the substance was obtained. After washing with ether and recrystallization from ethanol-petroleum ether in 1:1 ratio 0.16 g of pure D-arabitol was obtained; m. p. 100° (uncorr.). (Found: C 39.54; H 8.06. Calc. for $C_5H_{12}O_5$: C 39.47; H 7.95.) $[\alpha]_D^{21}$ + 7.66° (from saturated, aqueous boric acid solution, c = 2.2).

The comparison of X-ray diffraction patterns of L-arabitol and the substance from *Alectoria jubata* var. *chalybeiformis* crystallized from acetone gave the spacing values in Å, recorded in Table 1. The instrument used was one Philips X-ray Geiger Counter Spectrometer with Cu-$K\alpha_1$ radiation.

L-Arabitol was kindly provided by Farmasöytisk Institutt, Blindern-Oslo, Norway.

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Sialic Acid in Human Cervical Mucus, in Hog Seminal Gel, and in Ovomucin

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The presence of sialic acid as a characteristic constituent in epithelial mucus has been indicated by colour reactions [1]. This and/or closely related substances have also been isolated in crystalline form from certain materials of epithelial origin [2-8]. The isolation of a sialic acid from three additional similar sources, *viz.* human cervical mucus, the gelatinous lumps of hog semen, and ovomucin, will be reported here.

Using quantitative colorimetric methods Werner [1] has found, that the mucous plugs of the pregnant human uterine cervix contain considerable amounts of sialic acid in addition to glycoprotein material of the same general composition as the blood-group substances.

The carbohydrate components of ovomucin, the probably non-homogeneous, water-insoluble glycoprotein of hen's eggs, have been reported to be galactose and mannose in approximately equimolecular amounts [9], together with glucosamine and galactosamine [10]. By means of colour reactions the presence in ovomucin of fairly large amounts of substance of the sialic acid type has been indicated [10,11].

In hog semen a voluminous gel is formed shortly after ejaculation. No recent investigations on the composition of this material seem to have been published. From human seminal plasma Freudenberg *et al.*[12] have prepared a product showing the properties of the blood group substances. The preparation from the same material of an electrophoretically homogeneous glycoprotein, containing hexosamine but not uronic acid, has also been reported [13].

Experimental. Analytical methods. The same methods as those recently reported [8] were used. Separation of the aminosugars was performed by Gardell's method [14]. The single monosaccharide components in the glycoprotein hydrolysates were determined after paper-chromatographic separation. The X-ray powder diagrams were taken with a Guinier camera using copper $K\alpha$-radiation.

Materials. The cervical mucous plugs were collected immediately before delivery, and stored in 60 % aqueous ethanol. When enough material was obtained the plugs were cut up with scissors and kept in about 0.005 N hydrochloric acid for 24 hours at 4° C. After washing with aqueous ethanol the material was dried with ethanol and ether. The dry powder contained 9.7 % nitrogen, 6.6 % glucosamine, 3.9 % galactosamine, 7.5 % galactose, 5.0 % fucose, and 7.4 % sialic acid.

The colourless gelatinous mass from two ejaculates from a boar was thoroughly washed with saline and distilled water after decanting off the fluid seminal plasma. It was then cut in small pieces, treated with hydrochloric acid, and dried exactly as the cervical mucus. The analysis gave the following composition: 10.7% nitrogen, 2.0 % glucosamine, 8.5 % galactosamine, 2.7 % galactose, 1.3 % mannose, 0.7 % fucose, and 14.7 % sialic acid.

The ovomucin was prepared mainly according to the method of Gottschalk and Lind [15]. To cold homogenized egg-white, freed from chalazae by passing through a filtering cloth, cold distilled water was added. The mixture was centrifuged, and the gelatinous precipitate obtained dispersed in 10 % sodium chloride and reprecipitated by addition of distilled water. The precipitate was washed with saline and distilled water until the washings were free from protein. The material was then treated with hydrochloric acid, and dried in the

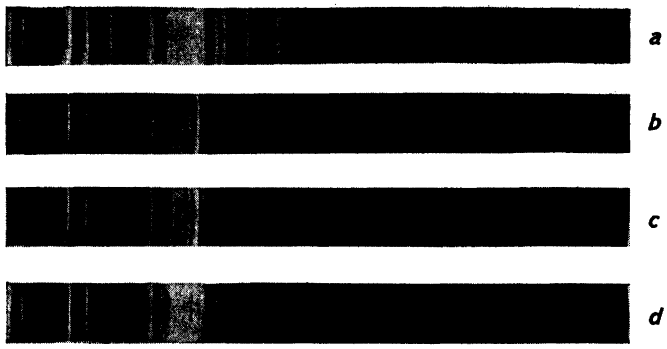

Fig. 1. X-ray powder diagrams of sialic acid isolated from (a) a human ovarian cyst gel, (b) human cervical mucus, (c) hog seminal gel, and (d) ovomucin.

usual way. The dry powder had the following composition: 13.3 % nitrogen, 4.7 % glucosamine, 2.4 % galactosamine, 4.4 % galactose, 2.2 % mannose, and 6.0 % sialic acid.

The *isolation of sialic acid* from these materials was performed according to the principles set out by Blix [2]. As only minute amounts of crystalline material were obtained from ovomucin by heating with water, weak sulphuric acid was used for the hydrolysis, but the yields remained small.

The dry powders from the cervical mucus (2 g) and hog seminal gel (10 g) were heated with distilled water on a boiling water-bath under reflux for one hour. The viscous fluids containing much undissolved gelatinous material were freeze-dried.

The ovomucin preparation (10 g) was heated with weak sulphuric acid (pH 2—3) in the same way. The hydrolysate was centrifuged. To the filtered supernatant barium hydroxide was added to pH about 8. After filtering, the solution was passed through a cation exchange column (Amberlite IRC-50, H), and then freeze-dried.

Crystalline products were obtained from the dried materials by the procedure recently described [3]. The crystallization appeared to be promoted by placing the specimens alternately in the refrigerator and at room temperature. From the cervical mucus about 30 mg of crystalline material was obtained, from the seminal gel about 150 mg, and from the ovomucin about 15 mg.

The X-ray diffraction patterns of the crystalline substances isolated from these three sources were all identical (Fig. 1). They were also identical with that of the sialic acid isolated from sheep submaxillary mucin by Blix *et al.*[2] and from some human sources by Odin [7,8]. Quantitative determinations with the Bial and Ehrlich reagents gave also the same extinction values for all the substances.

Identical sialic acids have thus been isolated from ovine, human, porcine, and avian sources. This substance is probably the naturally occurring sialic acid in these materials. Some changes in the molecule of an original substance occurring during the isolation procedure cannot, however, be excluded. It should be noted that the sialic acid, which has been isolated from hog submaxillary mucin [3], is not identical with the substance obtained from hog semen in the present investigation.

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Preparation of 2,5-Dimethyl-1,4-Dioxan

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In a recent paper it has been shown that Levene and Walti's method for the preparation of 2,6-dimethyl-1,4-dioxan yielded the cyclic acetal 2-ethyl-4-methyl-1,3-dioxolan [1]. 2,6-Dimethyl-1,4-dioxan, however, has been prepared by Nesmeyanov and Lutsenko [2]. In the course of studies related to addition compounds of ethers, which are being carried out in this laboratory it was found desirable to prepare 2,5-dimethyl-1,4-dioxan as well. Only one previous work is reported on the preparation of this compound. I. G. Farbenindustrie in a patent states that the compound is formed when propylene oxide vapours are passed over sodium bisulphate or other acidic substances at elevated temperatures [3]. The sodium bisulphate reaction, however, when repeated here was found to give a mixture of compounds.

Looking for another starting material for the preparation of pure 2,5-dimethyl-1,4-dioxan we discovered that *trans*-2,5-bis-(iodomethyl)-1,4-dioxan has been prepared through a few intermediates from allyl alcohol and mercuric nitrate [4]. This compound should be expected to give 2,5-dimethyl-1,4-dioxan on reduction.

However, experiments showed that the dioxan ring may be subject to easy cleavage under reducing conditions. By the use of zinc and ethanol or zinc and acetic acid as reducing agents allyl alcohol or allyl acetate were formed, respectively. This difficulty was overcome by using lithium aluminium hydride, which yielded the desired product. Physical constants of the purified reaction product: B.p. (750 mm) 121.5°; m. p. −4.5°; mol.wt. 113.9 (calc. for dimethyldioxan 116.2); d_4^{22} 0.932; n_D^{22} 1.4147; mol. refraction 30.61 (calc. 30.99).

1,4-Dioxan is known to form addition compounds with numerous inorganic salts. An X-ray structure investigation of the mercuric chloride addition compound has been carried out by Hassel and Hvoslef [5].

The 2,5-dimethyldioxan prepared in the present work forms a similar addition compound with mercuric chloride. An X-ray structure investigation of this addition compound has been started in order to confirm that the methyl groups are mutually "*trans*" situated.

Experimental. To 63 g of *trans*-2,5-bis-(iodomethyl)-1,4-dioxan prepared according to Summberbell and Stephens [4] were added 1 l of ether and 20.5 g of lithium aluminium hydride. The round bottom flask was equipped with a sealed stirrer and reflux condenser with a drying tube on top. The stirring was continued with gentle boiling for 8 days. The actual reaction time is probably shorter, but in a preliminary test experiment unreacted starting material was recovered after several days. Finally water was added drop by drop until the evolution of hydrogen had ceased. The etheral layer was separated from the precipitate, the latter washed several times with ether and the ether solutions combined. The main part of the ether was removed by distillation, rest *ca.* 200 ml. To remove iodine the solution was shaken until it became colourless with a few ml of a saturated aqueous sodium thiosulphate solution. After drying over anhydrous sodium sulphate the ether was distilled off and the remaining liquid distilled in a 24 inches Podbielniak column with a reflux ratio of 50 : 1. The liquid distilled at constant temperature .Yield *ca.* 15 ml. (Found: C 61.89; H 10.34. Calc. for $C_6H_{12}O_2$: C 62.01; H 10.41).