The impurity of radioiodinated triolein

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SYNOPSIS Commercially supplied radioiodinated triolein has been shown by thin-layer chromatography and silicic acid column chromatography to contain impurities, consisting mainly of diglycerides and monoglycerides, but also a small amount of free fatty acid. The effect of these impurities on the radioiodinated triolein absorption test requires further investigation.

Radioiodinated fats and fatty acids have been used to investigate steatorrhoea since the method was first described by Stanley and Thannhauser in 1949. While undertaking an investigation of a case of steatorrhoea using 131I triolein, an inconsistency in the results was found. Although many potential sources of error in this test have been described (Cox, 1961), it was thought that our error might have been due to our faulty labelling of triolein with 131I by the method of Lubran and Pearson (1958). On analysis of our end product by thin-layer chromatography a considerable amount of impurity was found and the source was traced to the synthetic triolein supplied commercially; commercially supplied 131I triolein was found to contain similar impurities. Consequently more detailed studies of 131I triolein, as supplied commercially in carrier olive oil, were performed using silicic acid column chromatography.

MATERIALS AND METHODS

ANALYSIS OF 131I TRIOLEIN IN OLIVE OIL Synthetic triolein previously purified by distillation and crystallization at a low temperature was labelled with 131I by the technique of Lubran and Pearson (1958) at the Radiochemical Centre, Amersham. The radioiodinated triolein was then diluted with commercial olive oil, yielding the preparation which is in general use for malabsorption studies.

Thin-layer chromatography was carried out using silica gel as described by Mangold (1961). Autoradiograms were then prepared. Silicic acid column chromatography was carried out using the method of Horning, Williams, and Horning (1960). The percentage of the total radioactive load recovered in the eluate using different solvents was found and the main fractions present in each eluate identified (Table). In addition, infra-red spectrometry was carried out on the synthetic triolein, the 131I triolein in olive oil, and the separated fractions.

For comparison with the thin-layer chromatography of the 131I triolein in olive oil, autoradiograms were also prepared after chromatography of a mixture of the 131I triolein in olive oil and 131I oleic acid and of 131I oleic acid alone. Stained chromatoplates of non-radioactive oleic acid and triolein were also prepared and are shown together with the autoradiograms as tracings in the diagram.

RESULTS

The autoradiogram (Fig. 1) of the chromatogram of the 131I triolein in olive oil shows radioactive impurities.

![Autoradiogram of radioiodinated triolein after thin-layer chromatography.](http://jcp.bmj.com/)

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Tentative identification is suggested from the known migration values and from the partial resolution of the triglycerides into three spots because of the variation in the degree of iodination of the double bonds present in the fatty acid side chains. The main impurity is present as diglycerides and little radioactivity appears to occur in the free fatty acid. This finding was confirmed by the stained chromatoplate as is shown in the diagram (Fig. 2) of the superimposed tracings. The nature of the unidentified spot in the autoradiogram remains obscure. It was thought that it might be due to a triglyceride with a polyunsaturated side chain, but this was not confirmed by liquid gas chromatography. It did not appear in analyses of subsequent samples of triolein.

The results of radioanalysis of the column fractions are tabulated below.

<table>
<thead>
<tr>
<th>Eluate Fraction</th>
<th>Predicted Output</th>
<th>Percentage Total Radioactivity Load in Fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>60% Benzene in hexane</td>
<td>Triglycerides</td>
<td>50.2</td>
</tr>
<tr>
<td>100% Benzene</td>
<td>Free fatty acid</td>
<td>7.2</td>
</tr>
<tr>
<td>100% Methanol</td>
<td>Diglycerides and monoglycerides</td>
<td>29.1</td>
</tr>
<tr>
<td></td>
<td>Unrecovered</td>
<td>13.5</td>
</tr>
</tbody>
</table>

Infra-red analysis failed to reveal the presence of lower glycerides in either synthetic triolein or in the $^{131}$I triolein in olive oil, although it verified the presence of lower glycerides in the methanol fraction of the silicic acid column. The reason for this finding is uncertain but it may be due to the insensitivity of infra-red spectrometry in this application.

**DISCUSSION**

About 30% of the total radioactivity of a commercial sample of $^{131}$I triolein was found to be present in a diglyceride impurity; there was little radioactivity in the free fatty acid.

Mattson and Volpenheim (1962) have reviewed the methods of synthesizing glycerides and commented on the difficulties in preparing pure triolein, describing some of the impurities that result. Lakshminarayana, Kruger, Cornwell, and Brown (1960) have also noted impurities in $^{131}$I triolein, and found in their analysis that the methyl esters, monoglycerides, and diglycerides, present as impurities, appeared to take up the $^{131}$I label to a greater extent than might have been expected from chemical analysis.

**FIG. 2.** Comparison of autoradiograms of radioiodinated preparations (shaded areas) and stained chromatoplates of unlabelled triolein and oleic acid (broken lines).
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It would be of interest to know whether the lower glyceride impurity in the triolein has any effect on the 131I triolein absorption test and this is being investigated using a purified 131I triolein for comparison.

We are grateful to Professor E. M. McGirr, Dr. J. H. Wright, Dr. J. Badenoch, and Dr. V. Lawrie for their advice and encouragement.

REFERENCES

ADDITIONAL

Since this paper was written, analysis of a purified triolein (99% triglyceride) labelled with 131I at the Radiochemical Centre, Amersham, has revealed 16% lower glycerides and 4% oleic acid in the end-product. This suggests that the mode of preparation (possibly heating to 60°C) to evaporate solvent) must contribute to the impurities of 131I triolein.

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