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[Received for publication March 6, 1962]

» A method for incorporation of radioactive carbon into either the 1- or 2-position of fatty acids was developed in our laboratory and was used to prepare 2-$C^{14}$-labeled erucic acid and nervonic acid for use in metabolic studies (1). When the labeled fatty acids were chromatographed on siliconized paper (2) and the paper scanned for radioactivity, it was found that the fatty acids contained appreciable amounts of radioactive impurities (Fig. 1). Commercial samples of palmitic acid-1-$C^{14}$, oleic acid-1-$C^{14}$, and linoleic acid-1-$C^{14}$ were also found to contain radiochemical impurities. A simple method involving chromatography on acid-treated Florisil was developed, which eliminated most of the impurities and permitted recovery of the fatty acids in a high state of radiochemical purity.

Acid-treated Florisil was prepared as follows: 3 liters of dilute hydrochloric acid (1 vol of concentrated hydrochloric acid to 10 vol of water) was added to 300 g of Florisil[1] (60–80 mesh, activated at 650°), and the mixture was allowed to stand overnight. The Florisil was then filtered and washed with water until the washings were neutral. This procedure was repeated and, after the second filtration and washing, the material was dried overnight in an oven at 110° and then stored in a stoppered flask.

Labeled erucic acid (0.19 mc/mmole) was purified by chromatographing 150 mg on a 12-g column of acid-treated Florisil. The column was prepared by mixing the Florisil with chloroform to make a slurry and adding this in several portions to a chromatographic tube 1.2 cm in diameter. This gave a column about 20 cm in height. The fatty acid was added to the column in a small volume of chloroform and elution was carried out with 300 ml of chloroform followed by 100 ml of methanol. The effluent was collected in 10-ml fractions. Radioactive impurities containing 7% of the total activity were eluted in fractions 2 and 3. These were followed by a main peak containing 83% of the total activity (fractions 4 to 15), which appeared to be radiochemically-pure erucic acid as judged by chromatographing it on siliconized paper and scanning the paper for radioactivity. Some labeled erucic acid was also present in fractions 15 to 30 of the chloroform effluent but these fractions contained radioactive impurities as well and their combined activity amounted to only 3% of the total. The radioactivity in the methanol effluent (7% of total) was all associated with impurities.

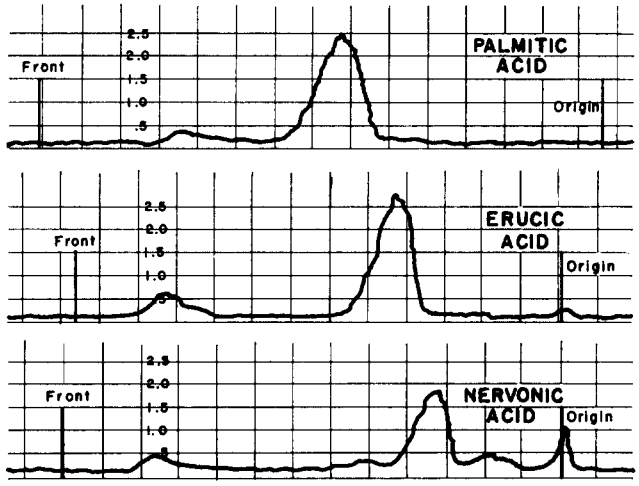


Fig. 1. Distribution of radioactivity on paper chromatograms of palmitic acid, erucic acid, and nervonic acid developed on siliconized paper with acetic acid–water 85:15. The papers were scanned with an Actigraph II, Model C100 B, using a D47 gas-flow counter with Micromil window (Nuclear-Chicago): Collimator, 1/8 in.; scan speed, 3 in. per hr; time constant, 40 sec; count-rate range, 1000 cpm.

This chromatographic method was also used to purify labeled nervonic acid (0.25 mc per mmole), palmitic acid (5 mc per mc/mole), oleic acid (7.5 mc per mmole), and linoleic acid (4.5 mc per mmole). The samples of palmitic acid, oleic acid, and linoleic acid were much smaller (4 to 6 mg) and were therefore chromatographed on 5-g columns of acid-treated Florisil, using smaller amounts of eluting solvents.

The pattern of emergence of the main component and impurities was very similar for each of the fatty acids. Impurities that remained at or near the origin on the paper chromatograms were eluted before the main peak

* This work was supported by the Life Insurance Medical Research Fund, the Multiple Sclerosis Society of Canada, and the Medical Research Council of Canada.

[1] Activated magnesium silicate obtained from the Floridin Co., P.O. Box 989, Tallahassee, Florida.

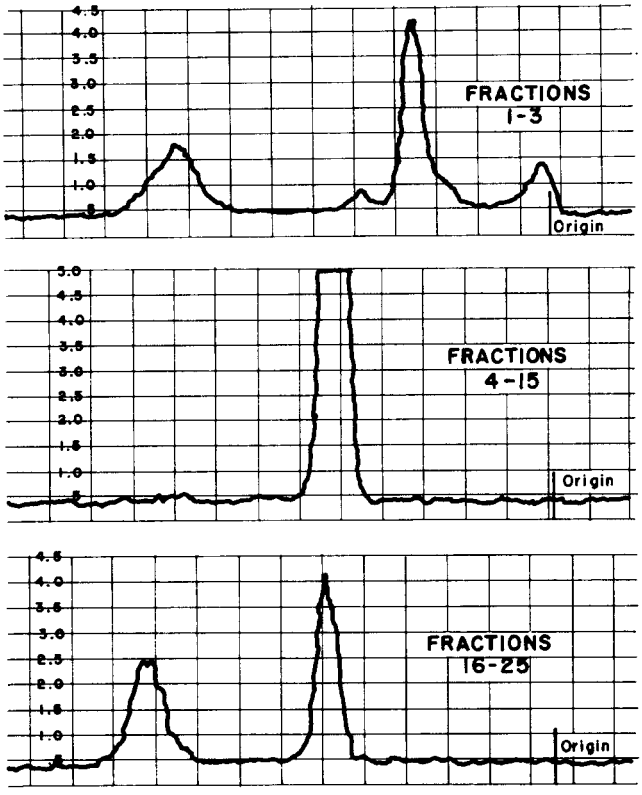


FIG. 2. Distribution of radioactivity on paper chromatograms of fractions from the Florisil column used to purify palmitic acid-1-$C^{14}$. The three chromatograms were run at the same time using the conditions described in Fig. 1. Fractions 1 to 3, which contained 8% of the total radioactivity, consisted mainly, if not entirely, of radiochemical impurities. Fractions 4 to 15 (74% of the total radioactivity) contained palmitic acid of high radiochemical purity. Fractions 18 to 25 (18% of the total radioactivity), which included the methanol effluent in this instance, contained labeled palmitic acid together with an impurity of higher $R_f$ value.

on the column chromatograms. Impurities that had relatively high $R_f$ values on the paper chromatograms were in general eluted from the columns in the later chloroform fractions or with methanol but components with high $R_f$ values were also eluted before the main peak. Results obtained with labeled palmitic acid are illustrated in Figure 2.

The method appeared to be highly successful for separating radioactive impurities from the labeled fatty acids but it probably would not separate mixtures of the fatty acids themselves. Although a direct comparison cannot be made between results obtained with the 5-g and 12-g columns, the three fatty acids purified on the 5-g columns were eluted in the same fractions and the two fatty acids purified on 12-g columns occupied the same position on the chromatograms.

It was found in other experiments that the amount of fatty acid added to the column could influence the results obtained. For example, when 4 mg of labeled oleic acid was chromatographed on a 12-g column, only about half of the total activity was eluted with 200 ml of chloroform and the remainder appeared in the methanol effluent. When it was rechromatographed on a 5-g column, nearly all of the radioactivity associated with oleic acid was found in tubes 5 to 10 of the chloroform fraction. Similarly, when trace amounts of purified nervonic acid were rechromatographed on a 5-g column of acid-treated Florisil, about half of the radioactivity was eluted in the chloroform fraction and about half in the methanol. If 0.1% acetic acid was added to the eluting solvents, the labeled nervonic acid was all eluted in the chloroform. It seems possible that this phenomenon was caused by a limited number of active adsorption centers on the treated Florisil that retarded the elution of fatty acids when the ratio of fatty acid to Florisil was very low.

The amount of radioactivity associated with contaminants in these fatty acid preparations was higher than one might think from casual inspection of the charts shown in Figure 1. Assay of the fractions from the column chromatograms indicated that each of the labeled fatty acid preparations contained four to six different radioactive impurities. These accounted for 10 to 15% of the total activity in the palmitic, oleic, and erucic acid preparations and about 45% in the nervonic acid preparation. There was some indication that the amount of radioactive impurity increased during storage of the fatty acid preparations. For example, the sample of linoleic acid appeared to be reasonably pure when first received; but two to three years later when these purification experiments were carried out, less than 50% of the total radioactivity was associated with linoleic acid and most of the remaining radioactivity was associated with impurities that ran near the solvent front. Similar, but less marked effects, were observed with other fatty acid preparations. Increases in radiochemical impurity may have been due to the formation of decomposition products formed by self-irradiation, although the fatty acids were stored in the cold in dilute solution in petroleum ether to minimize such decomposition.

The presence of radioactive impurities in commercial preparations of $C^{14}$-labeled fatty acids has also been noted by other workers, and techniques such as thin-layer chromatography and gas-liquid chromatography have been used for purification of the fatty acids (3, 4, 5). Both of these techniques are more limited in respect to the amount of fatty acid that can be conveniently purified, and the fact that gas-liquid chromatography is normally carried out with methyl esters

is a disadvantage if the purified material is required in the form of free fatty acid.

The technical assistance of Mr. E. Pedersen is gratefully acknowledged.

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