The results obtained in this investigation will be used in a calculation of the band structure of the zirconium-oxygen system which is proceeding.[4] Full details of the present investigation will be published by one of us (BR) in *Arkiv för Fysik*.

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Isolation of the Major Ganglioside of Human Spleen

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In a study of the ganglioside profile of human brain, a minor component was observed which constituted less than 1 % of the total ganglioside fraction. The small amount isolated did not allow a further characterization of this ganglioside. It was then observed that the major ganglioside of human spleen had the same R_F-value in two chloroform-methanol-water solvents and in 70 % aqueous 1-propanol [1] as the minor brain component mentioned. Because this spleen ganglioside had considerably higher R_F-value in all the three solvents than the normal basic brain ganglioside, acyl-sphingosine-N-tetrose-N-acetylneuraminic acid, it was assumed to have a more simple carbohydrate moiety and to be related to a ganglioside isolated by Yamakawa and Suzuki [2] from equine erythrocytes, termed hematoside, which contained fatty acid-sphingosine-hexose-sialic acid in the molar ratio 1:1:2:1.

Experimental. 1.8 kg of fresh spleen from 18 old-aged people who had died in cardiovascular diseases were homogenized in a Turmix blender, dehydrated and freed from simple lipids by extraction twice with 10 liters of acetone at + 4°. The remaining lipids were extracted twice with 3 liters of chloroform-methanol (C-M), 2:1, v/v, at room temperature and finally in a Soxhlet hot extractor with C-M 1:2. The combined extracts were evaporated, redissolved in C-M 2:1, and filtered. The extract was evaporated again and hydrolysed for 24 h at + 37° in aqueous 0.5 N KOH. After neutralisation to pH 5–6 the lipids were extracted with 6 volumes C-M 2:1. 90–95 % of the gangliosides were extracted together with the other lipids in the organic solvent phase. The gangliosides remaining in the water phase were isolated by extraction of the dialysed and lyophilized water phase with C-M 2:1. The total lipid extract, combined organic and solvent phases, was chromatographed on four columns of 60 g silicic acid.[3] The effluents, collected in 25 ml samples, were tested with thin-layer chromatography and the sphingolipids were combined into six main fractions: ceramide-monohexosides, -dihexosides, -trihexosides, -tetra-saccharides, gangliosides and sphingomyelins[4]. Lipid-bound sialic acid (gangliosides) occurred, except in the ganglioside fraction, also in the other fractions which is evident from Table 1:

Table 1.

Fraction	*N-Acetylneuraminic acid* in mg
Ceramide-dihexosides	14.4
» -trihexosides	10.3
» -tetrasaccharides	5.6
Gangliosides	50.0
Sphingomyelins	14.4

It was found that the major ganglioside of all the fractions had the same R_F-value in thin-layer chromatography (Fig. 1).

The major ganglioside has only been isolated from the ganglioside fraction. This was far from pure as it contained about equal amounts of ceramide-trihexosides, -tetrasaccharides, and gangliosides. Separation of the gangliosides from the other two glycolipids on columns with diethylaminoethyl (DEAE) cellulose and magnesium trisilicate (Florisil) failed. A very satisfactory separation was achieved on thin-layer plates of silica gel G with the technique recently described.[5] The recovery, calculated from sialic acid analyses, was about 90 %. A chromatographically homogeneous ganglioside was crystallized from methanol. P 0, N 2.15, hexose 28.1, N-acetylneuraminic acid

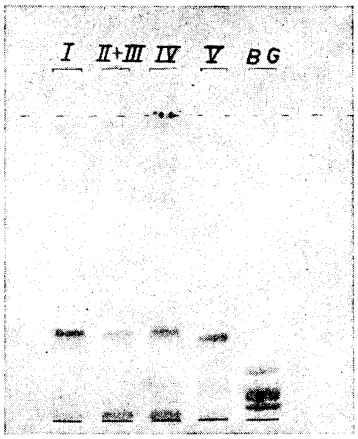

Fig. 1. Thin-layer chromatogram of the ganglioside-containing fractions of human spleen. I = ceramide-dihexoside, II and III = ceramide-trihexoside and, -tetrasaccharide, IV = ganglioside, and V = sphingomyelin fractions. B. G. = human brain gangliosides. Solvent: chloroform-methanol-water, 60:35:8, by vol. Spray: resorcinol reagent.[1]

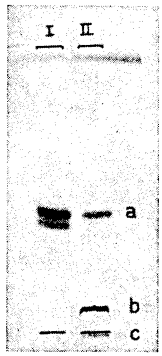

Fig. 2. Thin-layer chromatogram of sialidase treated spleen ganglioside. I = spleen ceramide-dihexosides, II = sialidase treated spleen ganglioside: ceramide-dihexoside(a), intact spleen ganglioside(b) and free N-acetylneuraminic acid(c). Solvent: chloroform-methanol-water, 65:25:4, by vol. Spray: perchloric acid-molybdate.

24.0, hexosamine 0.6 %. Molar ratio N-acetylneuraminic acid:hexose = 1.0:2.0.

The sialic acid was quantitatively released by sialidase (Behring-Werke, Marburg-Lahn), crystallized and shown to be N-acetylneuraminic acid. The sialic acid could also be split off to about 80 % by heating with 0.1 N H_2SO_4 at 80° for 1 h. At the enzymic hydrolysis an acyl-sphingosine-dihexoside was formed with the same R_F-value as the ceramide-dihexoside with normal fatty acids isolated from spleen [4] (Fig. 2). The disaccharide unit was composed of equal amounts of galactose and glucose. Galactose and a disaccharide which behaved as lactose at chromatography and paper electrophoresis in borate buffer,[5] were isolated from the dialysate after acid partial hydrolysis. In the dialysis residue was an acyl-sphingosine-monohexoside, which contained glucose as the only sugar. After periodate oxidation of the intact ganglioside and subsequent acid hydrolysis, galactose was the only sugar to be identified.

Discussion. The ganglioside isolated from human spleen shows great similarities to the ganglioside of equine erythrocytes,[2,6] but there are also slight differences. The sialic acid of equine red cell gangliosides [7,8] was N-glycolylneuraminic acid, while N-acetylneuraminic acid was found in the human spleen ganglioside as in the ganglioside canine erythrocytes[9]. In their careful study of the equine red cell gangliosides Klenk and Padberg [6] observed after acid partial hydrolysis small amounts of galactocerebrosides besides the glucocerebrosides but in our preparation, treated in a similar manner, only glucocerebrosides could be identified.

The data from the periodate oxidation and the rapid release of the sialic acid by sialidase makes it likely that the sialic acid is bound to C-atom 3 of galactose. The following structure of the ganglioside may then be suggested:

N-acetylneuraminic acid (2 → 3) galactosyl-β-(1 → 4) glucosylacyl (mainly nervonyl) sphingosine.

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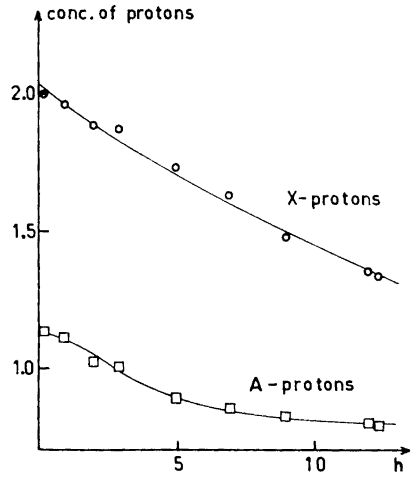

Fig. 1. Hydrogen exchange of indene showing the variation of the proton-concentration (in arbitrary units) with time. Temperature: 25°C.

Proton-Mobility in the Indene Ring-System

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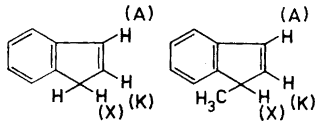
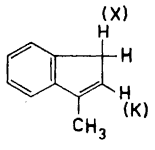
The proton-mobility in the five-membered ring of indene has been the subject of several investigations. Protium-deuterium or protium-tritium exchange experiments have been made in different ways,[1] and some scattered information is available concerning the isomerization of substituted indenes[2]. However, only a few quantitative rate studies under unified conditions have been reported.



I

II



III

In this communication we wish to report some preliminary results of an extensive series of investigations now in progress. Protium-deuterium exchange of indene (I) and isomerization of 1- to 3-methylindene (II and III, respectively) under the influence of basic catalysts have been studied with the NMR-technique.

The NMR-spectrum of indene was recently analyzed in detail by Elleman and Manatt,[3] and was shown to be of the AKX_2-type. The peaks for the non-equivalent protons in the 5-ring are sufficiently separated to permit a study of the protium-deuterium exchange in each position. A 2 M solution of freshly distilled indene in pyridine together with D_2O (8 moles litre^{-1}) and triethylamine (0.36 moles litre^{-1}) as a catalyst, was studied at various time intervals in a Varian A-60 NMR-spectrometer. The area of the peak due to the K-proton remained constant during the experiment (this was also true for the aromatic proton-peak), whereas the amount of X- and A-protons changed with time as shown in Fig. 1. In the absence of triethylamine there was no measurable hydrogen exchange. Protodedeuteration of indene deuterated in the 1- and 3-positions, using H_2O in the above medium has also been carried out with similar results. A mathe-