

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.

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## 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-<sup>14</sup>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-<sup>14</sup>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

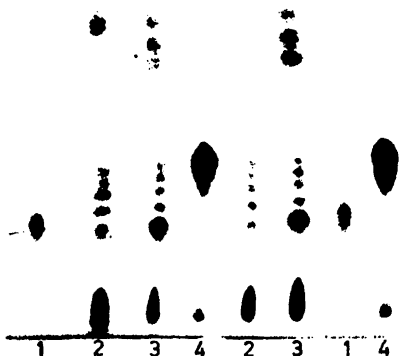


Fig. 1. Thin layer chromatogram. Developing solvent: 1.5 % Ethyl ether in normal hexane. Spraying solution: Saturated solution of phosphomolybdic acid in ethanol. Spot 1: Methyl oleate. 2: Lymph lipids before feeding methyl oleate. 3: Lymph lipids after feeding methyl oleate. 4: Cholesterol stearate.

was further subjected to thin layer chromatography as described previously<sup>6</sup>. The thin layer plates were sprayed with a saturated solution of phosphomolybdic acid in ethanol and heated at 100°C for a few minutes to bring about the color of the spots (Fig. 1). As this procedure cannot be used when recovery of the material is desired it was substituted by short exposure to iodine vapour to mark the spots, scraping off the silicic acid and extracting with ether. Experiments showed that this procedure did not alter the original compounds

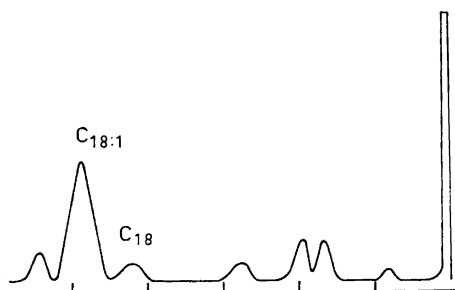


Fig. 2. Gas liquid chromatogram of methyl esters recovered from thin layer plate after feeding methyl oleate. Column: Polar polyester (LHC-R-296), 6 ft. Temp. 195°C.

and so the ether extract was directly used for gas liquid chromatography (Fig. 2).

*Comments.* It can be seen from Fig. 1 that the lymph lipids contained methyl esters even when no methyl esters were fed to the animals (Column 2). However, after feeding methyl oleate the same two spots were much more prominent (Column 3). Thus it was assumed that methyl oleate could be absorbed without complete hydrolysis. If this assumption was correct one would expect a pronounced peak of methyl oleate in the lymph lipid methyl esters after feeding methyl oleate. Examination by gas liquid chromatography (Fig. 2) showed that this was indeed the case. As a further confirmation the sample of methyl esters was hydrogenated using platinum oxide as catalyst and analysed by gas liquid chromatography. As predicted the original methyl oleate peak now merged into the stearic acid peak when retention times of known standard substances, analysed under similar conditions, were compared.

Thus it was concluded that rat lymph contained methyl esters and that at least a portion of the fed methyl oleate could be absorbed without complete hydrolysis. This observation is in accordance with the data on triglycerides and cetyl and chymyl alcohol reported earlier. Further work on the quantitative aspect is being undertaken.

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