. 1). The X-ray diagrams were also identical with that of ovine sialic acid [12].

Conclusion. N-Acetyl-sialic acid ($C_{11}H_{19}NO_9$) has been isolated from brain gangliosides and lipid extracted brain tissue.

The methods worked out for the quantitative estimation of gangliosides are based on determinations of sialic acid in lipid extracts. But a minor part of the non-lipid-bound sialic acid is extracted together with the lipids by methanol-chloroform mixtures containing some water. So when fresh tissues are extracted

directly with methanol-chloroform mixtures too high values for gangliosides are obtained.

Rittenberg, Howe and Chargaff [8] have suggested the existence of both gangliosides and mucolipids (strandin) in brain tissue, as they found that strandin contained amino acids, which was not the case of gangliosides. When strandin, prepared by the method used by them, was chromatographed on a cellulose column [9] with chloroform-methanol mixtures, gangliosides were isolated in the effluents, while on the top of the column a substance remained which gave strong positive reactions for sialic acid (Bial's reagent) and amino-acids (ninhydrin). After hydrolysis of the substance all the common amino acids were indicated by paper chromatography. A further evidence for the heterogenity of strandin is that Folch *et al.*[1] showed only amino acids in strandin prepared with the partition dialysis method (procedure C) but not in strandin prepared by two other procedures.

Until further data are given for strandin we have to consider it is a ganglioside to which other substances are associated. Gangliosides consist of a hydrophobic and a hydrophilic portion and may be able to act as protecting colloids for, *e.g.*, polypeptides in organic solvents and cerebrosides in water.

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Structure of the 1:1 Compound Pyridine — Iodo Monochloride

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It has been suggested that the 1:1 complexes probably present in the liquids containing pyridine and halogens are non-planar: The halogen atom directly linked to nitrogen is assumed to lie in the ring plane whereas the second halogen atom (bearing a negative charge) was expected to form an electrostatic link with the (positive) nitrogen atom and to lie outside the ring plane [1,2]. Unfortunately the yellow precipitate obtained by adding water to a solution of iodine in pyridine has been shown to contain water and the very unstable compound obtained in the absence of water does not appear to have the 1:1 composition [3]. Our X-ray work has therefore started with the iodo monochloride compound [4], the yellow, needle-shaped crystals of which (m. p. 132° C) turned out to be monoclinic — space group $P2_1/c$ — with the parameters $a = 4.25$, $b = 12.29$, $c = 14.07$, $\beta = 94.4°$. Using trial and error methods approximate y and z parameters of the iodine atoms (occupying a fourfold position) could easily be determined and signs of the structure factors evaluated which made possible a preliminary Fourier synthesis referring to the $(0kl)$-zone.

In Fig. 1a the refined electron density map is reproduced. The pyridine ring drawn in the figure is derived not from the direct synthesis but is slightly modified according to the results of a difference synthesis with subtraction of the contribution to structure factors from the halogen atoms. The $(h0l)$-synthesis in its final form is given in Fig. 1b. Here the overlapping of *two* pyridine rings forms an obstacle to a more precise determination especially of the carbon coordinates. A difference synthesis with subtraction of the contribution of the *iodine* atoms led to a z coordinate of the nitrogen atom which did not deviate more than 0.004 from the value obtained in the first projection. Neglecting not observed reflexions, the R factors for the two zones are 0.12 and 0.14, respectively.

From the above results it may safely be