lated from X-ray diffraction patterns[2], and the helical structure predicted from X-ray diffraction is strikingly revealed in the electron microscope.

Deoxycholate has been widely used to prepare deoxyribonucleic acid from bacteria[4] and infectious "ribonucleic acid" from animal viruses[5]. Since the steroid may form long helical fibers under certain conditions, and hence dialyzes slowly, electron micrographs taken after using it should be interpreted cautiously.

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Type of attachment of sialic acid in ox-brain mucolipid

Sialic acid is easily liberated from brain mucolipid preparations under mildly acid conditions[1,2]; it may thus partly be attached terminally to the rest of the glycolipid polymer in a comparatively labile glycosidic linkage. Since structural models proposed heretofore for these interesting and complex substances are entirely hypothetical, we have undertaken a study of the various linkage types supporting the architecture of the polymer. We limit ourselves here to a consideration of sialic acid and to the behavior of a purified ox-brain mucolipid, both intact and after hydrolytic removal of sialic acid, towards oxidation by periodate (Fig. 1).

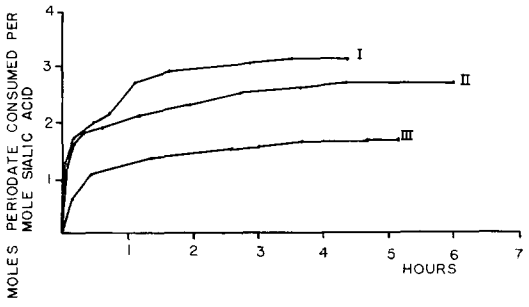

Fig. 1. Oxidation with metaperiodate at pH 4. I, free sialic acid; II, mucolipid after treatment with 0.1 *N* H_2SO_4 at 80° for 2 h; III, intact mucolipid.

The preparation of the mucolipid and, from it, of crystalline ovine sialic acid (5-N-acetylneuraminic acid) were described previously[2]. The reactions were carried out in 5 m*M* aqueous solutions of sodium metaperiodate around pH 4. Oxidant

consumption was measured by titration of residual periodate to iodate at pH 7 with thiosulfate.

The theoretical consumption by one mole of the free sialic acid isolated from the mucolipid is 2 moles of periodate. Actually, there is some overoxidation (Fig. 1), 2.7 moles being consumed, most likely owing to the active methylene group in position 3[3,4]. The intact mucolipid, containing 26 % sialic acid (w/w), reduced 1.7 mole of periodate per mole of polymer-bound sialic acid, whereupon its sialic acid content (by direct Ehrlich reaction[5]) dropped to one half. Only one half of the sialic acid molecules would hence appear to be strictly terminal, *i.e.*, bound only glycosidically at carbon-2, whereas the other half must be also substituted at least at the 8 hydroxyls, thus escaping destruction by periodate. The quantity of sialic acid attacked would have required the uptake of 1 or, if the explanation given above also holds for bound sialic acid, of 1.35 moles of oxidant. The small amount of additional periodate reduced is probably attributable to the cleavage of the few galactose units that, as shown in the following note[6], are terminal in the polymer.

The results are compatible with the provisional assumption that in the lipid polymer the bulk of the hexose molecules must be substituted, in addition to glycosidic attachment at carbon-1, either at position 3 or doubly at 2, 3 or 3, 4, and the N-acetyl hexosamine fraction similarly in position 3 or 4, in order to withstand attack by periodate. The following note[6] reports on methylation studies designed to clarify the situation.

Initial degradation of the mucolipid is not without influence on the subsequent course of oxidation. Brief pretreatment at pH 9 and room temperature augmented both the reaction rate and the extent of uptake of periodate. This effect on the polymer appears, however, to be complex, a portion of the sialic acid being liberated in form of a dialyzable degradation product. Following the treatment of the mucolipid with 0.1 *N* H_2SO_4 at 80° for 2 h, at which point the sialic acid is liberated almost completely without accompanying loss of hexose and hexosamine, the total hydrolysate consumed 3.2 moles of periodate (Fig. 1). The oxidation is, in this case, accompanied by the loss of about one third of the hexose (anthrone reaction) which must be responsible for the uptake of an additional 0.5 mole of oxidant over that reduced by an equivalent amount of free sialic acid.

The molar ratio of sialic acid to hexose in the mucolipid is about 2:3[2]. The results of the oxidation experiments described here may be taken to imply that, statistically, one out of three hexose moieties carries both sialic acid molecules, probably as a 2 → 8 linked dimer. The hexose molecule carrying the latter may, furthermore, be assumed to be doubly substituted along the 2, 3, and 4 positions, as the complete removal of sialic acid makes only a single vicinal diol of the hexose available for periodate attack. Experimental evidence points to galactose being the major sugar to which the sialic acid is attached.

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Methylation studies on ox-brain mucolipid

A large proportion (nearly 60 %) of the mucolipids consists of sugars (hexoses, hexosamine) and sialic acid[1]. In order to gain an insight into the backbone of these complicated structures it is, therefore, of interest to study the types of linkage supporting the various constituents. Preliminary information on the mode of attachment of sialic acid is presented in the preceding note[2]. Here, we discuss the results of methylation experiments designed to provide an understanding of the types of linkage involving the hexose moieties.

Purified mucolipid preparations from ox brain[1] were employed. For exhaustive methylation it was found convenient to use the methyl iodide–silver oxide arrangement with dimethylformamide[3] as the solvent. Methylation was repeated 6 to 8 times, with agitation of the mixture in the dark, but with decreasing quantities of the methylating reagent. The first treatment was performed in the cold (3°) for 40 h, the subsequent ones at room temperature, each time for 16 h. After each treatment the precipitate was washed with dimethylformamide and 4 times with chloroform; the combined extracts were, after dilution with more chloroform, clarified by filtration and concentrated to a small volume *in vacuo* at 35°. After every second or third methylation treatment the concentrated solution was first subjected to dialysis against running and distilled water (for 24 h each) and lyophilized.

The progress of the methylation was followed both by methoxyl determinations and by the infrared spectra (KBr plate) of the purified reaction products. The initially strong OH peak around 3600 cm^{-1} of the untreated mucolipid disappeared gradually until it reached the level of the control KBr plate. The spectroscopic test is more sensitive than the analytical one: even before the final stage the methoxyl content was already at a plateau of 25.3 %.

Measurements of osmotic pressure[4] indicated a molecular weight of 180,000 for the untreated mucolipid, in agreement with the value obtained previously by means of the ultracentrifuge[1], and of 200,000 for the fully methylated product. It is obvious that no appreciable degradation of the polymer had occurred during methylation.

The fully methylated mucolipid was methanolysed with 4 % methanolic HCl for 20 h at 105° in a sealed tube and then treated with 6 % aq. HCl for 100 min in a boiling-water bath. The solution was neutralized with Ag_2CO_3 and the filtrate, after being treated with H_2S and clarified, was passed through a short Amberlite IR-4B column and lyophilized. The residue, dissolved in little water, was examined chromatographically. First, separation on Whatman No. 1 filter paper was employed with several solvent systems and spraying reagents. *n*-Butanol-ethanol–water (4:1:5, v/v/v, top layer) and an aniline *p*-diodosalicylate spray reagent[1] were used routinely. After the preliminary identification of the spots by paper chromatography, a cellulose