absteigende Chromatographie wird der Bogen gemäss Fig. 2b in eine Glaswanne eingelegt und mit einem Glasstab festgehalten. Nach dem ersten Lauf und Abdampfen des Laufmittels wird der Bogen senkrecht zur ersten Richtung gefaltet und der zweite Lauf erfolgt in analoger Weise. Durch Vergleich gleichgrosser Substanzmengen an den korrespondierenden Stellen der vier Teilchromatogramme konnte eine sehr gute Reproduzierbarkeit der Farbbildung festgestellt werden. Während die Leerwerte des Papiers an verschiedenen Stellen *eines* Teilchromatogrammes recht charakteristisch verschieden sind, stimmen sie an korrespondierenden Stellen der vier Teilchromatogramme gut überein. Die Genauigkeit der Methode hängt natürlich davon ab, in welcher relativen Konzentration die zu bestimmende Aminosäure im Gemisch vorliegt. Unter Optimalbedingungen wurden bei jeweils vier Einzelbestimmungen Maximalabweichungen von etwa 5 % gefunden.

Das Verfahren hat sich in umfangreichen Serienuntersuchungen bewährt. Eine genaue Beschreibung der Methode, sowie Angaben über die Reproduzierbarkeit und über ein geeignetes Laufmittelsystem sollen einer grösseren Arbeit vorbehalten bleiben.

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Detection of chitin oligosaccharides on paper chromatograms

The chromatography of oligosaccharides in general has been reviewed by BAILEY AND PRIDHAM[1], and BARKER *et al.*[2] have presented some data on chromatography of chitin oligosaccharides using a pentan-2-ol–pyridine–water mixture followed by detection with alkaline silver nitrate. Many of the reagents which have been described for the detection of sugars on chromatograms depend upon a reaction with

the reducing groups of the sugar. Although these reagents are satisfactory with monosaccharides, the amount of colour obtained with an oligosaccharide series decreases rapidly with the increase of molecular weight. The detection of sugars with silver depends upon a rather generalised reaction, since it can be used for sucrose and trehalose, but it is not satisfactory for oligosaccharides from chitin (Fig. 1). JEANES, WISE AND DIMLER[3] were presumably forced to apply large samples of starch digests to their chromatograms because of the low sensitivity of dinitrosalicylic acid with the oligosaccharides. With a view to increasing the sensitivity and specificity in detection of oligosaccharides in chitin digests, the chlorination reaction of RYDON AND SMITH[4] and the Morgan-Elson reaction as described by SALTON[5] were examined as follows.

All chromatograms were prepared on Whatman No. 1 paper by ascending development with isoamyl alcohol (BDH)–pyridine–water (1:1:0.8). This solvent was found to give better results than pentan-2-ol–pyridine–water (1:1:1) (BARKER

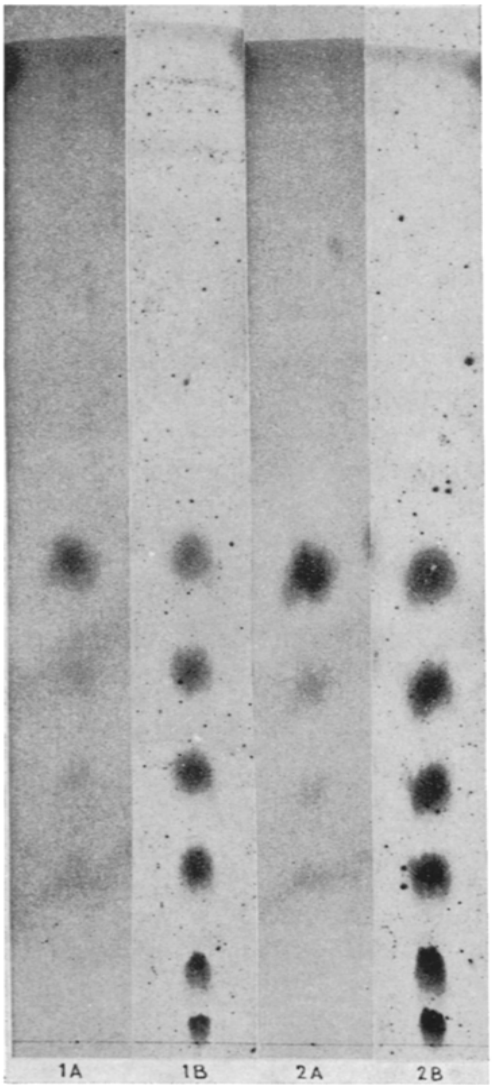

Fig. 1. Chromatograms of chitin oligosaccharides. Solvent: isoamyl alcohol–pyridine–water (1:1:0.8). (A) silver detection; (B) chlorine–potassium iodide–starch detection; (1) 2 μg and (2) 5 μg of saccharide each. The spots are hexasaccharide to acetylglucosamine, in ascending order.

et al.[2]). Chlorine was prepared in a generator from 100 ml concentrated hydrochloric acid and 50 g potassium permanganate, then washed by passing through water and concentrated sulphuric acid and absorbed in 1 l carbon tetrachloride. About 5 g barium carbonate and 5 g anhydrous calcium chloride were added to the flask. Starch-iodide solution was freshly prepared by boiling 1 g starch, 0.25 g potassium iodide and 1 ml 5 *N* HCl in 100 ml water.

The chromatograms were air dried to remove the solvent, then humidified for 2 h in a cylinder with a water-saturated atmosphere. The papers were then rolled and soaked in the chlorine reagent for 20 min. The use of chlorine solution as a dip was more convenient than the use of chlorine gas (RYDON AND SMITH[4]) or spraying techniques (MAZUR *et al.*[6]). After aeration for $^1/_2$ to 1 h to remove the excess chlorine, the papers were sprayed with the starch-iodide reagent. The resulting blue colour in the spots was stable for months and the technique is simpler than that described by BAROLLIER[7], in which the colour is stabilised by treatment with ammonium molybdate. The limit of sensitivity was less than 1 μg and the colour values permitted a roughly quantitative estimation by eye. Fig. 1 illustrates the results using purified chitin oligosaccharides (BARKER *et al.*[2]) and demonstrates the sensitivity of the chlorination method compared with silver treatment.

The Morgan-Elson reaction may be used for detection of acetylamino sugars and also for compounds of acetylglucosamine and acetyl muramic acid in digests of cell walls of microorganisms[5]. The sensitivity of the reaction is very low for chitin oligosaccharides, and DIERICKX AND GHUYSEN[8] found that the limit of detection with the Ehrlich reagent for chitobiose and chitotetraose was 100 μg. This phenomenon was discussed by KUHN *et al.*[9], who showed that 3- or 6-substituted acetylglucosamine compounds give colours with Ehrlich's reagent, but substitution in the 4-position (as in chitin) strongly depresses the colour yield. A simple technique to permit the use of the fairly specific Ehrlich reagent with chitin oligosaccharides is to digest the oligosaccharide spots on the chromatogram with a crude chitinase which produces acetylglucosamine. Chromatograms were lightly sprayed with a solution of a freeze-dried preparation from the puff-ball *Lycoperdon perlatum*[10] (2 mg/ml in 0.05 *M* citrate buffer, pH 4.5), then held in a moist atmosphere for 1 h before applying the SALTON[5] method. Under these conditions good spots with similar colour densities per unit weight of oligosaccharide were obtained.

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