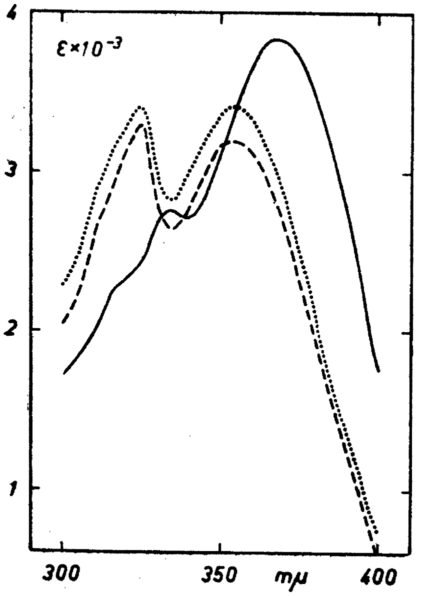

Fig. 2a. The molar extinction of $Co^{II}(C_9H_4O_4NIS)$ *in distilled water* ———, *in 1* M *HCl* — — — — *and the molar extinction of* $C_9H_6O_4NIS$ *in 1* M *HCl* ......

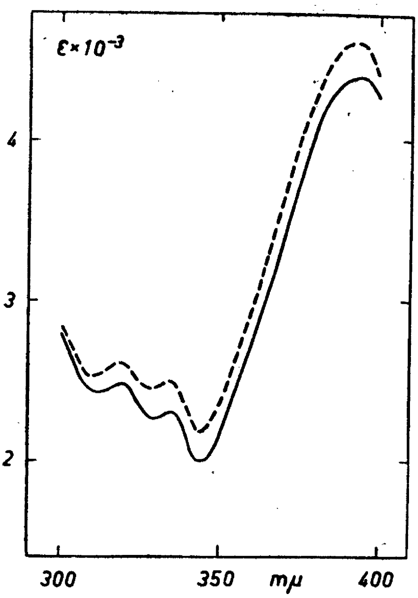

Fig. 2b. The molar extinction of $Na_2[Co^{II}(C_9H_4O_4NIS)_2]$ *in distilled water* — — — *and in 1* M *HCl* ———

out more rapidly but not quantitatively. After repeated evaporations an almost black precipitate can be isolated. According to magnetic and spectrophotometric measurements the composition and structure of the precipitate is given by the formula $Na_2[Co^{II}(C_9H_4O_4NIS)_2]$. The compound is very soluble in water and soluble in alcohol. The solubility is not incompatible with the covalent bond because of the SO_3 groups [5]. The corresponding acid $H_2[Co^{II}(C_9H_4O_4NIS)_2]$ can be prepared by exchange reaction from the sodium salt. Potentiometric titration of the acid dissolved in water has shown it to be a strong acid.

Similar compounds with 8-quinolinol-5-sulphonic acid and its other derivatives as chelating agent are expected.

Further investigations are in progress.

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Received May 9, 1955.

Sialic Acid in Human Serum Protein and in Meconium

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The presence of sialic acids or closely related substances in many glycoproteins and in gangliosides has been indicated by more or less specific colour reactions. In some materials the presence of these substances has been definitively proved by their isolation in pure form and identification by chemical and physical methods. In this paper the isolation of a sialic acid from human serum protein, an acid serum glycoprotein (orosomucoid [1]), and meconium will be reported.

In 1952 Werner and Odin [2], using *i. a.* the colour reactions with Bial's orcinol and Ehrlich's *p*-dimethylaminobenzaldehyde reagents, adapted for quantitative determination, showed that sialic acid most probably occurred in considerable amounts in certain serum glycoproteins, as human α_2-globulin, orosomucoid, and ox seromucoid. It was further observed, that the colour reactions with the diphenylamine and tryptophane-perchloric acid reagents, used by some authors in studies on normal and pathological sera (for references see Winzler [1]), as applied to blood serum probably were almost entirely due to the presence of sialic acid. In 1954 Böhm *et al.*[3], also using a quantitative adaption of the 'Bial' method, suggested the presence of neuraminic acid (Klenk) in human serum protein. Somewhat later Böhm and Baumeister [4] reported the isolation of methoxy-neuraminic acid in crystalline form from the same material.

Meconium has been reported to contain considerable amounts of glycoproteins with the chemical and serological properties of the blood group substances [5]. Using colour reactions Werner [6] has shown, that in addition to the blood group carbohydrates, sialic acid is present in varying amounts in all the mucous secretions investigated by him.

Experimental. Analytical methods. Nitrogen was determined by the Kjeldahl micromethod. For the hexosamine determinations Blix's modification [7] of the Morgan-Elson method was used. Hexose was determined by Vasseur's modification [8] of the Tillmanns-Philippi orcinol reaction. The values were corrected for the fucose content, determined by the method of Dische [9]. Sialic acid was assayed by the colour reactions with Bial's and Ehrlich's reagents [2]. Paper chromatographic analysis was performed with butanol-acetic acid and ethyl acetate-pyridine as solvents. The papers were sprayed with the orcinol-trichloroacetic acid reagent of Klevstrand and Nordal [10]. It gives violet spots with sialic acids. The X-ray powder diagrams were taken with a flat film camera, with a distance of 10.0 cm between the specimen and the film.

Materials. 2 l of pooled *human ACD-plasma* was dialysed for 96 hrs at 4° C, first against several changes of 0.01 *N* hydrochloric acid and then against distilled water. The proteins were precipitated by addition of 5 volumes of ethanol and dried with ethanol and ether. The dry powder contained 14.2 % nitrogen, 1.4 % hexosamine, 1.3 % hexose, <0.1 % fucose, 1.1 % sialic acid.

Orosomucoid was prepared as described by Weimer *et al.*[11] 2 g of the substance was obtained from 12 l of human ACD-plasma. The results of analysis corresponded well with those of earlier reported preparations [1,2]. By the chromatographic method of Gardell [12], however, glucosamine only, but no galactosamine, could be shown to be present. Analysis: 10.7% nitrogen, 12.2 % glucosamine, 13.5 % hexose, 1.4 % fucose, 10.6 % sialic acid.

Pooled human *meconium* was suspended in 0.01 *N* hydrochloric acid. After 48 hrs at 4° C under occasional stirring, the suspension was centrifuged. (Considerable amounts of carbohydrate were evidently still n suspension or dissolved in the supernatant.) The undissolved residue was resuspended in distilled water and dialysed. 5 volumes of ethanol were then added and the precipitate was dried with ethanol and ether. The greenish powder had the following composition: 10.1 % nitrogen, 11.8 % hexosamine, 7.9 % hexose, 4.9 % fucose, 5.0 % sialic acid.

For the isolation of sialic acid from these materials the method of Blix [13] was followed in principle. For the hydrolysis of the serum proteins weak sulphuric acid was used; simple heating with water apparently did not give sufficiently acid conditions for the splitting off of sialic acid. Sulphuric acid has earlier been used for the isolation of the lactaminic [14] and gynaminic [15] acids.

The serum protein preparation (90 g) and the orosomucoid (2 g) were heated for one hour on a boiling water bath with weak sulphuric acid (pH about 2). To the filtered solutions barium hydroxide was added in a little excess (pH about 8). After filtering the solutions were passed through cation exchange columns (Amberlite IRC-50, H) and then freeze-dried. The meconium dry powder (10 g) was heated with distilled water only, and the solution filtered and freeze-dried.

The dried materials were extracted with methanol and the extracts evaporated *in vacuo*. The residues were dissolved in methanol after addition of a little water. Ether was added in small portions for several days, the amorphous precipitates, which formed, being filtered off. When no more of these appeared, petroleum ether was added, and in a few days crystalline deposits were obtained. These were recrystallized from a water-methanol mixture.

The crystalline materials from all three sources showed X-ray diffraction patterns identical with that of the sialic acid isolated by Blix *et al.*[16] from sheep submaxillary mucin and by Odin [17] from human ovarian cyst gels. The colour intensities with the 'Bial' and 'Ehrlich' reagents and the paper chromatographic R_F-values were also the same for all the substances.

Identical sialic acids have thus been isolated from three human sources, *viz.* pseudomyxomatous gels, serum proteins, and meconium, and from the submaxillary mucin of sheep. This substance differs from the sialic acids of ox, swine, and horse origin, which also differ between themselves [16].

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Received May 28, 1955.

Separation of Saturated Straight Chain Fatty Acids. Qualitative Paper Chromatography

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Several different paper-chromatographic methods for the separation of saturated straight chain fatty acids or their alkali salts have been described in the literature. The methods published may be divided into two groups, the first of which involves the volatile acids (from formic to caproic acid) (Brown [1], Hiscox [2], Kennedy [3], Long [4], Isherwood [5]) and the second the long chain acids (from caprylic acid upwards) (Kaufmann [6], Inoyue [7], Spiteri [8], Baker [9], Wegman[10], Holasek[11], Kaufmann[12], Kobrle[13]). In the following, procedures now found suitable for the separation of fatty acids from formic to cerotic acid are described. Because of the volatility of the short chain acids and the poor solubility of the long chain acids not all the acids in question can be separated by one method, but they have to be analysed in separate groups. Suitable groups are: formic — caproic acid, caproic — capric acid, and capric — cerotic acid.

A very suitable method for the analysis of the first group, from formic to caproic acid, is that of Hiscox [2]. According to his method the acids are separated as their ammonium salts, using *n*-butanol saturated with water as solvent. The hydrolysis of the salts during the elution is prevented with ethylamine vapour. The elution is completed in a few hours when the circular chromatography method described by Rutter [14] is employed. A suitable chamber for this purpose was made by grinding two shallow glass bowls against each other and placing the paper between them. The inner diameter of the chamber was 33 cm and the inner height 4 cm. The acids are suitably detected by dyeing with the indicator mixture methyl red-bromothymol blue as described by Duncan [15]. Formic and acetic acid cannot be separated from each other by the method of Hiscox, but according to Lindqvist [16] they may be distinguished by using silver nitrate, which is reduced by formic acid, in connection with the identification. By these methods 0.05 mg of acid can easily be detected. The following approximate R_F-values were obtained: