tabolism. An early increase in phospholipid synthesis as indicated by $P^{32}$ incorporation may be a general phenomenon occurring in all virus infected cells but sufficient data are not available to bear this out.

The significance of the elevated specific activity of phosphatidyl inositol 24 hours after infection is not clear but may be associated with the egress of mature virus from the cell or with an increase in transport of nutrients into the severely damaged cells.

*Summary.* The lipid composition of HeLa cells infected with vaccinia virus as well as non-infected cells is described. The results indicate that virus infection in a serum-free medium does not alter the lipid composition of the cells. However, the incorporation of $P^{32}$ into the phospholipids of virus infected HeLa cells was greater than that observed in non-infected cells, a finding that appears to be generally applicable to virus infected cells.

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## Age-Related Changes in the Behavior of Diethyleneglycol Succinate Polyester Columns. (28258)

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During the course of gas-liquid chromatographic studies with fish oil fractions(1) containing considerable amounts of long chain highly unsaturated fatty acids, it became apparent that as the succinate polyester column aged, progressive changes occurred in the fatty acid patterns.

Magidman(2), using butter fat, reported that as the succinate polyester column was used, the stationary phase appeared to become less polar in character and the retention time for the unsaturated esters changed at different rates than for the saturated esters.

A more precise definition of the changes which occur in relation to aging seemed desirable. Therefore the following studies were carried out.

The polyester diethyleneglycol succinate was prepared according to the procedure of Koroly and Beavers(3) as modified by Craig and Murty(4). The cross-linking agent, diglycerol, was omitted since it was not necessary for the synthesis of this polyester. We also eliminated the catalyst, zinc chloride, because trace amounts left after treatment with Amberlite IR-120 resulted in shorter column life. Elimination of the catalyst made it necessary to increase the initial heating time at 160°C from 4 to 6 hours. The columns were prepared as follows: the polyester was dissolved in acetone and the solid support Celite 60-100 mesh was added in a ratio of 1:4. This was then dried in a rotary evaporator under reduced pressure. The dried material was then added to the column. Good packing

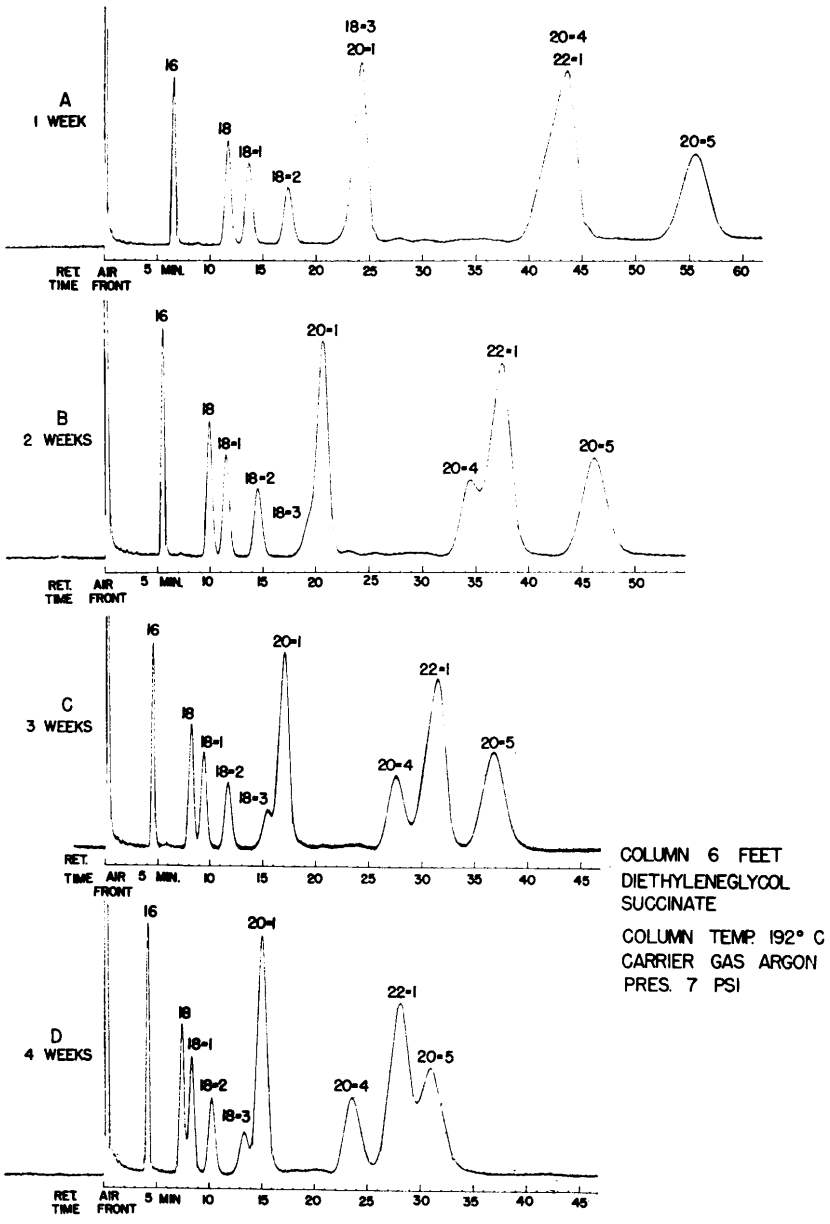


FIG. 1. Changes in retention time of unsaturated fatty acids in relation to progressive aging of a diethylene glycol succinate polyester column.

was obtained by tamping the column while the material was added.

The instrument used was a Barber Coleman Model No. 10 with a $Sr^{90}$ ionization detector. 500 V. was supplied for the detector with a 300 megohm linearizing resistor. The operating condition is shown in the Figure. Initial conditioning of the column was carried out at 197°C for a period of 3 days; thereafter the column temperature was maintained at 192°C.

The material used for evaluation of column behavior changes with relation to age consisted of a mixture of palmitic, stearic, oleic, linoleic, linolenic, 11-eicosenoic, arachidonic, 13-docosenoic, and 5, 8, 11, 14, 17-eicosapentaenoic acids. This "standard solution" was submitted to gas-liquid chromatography at

periods of 1, 2, 3, and 4 weeks, respectively, during which time the column was in constant use. The results are shown in Fig. 1. It is apparent that as the column aged, retention times of all esters decreased. However, the decrease was more rapid in the more unsaturated as compared to the less unsaturated acids. These changes were of such magnitude as to cause a change in sequence in some instances.

From the findings in the Figure, it is apparent that if one wishes to have optimal separation of C18:0 from C18:1, or C22 from C20:5 that A or B conditions are optimal. (Other studies have indicated that this same statement applies to separation of C20:0 from C18:3, or C22:0 from C20:4.) If, however, one wishes to obtain adequate separation of C20:1 from C18:3, or C22:1 from C20:4, a C or D condition will give dependable results.

Column temperature appeared to be the major factor which influenced the rate of aging. Some differences were observed in different lots of polyester. The changes associated with aging bore no relationship to the total number of methyl fatty acid ester samples which had been placed on the column during the period of aging; in other words, identical aging changes occurred in columns which had been subjected only to heat as compared to columns which had been used extensively but maintained at the same temperature for the same period.

Identification of unknown peaks is frequently quite difficult. Hydrogenation usually provides adequate clarification insofar as the major component peaks are concerned; however, in the case of some minor components, hydrogenation may not be particularly helpful. In such cases, comparison of the gas-liquid chromatographic pattern in new and aged columns, respectively, may provide most helpful information in this regard.

*Summary.* Aging of a succinate polyester column at a temperature of 192°C for periods of several weeks, results in progressive changes in retention time for unsaturated methyl fatty acid esters. These changes are reasonably predictable.

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