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 Odin¹⁷ 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 themselves¹⁸.

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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) (Brown¹, Hiscox², Kennedy³, Long⁴, Isherwood⁵) and the second the long chain acids (from caprylic acid upwards) (Kaufmann⁶, Inoyue⁷, Spiteri⁸, Baker⁹, Wegman¹⁰, Holasek¹¹, Kaufmann¹², Kobrie¹³). 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 Hiscox². 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 Rutter¹⁴ 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 Duncan¹⁵. Formic and acetic acid cannot be separated from each other by the method of Hiscox, but according to Lindqvist¹⁶ 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:

formic 0.37, acetic 0.38, propionic 0.52, butyric 0.65, valeric 0.75, caproic (hexoic) 0.86 and δ -nonylic (heptoic) acid 0.88.

The acids of the second group (from caproic to capric acid) may conveniently be separated by the method of Holasek¹¹, who used as solvent carbon tetrachloride-methanol-ammonia (81:18:1 v/v). Thus the acids migrate as ammonium salts. If a paper of 90 g/m² weight is used, not more than 0.05 mg of each acid can be separated. The acids are detected according to the method of Kaufmann⁶ with a solution of Rhodamin-B; 0.01 mg of acid is still detectable. The best separation is achieved if the elution is continued until the undecic acid has reached the edge of the paper. By the usual descending method the following R_F and R_U -values (R_U = undecic acid as reference substance) were obtained: caproic (hexoic) 0.28 (R_F) and 0.57 (R_U), δ -nonylic (heptoic) 0.34 and 0.69, caprylic (octoic) 0.39 and 0.79, pelargonic (nonoic) 0.43 and 0.87, capric (decoic) 0.46 and 0.94, and undecic acid 0.49.

Kaufmann¹² describes a reversed phase method for the separation of the even-numbered acids from capric to stearic acid. The paper is first saturated with hydrocarbon (bp. 190–220° C) and the elution is then carried out with acetic acid-water (9:1 v/v) using the descending method. This procedure was found in the present study to be easier to carry out when circular chromatograms are used. In these the paper need not be so evenly saturated as in the descending method and, of course, the process is shortened. With acetic acid-water all the even-numbered acids from capric to cerotic acid separated. Melissic (triacontic) acid was not sufficiently soluble in these conditions and did not migrate on the chromatogram. Of the odd-numbered acids nonoic and undecic acid were investigated and found to separate easily from each other and also from the even-numbered acids. Hence it is to be expected that all the other odd-numbered acids may likewise be separable from the even-numbered acids. On account of the small R_F -values the chromatograms must be eluted considerably longer than is needed for the solvent front to reach the edge of the paper. This is quite possible with the circular method now employed, because the solvent continuously evaporates from the edge of the paper and the elution may be prolonged indefinitely.

Since the third group includes a great number of acids, they could not be analysed

on one chromatogram, because the size of the paper was limited by the size of the chamber used. When both even-numbered and odd-numbered acids were present, this group was further divided into three groups: capric–palmitic, palmitic–behenic, and behenic–cerotic acid. When only even-numbered acids were present, the division was made into two groups: capric–stearic, and stearic–cerotic acid.

After the hydrocarbon was evaporated from the paper, the acids were detected by converting them into their copper salts and then locating the copper as the ferrous cyanide complex (Kaufmann¹²); 0.1 mg of acid could thus easily be detected. In the conditions used, the R_F -values and R_C -values (capric acid as reference substance) of the acids were as follows: pelargonic (nonoic) 0.65 (R_F), capric (decoic) 0.51, undecic 0.39 (R_F) and 0.77 (R_C), lauric (dodecoic) 0.32 and 0.63, myristic (tetradecoic) 0.24 and 0.48, palmitic (hexadecoic) 0.18 and 0.35, stearic (octadecoic) 0.13 and 0.26, arachidic (eicosoic) 0.10 and 0.19, behenic (docosoic) 0.07 and 0.14, lignoceric (tetracosic) 0.06 and 0.11, and cerotic (hexacosic) acid 0.05 and 0.09.

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