The prorennin peak has disappeared in Fig. 2b, and in the peak which contains the rennin activity it is possible to recognize three components similar to the A, B, and C rennins obtained from rennin crystals. Furthermore, the specific activity (*i.e.* RU/ml/E_{278}) varies over the rennin peak in a similar way as in chromatography of rennin crystals (Foltmann [4]).

An experiment was carried out after only 15 min. of activation at pH 2. Chromatography of the reaction mixture thus obtained revealed approximately equal amounts of A and B rennins and only traces of C. On the basis of this experience it may be surmised that the different rennins are formed from the same prorennin, perhaps by consecutive reactions as in activation of chymotrypsinogen.

The chromatographically purified prorennin was labelled with fluorodinitrobenzene by the Sanger method [5]. After 7 h of hydrolysis in constant boiling, glass-distilled HCl at 110°C, the DNP-amino acids formed were analysed by paper chromatography according to Levy [6]. In this way alanine was found as NH_2-terminal. With the use of an internal standard of DNP-alanine which was treated in the same way as the DNP-prorennin, a semi-quantitative assay indicated 1 mole of DNP-alanine per 60 000 g of DNP-prorennin. Allowing for the weight increase due to DNP groups, this corresponds to a molecular weight of 40 000—50 000 for the prorennin.

In ultracentrifugation the chromatographically purified prorennin showed one slightly asymmetrical peak. The sedimentation constant was increased by increasing concentrations of prorennin, suggesting the formation of dimers in the more concentrated solutions. By extrapolation to zero concentration, $S_{20,w}$ was found to be 3.5.

The chromatographic behaviour of prorennin and rennin is consistent with the earlier observation (Foltmann [1]) that the isoelectric point of prorennin is higher than that of rennin since compounds with higher isoelectric points would be expected to be eluted first in the technique used.

Further studies on prorennin and its activation will appear in the *Compt. rend. trav. lab. Carlsberg.*

Acknowledgements. The author thanks Chr. Hansen's Laboratory, Ltd. for a generous supply of material. The author is indebted to the staff of the Carlsberg Laboratory, especially to the department head, Professor M. Ottesen, for valuable discussions.

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Received December 2, 1960.

Chromatographic Analysis of Sugars as Complex Borate Ions

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As demonstrated by Khym and Zill [1], neutral sugars can be separated chromatographically on an anion exchange resin as borate complexes, when elution is performed with dilute borate buffers. However, although they used small columns (0.5 × 11 cm) the effluent volumes of the monosaccharides became very large (500 — 1 500 ml). This is obviously a disadvantage in the analysis of small amounts of sugars. A modification involving elution at a higher ionic strength has therefore been worked out. Fig. 1 shows a typical chromatogram, including galacturonic acid and glucuronic acid, of which the separation has been reported by Khym and Doherty [2].

The components were obtained in 100 % yield (error of method ± 10 %). In parallel with the relative rates of migration of the sugar-borate complexes in high-voltage paper electrophoresis [3,4], lyxose was eluted together with mannose, ribose together with fucose, arabinose together with galactose, and xylose together with glucose. It thus seems necessary to use specific colour reactions [5,1] for the differential analysis of such sugar pairs. Keto sugars give a low colour yield in the aniline reaction used.

Experimental. The sugars (0.2—0.8 mg of each component) were dissolved in a total

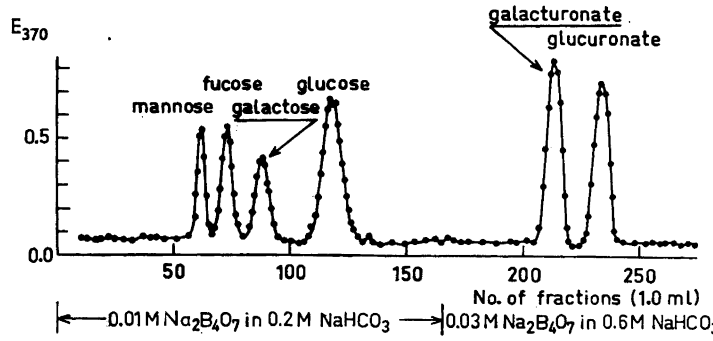

Fig. 1. Chromatography of monosaccharides on a 0.6 × 150 cm Dowex 2 column with borax-bicarbonate solutions. Test mixture: D-mannose (285 μg), L-fucose (298 μg), D-galactose (280 μg), D-glucose (544 μg), D-galacturonic acid (828 μg) and D-glucurone (795 μg).

volume of 0.3 ml of 0.05 M borax (19.1 g $Na_2B_4O_7 \cdot 10\ H_2O$ per liter), and put on the column in the usual way.

The resin, Dowex 2 X 8, 200–400 mesh, was treated with 2 M hydrochloric acid, 2 M sodium hydroxide, 2 M sodium carbonate, and finally 0.03 M borax in 0.60 M sodium bicarbonate, in which solution the resin was stored until used. It was then suspended in a suitable amount of 0.01 M borax in 0.20 M sodium bicarbonate, and was allowed to sediment spontaneously to form a 0.6 × 150 cm column. The column was washed overnight with 0.01 M borax in 0.20 M sodium bicarbonate before application of the sugar mixture. The air present in the solvents was removed by treatment in a suction flask under reduced pressure. Toluene was added to a concentration of 0.5 ml /liter to prevent growth of microorganisms.

The neutral sugars were eluted at room temperature with 0.01 M borax in 0.20 M sodium bicarbonate and, after the appearance of the last neutral sugar peak (glucose), the uronic acids were eluted with 0.03 M borax in 0.60 M sodium bicarbonate. The pH of both solutions was found to be 8.7. The flow rate was adjusted to 1.5 ml/h, and the effluent was collected in 1.0 ml fractions.

A set of standards was prepared for each component. The standards contained 10–100 (150) μg of sugar per ml, dissolved in 0.01 M borax in 0.20 M sodium bicarbonate.

Analysis was performed with a modification of Gardell's aldo sugar method [6]: 3 ml of a freshly prepared mixture of 10 ml of aniline (distilled) and 500 ml of acetic acid was added with an automatic pipette to unknowns and standards. After thorough shaking, the test tubes were provided with aluminium caps, heated for 20 min in a vigorously boiling water bath, and cooled with tap water. The yellow-brown colour was read at 370 mμ in a Beckman B spectrophotometer with water as a blank

The investigation was aided by grants from *Karolinska Institutets reservationsanslag.*

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Received December 1, 1960.