TABLE XIV

Changes in Goldfish Phospholipid Fatty Acids after 76 Days on Test Diets (Second Experiment)

| Fatty acid | Stock diet (92 days) | Depletion diet (267 days) | Low fat diet (76 days) | | | Linoleic 5% (76 days) | | | Linolenic 5% (76 days) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 13C | 19C | 25C | 13C | 19C | 25C | 13C | 19C | 25C |
| | % | % | % | % | % | % | % | % | % | % | % |
| 18:0 | 11.0 | 7.7 | 4.9 | 10.5 | 3.8 | 8.2 | 5.8 | 6.3 | 6.9 | 5.5 | 8.9 |
| 18:1 | 34.7 | 36.4 | 37.7 | 27.7 | 35.7 | 30.3 | 35.3 | 32.7 | 35.2 | 34.4 | 31.5 |
| 18:2 | 1.7 | 3.2 | 2.3 | 3.0 | 2.9 | 3.0 | 6.0 | 6.5 | 2.3 | 3.3 | 1.7 |
| 18:3 | 1.4 | 1.2 | 0.3 | 0.0 | 0.0 | 0.3 | 0.1 | 0.5 | 1.4 | 2.1 | 1.4 |
| 18:4 & 20:4 | 0.6 | 1.8 | 2.4 | 1.9 | 2.3 | 2.2 | 2.1 | 2.2 | 0.7 | 2.4 | 2.2 |
| 20:2 | 2.7 | 1.4 | 2.1 | 1.1 | 2.1 | 4.0 | 1.4 | 4.4 | 1.6 | 3.0 | 1.1 |
| 20:3 | 2.4 | 6.6 | 5.9 | 12.7 | 7.0 | 3.9 | 3.5 | 3.3 | 3.8 | 2.8 | 5.2 |
| 20:4 | 1.1 | 2.4 | 1.2 | 2.7 | 3.7 | 2.2 | 4.8 | 4.7 | 1.2 | 0.8 | 4.9 |
| 20:5 | 3.6 | trace | 0.3 | 0.0 | 0.1 | 0.4 | 0.2 | 0.0 | 1.4 | 0.4 | 0.9 |
| 22:1 | 0.0 | 0.0 | 0.4 | 0.0 | trace | 0.0 | 0.0 | trace | 0.2 | 0.1 | 0.0 |
| 22:2 | trace | 0.7 | 1.1 | 1.3 | 1.0 | 0.6 | 0.5 | 0.6 | 0.8 | 0.8 | 0.6 |
| 22:3 | trace | 0.3 | 0.5 | 1.4 | 0.8 | 0.2 | 0.0 | trace | 1.0 | trace | trace |
| 22:4 | 1.4 | 0.1 | 0.6 | 0.3 | trace | 0.4 | 0.2 | 0.1 | trace | trace | trace |
| 22:5 | 1.9 | 1.0 | 0.4 | 3.0 | 1.1 | 1.7 | 1.2 | 0.8 | 6.1 | 2.9 | 2.0 |
| 22:6 | 1.3 | 0.7 | 0.5 | 1.9 | 0.9 | 0.9 | 1.0 | 0.8 | 3.2 | 2.4 | 6.1 |

positions in the triglyceride molecule.

It is thus apparent that fresh water and marine fish probably do not differ in any basic way in their mechanism for the deposition, synthesis and interconversions of fatty acids, nor do they differ from other animals.

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# Separation of the Methyl Esters of Oleic, Linoleic, and Linolenic Acids by Column Chromatography Using Cation Exchange Resin Containing Silver Ion

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## Abstract

By taking advantage of the tendency of silver ion to form complexes with unsaturated compounds, the methyl esters of oleic, linoleic, and linolenic acids have been cleanly separated in good yield by column chromatography. The silver ion was supported by means of a cation exchange resin; no silver was ever eluted from the column. Aqueous methanol, pure methanol, and ethanol saturated with butene-1 were employed as solvents.

## Introduction

IT HAS BEEN known for many years that silver ion undergoes a reversible reaction with unsaturated compounds to form rather weak and unstable complexes. In 1938 Winstein and Lucas (1) studied these complexes in some detail, including the determination of equilibrium constants between silver ion and a number of olefins. Subsequent investigations of these compounds were performed by Lucas, et al. (2), and Traynham (3), while Andrews (4) and Keefer (4b) studied the more unstable complexes of silver ion and aromatic compounds. Recently Muhs and Weiss (5) determined the equilibrium constants of a great many silver-olefin complexes and discussed the factors affecting their stability.

With the increased use of chromatography, advantage has been taken of these silver complexes to effect the separation of various unsaturated compounds. Bednas and Russell (6), Bradford et al. (7), Tenny (8), and Smith and Ohlson (9), used silver nitrate solutions in gas chromatography to separate low molecular weight olefins. Muhs and Weiss (5), used a similar gas chromatographic system. But while these systems are excellent for the separation of low molecular weight materials, the elevated temperatures needed for gas chromatographic analysis of substances of higher molecular weight cause this method to be definitely limited; this is due to the non-volatility and thermal instability of many such substances, as well as possible thermal decomposition of the column itself (6).

## Discussion

Since we in this laboratory are interested in the separation of various phospholipids according to the degree of unsaturation of their fatty acid substituents, column chromatography involving a cation exchange resin containing silver ion was investigated,

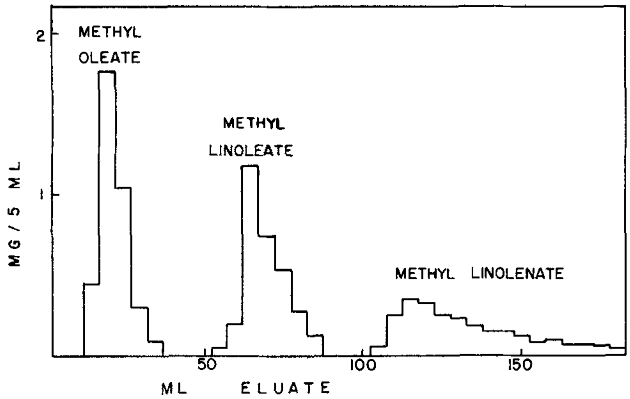

FIG. 1. Separation of methyl oleate, methyl linoleate, and methyl linolenate by column chromatography using cation exchange resin containing silver ion. Solvents: 75% methanol, 25% water (40 ml); methanol (50 ml); ethanol saturated with butene-1 (100 ml).

using fatty acid methyl esters as model compounds. Employing a cation exchange resin as support for silver ion, the methyl esters of oleic, linoleic, and linolenic acids have been cleanly separated as shown in Figure 1, using aqueous methanol, pure methanol, and ethanol saturated with butene-1 as solvents.

Recently de Vries (10) reported the separation of the methyl esters of oleic, linoleic, and linolenic acids, as well as several other unsaturated lipids, using a column containing silica impregnated with silver nitrate. Unlike the de Vries column, use of ion exchange resin as support for the silver ion permits the use of polar solvents, since the silver is tightly held and therefore not removed from the column. This system, in conjunction with the de Vries technique, should prove of considerable versatility in the separation of unsaturated lipids.

Upon elution of methyl oleate and methyl linoleate, methyl linolenate was displaced from the resin column by employing a solvent consisting of ethanol saturated with butene-1. Since butene-1 has a stronger affinity for silver ion (5) than does methyl linolenate, the latter is displaced. Tailing of the methyl linolenate peak may be caused by an insufficient concentration of butene-1, due to its limited solubility in ethanol. This difficulty might be eliminated by use of a pure olefin, such as pentene-1, as displacing agent, but this was not attempted. Samples consisting of a few milligrams of each ester were used, and their emergence was detected by microdetermination of iodine uptake (11). No silver was ever observed to be eluted from the column.

The identity of each peak in Figure 1 was established by introduction of each of the above esters individually into the column under conditions comparable to a normal run. That separations were caused by silver ion-olefin complexing was demonstrated by preparation of a column containing only sodium ion. All three esters passed directly and quantitatively through this column.

It seems apparent that the separation of unsaturated compounds by silver ion complexing involving various chromatographic techniques is an important and general method of wide applicability.

## Experimental Procedure

Analytical grade cation exchange resin AG 50W-X8, 200–400 mesh, hydrogen form, was stirred for one hour with aqueous silver nitrate. The silver nitrate was present in excess such that after stirring, settling, and decantation, the supernatant gave a copious precipitate of silver chloride when treated with hydrochloric acid. The supernatant was decanted. The resin was washed repeatedly with distilled water until the supernatant no longer gave a precipitate with hydrochloric acid and its pH value had risen above five. The resin was then washed several times with the initial solvent, i.e., 75% methanol, 25% water. It was then poured as a slurry into an 8 mm I.D. glass column, fitted with a stopcock at the bottom and a 100-ml reservoir at the top, to a height of approximately 425 mm. A glass wool plug supported the resin.

A mixture of 4.12 mg (0.0139 double bond(DB) meq.) of methyl oleate, 3.41 mg (0.0232 DB meq.) of methyl linoleate, and 2.59 mg (0.0266 DB meq.) of methyl linolenate (total, 0.0637 DB meq.) in 1 ml of methanol was placed on the column, which was then eluted with 40 ml of 75% aqueous methanol, 50 ml of methanol, and finally with 100 ml of ethanol saturated with butene-1. The eluate was collected automatically in 5-ml portions. Fractions 1–8 contained methyl oleate, 9–19 contained methyl linoleate, and 20–38 contained methyl linolenate. When the composition of the initial solvent, 75% aqueous methanol, was changed to higher methanol content in the range 85–100%, methyl oleate and methyl linoleate were not separated, while methyl linolenate did not elute with pure methanol within 300 fractions. When optimum solvent composition was used, the separation was not sensitive to flow rate.

Aliquots of each fraction were analyzed for double bond content by the addition of a known excess of iodine, followed by spectrophotometric determination of the unreacted halogen (11). Recovery of each ester in milligrams was then calculated from the known iodine values. These results have been plotted in Figure 1. Recoveries of methyl oleate, methyl linoleate, and methyl linolenate were 88%, 89%, and 97%, respectively.

That the silver-treated resin actually contained silver was demonstrated by treatment of the resin with dilute nitric acid; subsequent addition of hydrochloric acid to the supernatant produced a copious precipitate of silver chloride.

A column was prepared from resin treated exactly as described above, but replacing the silver nitrate with sodium chloride. None of the three esters was retained in this column containing sodium ion; recovery was rapid and quantitative.

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