

$C_{14}H_{10}N_4O_6$ : C 50.9; H 3.3; N 17.0) and through its *p*-nitrophenylhydrazone, m.p. 206.5°C. (Found: C 58.5; H 3.9; N 15.1. Calc. for  $C_{14}H_{11}N_3O_4$ : C 58.9; H 3.9; N 14.8). For the quantitative determination the precipitation with dinitrophenylhydrazine was utilised after a small correction for the solubility of the derivative had been applied.

Phthalic anhydride was found as such in the chloroform phase, especially if the hydrolysis had been of short duration. By thermal decomposition of the ozonisation solution or by reduction with sodium iodide the formed phthalic anhydride can be isolated almost quantitatively while the content of phthalic acid is practically nil. M.p. and mixed m.p. 130°C (Found: C 64.3; H 2.9. Calc. for  $C_8H_4O_3$ : C 64.7; H 2.7). Quantitatively the anhydride was determined by two methods:

(a) After the hydrolysis the chloroform is evaporated from the heterogeneous mixture whereby the anhydride dissolves in the water as phthalic acid. The water is removed at room temperature and the residue (A) is extracted repeatedly with chloroform leaving almost pure phthalic acid which is then weighed. This method gives probably somewhat low results.

(b) The residue (A) is dissolved in water again and titrated with standard sodium hydroxide (indicator: phenolphthalein). From the titration values is then deducted the amount due to phthalaldehydic acid.

In all quantitative work the ozonisation batches consisted of 1.00 g of 1,2-naphthoquinone in 100 ml of chloroform.

#### Results:

Carbon monoxide during ozonisation 8.1, 8.6, 9.6 mg. Mean 8.8 mg. During hydrolysis 6.2, 6.8, 5.6 mg. Mean 6.2 mg.

Carbon dioxide during ozonisation 102, 86, 78, 94 mg. Mean 90 mg. During hydrolysis 302, 276, 307, 283 mg. Mean 290 mg.

Formaldehyde 14.2, 14.6 mg. Mean 14.4 mg. Formic acid 182, 224, 215, 232 mg. Mean 213 mg.

Phthalaldehydic acid 733, 745, 711 mg. Mean 730 mg.

Phthalic anhydride. Method (a) 173, 161, 182 mg. Mean 172 mg.

Method (b) 191, 194, 200 mg. Mean 195 mg. Mean for both methods 183 mg.

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## Partition Chromatography on Ion Exchange Resins.

### Separation of Sugars

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In earlier papers it has been demonstrated that strongly polar non-electrolytes such as sugars can be taken up effectively from ethanol-water mixtures by means of ion exchange resins. Only a few chromatographic separations have been carried out based upon this sorption mechanism<sup>1,2</sup>. In separations of monosaccharides overlapping curves are obtained in many systems. A systematic study of the factors which govern this sorption has shown that the ethanol concentration, and the rate of diffusion inside the resin particles are critical factors<sup>3,4</sup>. For this reason it is necessary to use an appropriate ethanol concentration, a small resin particle size, and a low flow rate in order to achieve satisfactory separations. In the present work some results are given demonstrating applications of this method to sugar separations.

*Experimental.* The resin (Dowex 1 X-8) was classified hydraulically to obtain the fraction 45–75  $\mu$  and transformed into its sulfate form. The column was prepared and operated in the normal way, and precautions were taken to avoid gas bubbles in the column<sup>5</sup>. The dimensions of the resin bed were 10  $\times$  840 mm. The sample solutions which had a volume of 5 ml contained 75 % ethanol (by weight). The eluant was fed on to the column with a pump at a constant flow rate (0.8 ml cm<sup>-2</sup>min<sup>-1</sup>). The temperature was kept constant at 28°C.

The eluate was collected in a fraction collector and analyzed using the Technicon Auto-Analyzer<sup>6</sup>.

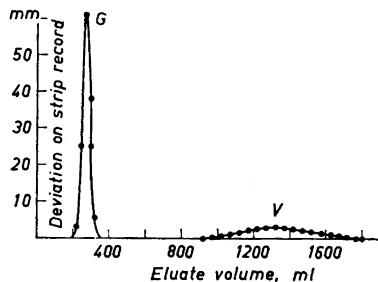


Fig. 1. Separation of 10 mg glucose (G) and 20 mg verbasose (V). Eluant: 65 % ethanol.

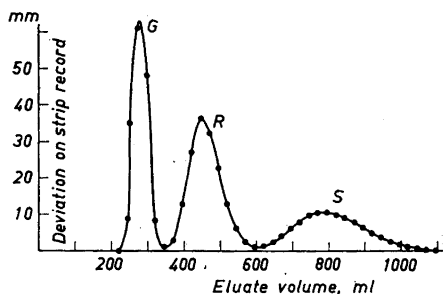


Fig. 3. Separation of 10 mg glucose (G), 20 mg raffinose (R), and 20 mg stachyose (S). Eluant: 65 % ethanol.

*Results and discussion.* A low ethanol concentration facilitates the diffusion inside the resin and is, therefore, desirable. The results given in Figs. 1 and 2 show that glucose is separated quantitatively from the pentasaccharide verbasose and from the tetrasaccharide stachyose in 65 % ethanol. An almost complete separation from raffinose is also achieved in this medium. As shown in Fig. 3, the separation of a mixture containing glucose, raffinose, and stachyose is almost complete under the same conditions.

An increased ethanol concentration results in increased distribution coefficients, *i.e.*, in increased peak elution volumes. The results given in Fig. 4 show that an increased separation factor is obtained. In agreement with theory (*cf.* Ref.<sup>5</sup>) a broadening of the elution bands occurs. An ethanol concentration of 72–74 % is suitable for the separation of monosaccharides from various disaccharides such as maltose (Fig. 5), cellobiose, sucrose, and

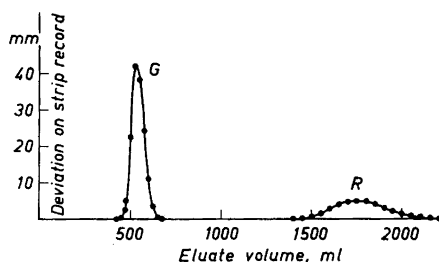


Fig. 4. Separation of 10 mg glucose (G) and 10 mg raffinose (R). Eluant: 74 % ethanol.

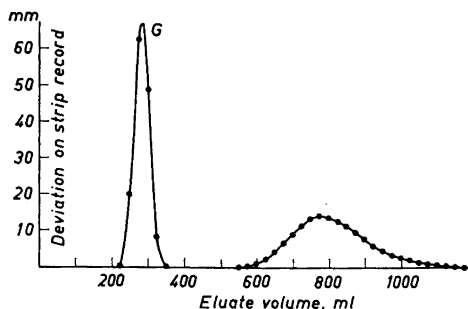


Fig. 2. Separation of 10 mg glucose (G) and 20 mg stachyose (S). Eluant: 65 % ethanol.

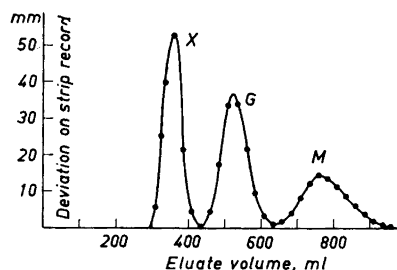


Fig. 5. Separation of 10 mg xylose (X), 10 mg glucose (G), and 20 mg maltose (M). Eluant: 74 % ethanol.

lactose. Among the sugars hitherto studied, all monosaccharides appear ahead of the polysaccharides, whereas the lower polysaccharides appear before those of a higher molecular weight. With complicated mix-

tures it is advantageous to use stepwise elution or gradient elution with eluants containing lower ethanol concentration in order to avoid an excessive broadening of the bands corresponding to higher saccharides. As an example it can be mentioned that a satisfactory separation of a mixture containing glucose, sucrose, raffinose, stachyose, and verbascose can be obtained by eluting the first two components with 74 % ethanol and then decreasing the ethanol concentration to 65 %.

A separation of monosaccharides from each other is also possible. The separation of xylose from glucose is demonstrated in Fig. 5. Similarly, it is possible to obtain a quantitative separation of mannose from glucose, whereas no satisfactory separation of galactose from glucose has been obtained under the experimental conditions chosen in the present work.

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## Correction to "Conversion of $\Delta^5$ -Cholestene- $3\alpha$ - $12\alpha$ -diol to Cholic Acid in the Rabbit" \*

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In the title above for  $\Delta^5$ -cholestene- $3\alpha$ - $12\alpha$ -diol read  $\Delta^5$ -cholestene- $3\beta$ - $12\alpha$ -diol.

\* *Acta Chem. Scand.* **16** (1962) 1534.

## Occurrence of Methyl Esters in Lymph

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Absorption of dietary triglycerides from intestines in a partially hydrolysed form has been established. Intestinal absorption of oleic acid  $1-^{14}\text{C}$  has been studied by Bergström *et al.*<sup>1</sup> with cannulated thoracic duct in rats. They reported that oleic acid was transported *via* the lymph and incorporated into triglycerides and phospholipids whether fed as oleic acid or triolein. Blomstrand and Rumpf<sup>2</sup> have reported that when cetyl alcohol  $1-^{14}\text{C}$  was fed to rats with thoracic duct fistula about 15 % of it was present as unchanged alcohol in the lymph. Bergström<sup>4</sup> fed ethyl esters of fatty acids but found only traces of unhydrolysed esters in the lymph. Blomstrand<sup>5</sup> noticed that chimyl alcohol could be absorbed unchanged but was extensively metabolised already in the mucosa cells. Dhopeshwarkar and Mead<sup>6</sup> have shown evidence for occurrence of methyl esters in body and blood lipids. In another study<sup>7</sup> they also showed that when methyl elaidate was fed to fat deficient animals a part of it was found unhydrolysed in body lipids. The purpose of this short study was to determine whether methyl esters were present in rat lymph and to find out if methyl oleate could be absorbed without undergoing complete hydrolysis.

*Experimental.* Cannulation of lymph duct was performed as described before<sup>1</sup> on two male albino rats maintained on a regular chow diet. Lymph was collected in suitable containers under ethyl alcohol, before and after feeding methyl oleate. Methyl oleate was purified by vacuum distillation and was found to contain 85 % oleate, 15 % palmitate and a small amount of palmitoleate. No attempt was made to remove these impurities. The total lipids were extracted as usual from lymph using ethanol-ether (3:1) and subjected to silicic acid chromatography. The fraction that was eluted with 2 % ether in petroleum ether