tion. Washing of the ether extract with HCl does not remove all the excess dinitrophenylhydrazine, and this forms five spots, of which one travels about as far as 2-hexanone. The method of Pool and Klose could most probably be used, however, for purification of the ether extract.

Acknowledgements. The authors wish to express their gratitude to the Head of the Department of Nutritional Chemistry, Professor Paavo Roine, for his interest and criticism during the work. Financial support was given by The State Scientific Council. One of the authors (H.W.) has performed additional experiments especially with the low molecular compounds at the Research Laboratories of the State Alcohol Monopoly, Helsinki. In these experiments, Miss Ingrid Hanika has assisted.


Received November 7, 1956.

A Low-Resistance Vapour-Phase Chromatograph Column

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In vapour-phase chromatographic separation of high-boiling compounds, as for instance methyl esters of higher fatty acids, it is convenient to apply low pressures as this enables the use of correspondingly low column temperatures. It is not possible, however, fully to utilize such a pressure reduction in columns of the usual type, as for instance those in which Celite is used as the inert carrier of the static phase, as the pressure drop is very great, about 400 mm mercury pressure in the case of a column length of 1 m. Cropper and Heywood have suggested the substitution of sodium chloride crystals for Celite, enabling a reduction of the pressure drop to 20 mm mercury per metre of column length, and column packing of this type has been used successfully in this laboratory. However, it would be convenient — for several reasons — to be able to reduce the pressure drop further, and since the technique of vapour-phase chromatography offers many points of resemblance to that of fractional distillation, it would be reasonable to assume that packing elements of the types used in low-pressure fractionating columns might also be applied in a vapour-phase chromatograph column. This assumption has been confirmed by experiments with gauze helices of stainless steel, so-called Dixon Gauze Rings, made from fine-mesh steel gauze.

A column packing was prepared with helices of the above-mentioned type, Griffin & George, Ltd., Cat. No. B34-420, dimensions 1/16" x 1/16", the helices being carefully mixed with a solution of Dow Corning High Vacuum Silicone Grease in ethyl acetate. After evaporation of the solvent, the grease was left as a film which covered the gauze meshes, but did not fill out the interior of the helix. The helices were packed into a 2 m column of stainless steel tubing with an inside diameter of 8 mm. At 200°C, 20 mm outlet pressure and a rate of flow of carrier gas of 8 ml N2 per min a pressure drop from one end of the column to the other of 4.5 mm mercury was measured. For purposes of comparison it may be mentioned that the pressure drop measured across the same column when packed with sodium chloride crystals, but otherwise under identical working conditions, was found to be 52 mm.

For our experiments a mixture of methyl esters of C18-C30 fatty acids was used, and it was ascertained that the degree of separation obtained by means of the column was practically the same for both types of packing (see Table 1), but that the elution peaks yielded by the steel gauze packing were of a more perfect shape and more symmetrical. The low pressure drop

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Table 1.

<table>
<thead>
<tr>
<th>200°C, 8 ml N₂/min</th>
<th>Steel gauze</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outlet pressure, mm Hg</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Pressure drop, g</td>
<td>4</td>
<td>4.5</td>
</tr>
<tr>
<td>Inlet pressure, g</td>
<td>54</td>
<td>24.5</td>
</tr>
<tr>
<td>HETP* for C₁₆, cm</td>
<td>2.2</td>
<td>4.2</td>
</tr>
</tbody>
</table>

* HETP has been calculated according to a formula given by van Deemter et al. in "Symposium on Vapour Phase Chromatography, London, 30th May—1st June 1956", Butterworth's Scientific Publications, p. A 21.

Further enables the use of lower column temperatures or higher outlet pressures, and in both cases the degree of separation obtained from a column is increased considerably. It is also possible to use long columns, the pressure drop in the case of a 4 m column being for instance 8 mm mercury.

It remains to be ascertained whether the type of packing described is also applicable for vapour-phase chromatography at normal pressures, but considering the great similarity between vapour-phase partition chromatography and fractional distillation there is every probability that this is the case.


Received November 15, 1956.

Distribution of Enzymes Involved in the Breakdown of Uracil to β-Alanine in Rat Liver Cells

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Previous studies of the catabolism of [¹⁴C]-labeled uracil, dihydrouracil (DHU) and β-ureidopropionic acid (BUP) in the rat demonstrated the following reactions in rat liver slices in vitro and in the intact rat:

Uracil → DHU ⇌ BUP → β-alanine

The results further indicated that the liver is the main site of these reactions. The localization of the enzymes responsible for the different reactions in this process in the liver cells, has now been investigated.

White rats were decapitated, and the livers were homogenized in 0.25 M sucrose as described by Ernst and Low. Uracil-6-¹⁴C, DHU-8-¹⁴C and BUP-3-¹⁴C were incubated with the homogenate and with three fractions of the homogenate obtained by successive removal of nuclei, mitochondria and microsomes by fractional centrifugation at 1600 × g for 10 min, 4100 × g for 15 min, and 105 000 × g for 45 min, respectively. The reaction mixtures were analyzed for radioactive compounds by paper strip chromatography in n-butanol-acetic acid-water (4:1:5) and in collidine saturated with water, and the distribution of radioactivity along the strips was determined, as described previously. The total radioactivity of the incubation mixtures before and after incubation was determined by plating aliquots on planchets and subsequent counting, as described by Canelliak. The results showed, within the limits of experimental error, that no volatile, radioactive compounds were formed during incubation, indicating that all the radioactive products can be detected in the chromatograms. The results are recorded in Table 1.

The incubations with BUP as substrate show that the yield of β-alanine is the same in the presence of the homogenate and the particle-free supernatant fluid. The removal of nuclei and mitochondria has no effect on the enzymatic activity. These facts clearly demonstrate that the enzyme catalyzing the conversion of BUP to β-alanine is present entirely in the supernatant fraction. The data from the incubations of DHU with homogenate and supernatant fluid are consistent with the finding that the enzyme catalyzing the interconversion of DHU and BUP was present entirely in the supernatant fraction of isotonic KCl homogenates of rat liver.

The incubation of uracil with the supernatant fluid demonstrates that β-alanine is the only radioactive product which can be clearly detected in the chromatograms.

* The numbering system is that in which uracil is 2,4-dihydroxy-pyrimidine. The preparation and tests of purity of the labeled compounds are described elsewhere.

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