INTRODUCTION

Dietary fatty acids absorbed from the intestinal tract are in most part biosynthesized to form triglycerides in the intestinal mucosa. They are then complexed with protein, cholesterol ester, and phospholipid to form chylomicrons, which enter the lymphatic ducts whereby they are transported into the blood. It is said that the chylomicrons brought into the blood are cleared from the plasma into the fat deposits (adipose tissues) chiefly via the two pathways, viz. directly from the plasma into adipose tissues\textsuperscript{1-3} or by way of the liver into the fat deposits.\textsuperscript{4}

This communication reports the results of a study we performed in rats to determine whether the two pathways to adipose tissues might differ with sorts of dietary fatty acids.

EXPERIMENTAL

1. Animals used were male white rats, each weighing about 200 g, which had been starved for 48 hours. They were divided into 80 groups of 2 to 20 rats each. Rats in groups 1 through 10 were fed 0.8 g = 2.5μC (4.28 × 10^5 c.p.m.) of 1-C\textsuperscript{14}-oleic acid per animal along with small amounts (about 3 g) of fat-free fishmeal, starch, sodium chloride and water made into soft pellets (totally consumed in 0.5 to 2 hours), followed by feeding with a commercial diet. Animals in these groups were sacrificed at 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 hours after the initial ration, and total fats in the liver and depot fats separated and meas-
ured of respectively specific radioactivities with a gas Geiger-Müller counter.

Rats in groups 11 through 20 were fed 0.8 g = 5.9 μCi (2.47 × 10^5 c.p.m.) of 1-C^14-palmitic acid, those in groups 21 through 30 were given 1-C^14-oleic acid 0.54 g of 1.7 μCi (2.85 × 10^5 c.p.m.) plus 0.26 g of palmitic acid, those in groups 31 through 40 received 0.8 g = 1.2 μCi (1.86 × 10^5 c.p.m.) of triolein (C^14-oleic acid labeled), those in groups 31 through 50 0.8 g = 4.3 μCi (2.04 × 10^5 c.p.m.) of tripalmitin (C^14-palmitic acid labeled), those in groups 51 through 60 0.8 g = 2.1 μCi (2.36 × 10^5 c.p.m.) of α, β-dioleyl-α'-palmitin (C^14-oleic acid labeled), those in groups 61 through 70 0.8 g = 1.9 μCi (1.98 × 10^5 c.p.m.) of α, α'-dioleyl-β-palmitin (C^14-oleic acid labeled), and those in groups 71 through 80 1 g = 0.72 × 10^5 c.p.m. of rat depot fat, respectively. Total fats in the liver and in the adipose tissue were measured subsequently in the same manner as in groups 1-10. Samples of blood were drawn from rats in group 1 and from those in group 11 and fatty acids were separated from serum triglyceride fractions and their specific radioactivities measured.5,6

2. Synthesis of triglycerides

α, β-Dioleyl-α'-palmitin and α, α'-dioleyl-β-palmitin were synthesized by the following procedures.

To α, β-glyceroldichlorohydrin or α, α'-glyceroldichlorohydrin, 1-C^14-oleic acid sodium was added and the resultant mixture heated at 120-130°C for an hour to obtain α, β-diolein or α, α'-diolein, which were isolated by the use of sodium chloride. They were then desiccated and purified through a chromatographic column of silica gel.5 α, β-dioleyl-α'-palmitin or α, α'-dioleyl-β-palmitin was thereby synthesized by addition of palmitic acid chloride to the purified α, β-diolein or α, α'-diolein. The products were washed with diluted sulfuric acid, potassium bicarbonate and water, desiccated and purified by silica gel chromatography.5

3. Preparation of rat depot fats labeled with C^14-fatty acids.

Four-eight hour-starved albino rats were fed for 5 days basal diet with 10% 1-C^14-oleic acid added to it, and they were sacrificed and depot fats extracted with ether and subsequently with lukewarm methanol.

RESULTS

Figures 1(a), (b), (c), (d), (e), (f), (g) and (h) show the time-courses of total fat contents in the liver and depot fat of albino rats receiving dietary vari-
PATHWAYS OF DIETARY FATTY ACID DEPOSIT

(a) Administration of 1-C\(^14\)-oleic acid

(b) Administration of 1-C\(^14\)-palmitic acid

(c) Administration of 1-C\(^14\)-oleic acid +
    palmitic acid (2:1)

(d) Administration of triolein (C\(^14\)-oleic
    acid labeled)
Fig. 1 Change in the specific radioactivity (average % of g) of liver and depot fat of albino rats starved for 48 hours after the administration of various C¹⁴-labeled fats (0.8 g).
PATHWAYS OF DIETARY FATTY ACID DEPOSIT

ous fats after 48 hours of fasting.

Intrahepatic total fat began increasing conspicuously at 6 hours after oral administration of 1-C$^{14}$-oleic acid or 1-C$^{14}$-palmitic acid, reached a maximum level at 12 hours post administration, and thereafter declined. Total fat content in the depot fat increased gradually in reverse proportion to the decrease in hepatic specific radioactivity and reached peak levels at 21 and 27 hours after administration. In response to oral administration of a mixture of 1-C$^{14}$-oleic acid and palmitic acid (2:1), similarly, total fat in the liver became maximal at 18 hours and subsequently diminished with concomitant elevation of total fat in the depot fat which became maximal at 30 hours. Animals which had received triolein, tripalmitin or $\alpha$, $\alpha'$-dioleyl-$\beta$-palmitin also showed practically comparable trends; hepatic total fat content became maximal at 9, 15, and 12 hours post administration respectively and declined thereafter with a parallel gradual elevation of total fat content in the depot fat which eventually reached peak levels at 21, 24, and 21 hours respectively.

Animals fed $\alpha$, $\beta$-dioleyl-$\alpha'$-palmitin or rat depot fats showed slightly different time-courses of total fat contents in the liver and adipose tissue. In both the liver and adipose tissue, the total fat content increased drastically from 3–6 hours after administration onward and reached peak levels at 12–15 hours, respectively.

DISCUSSION

The depot fat is composed primarily of triglycerides. Although the constituent fatty acid radix of triglycerides in the fat depots of rats is as yet unknown, it has been described to be UUU and UUS (where U stands for unsaturated and S for saturated, respectively) in most instances. In a previous report the authors had described that the fatty acid compositions of fat depots are consistently U:S = 2:1 approximately irrespectively of kinds of dietary fatty acids given in large doses for prolonged periods. The fatty acid in fat depots are predominantly oleic acid and palmitic acid.

In view of these findings the investigation reported herein was performed with oleic acid, palmitic acid and triglycerides.

Dietary fatty acids and fatty acids liberated by hydrolysis of triglycerides in the intestine are absorbed into the lymphatic ducts and are in most part biosynthesized to form triglycerides in the intestinal wall. We conducted experiments on saturation, unsaturation, etc. of the fatty acids thereof. Tables 1 and 2 summarize the serum lipid analytical data from animals which were fed 1-C$^{14}$-oleic acid (group I) or 1-C$^{14}$-palmitic acid (group II). As can be noted, the results
Table 1

Incorporation of $1^{-}$-oleic acid and $1^{-}$-palmitic acid into serum fat at 3 hours after the administration in albino rats, starved for 48 hours

<table>
<thead>
<tr>
<th>Administration</th>
<th>Specific activity of serum fat (each % per 100 of total fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
</tr>
<tr>
<td>$1^{-}$-oleic acid</td>
<td>0.2</td>
</tr>
<tr>
<td>$1^{-}$-palmitic acid</td>
<td>—</td>
</tr>
</tbody>
</table>

— = absence of activity

Table 2

Specific radioactivity of individual fatty acid constituents of serum triglyceride in Table 1 comparison with the control

<table>
<thead>
<tr>
<th>Administration</th>
<th>Triglyceride fatty acid¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saturated fatty acid</td>
</tr>
<tr>
<td>$1^{-}$-oleic acid</td>
<td>Quantity (%)</td>
</tr>
<tr>
<td></td>
<td>Specific activity (%)</td>
</tr>
<tr>
<td>$1^{-}$-palmitic acid</td>
<td>Quantity (%)</td>
</tr>
<tr>
<td></td>
<td>Specific activity (%)</td>
</tr>
<tr>
<td>None</td>
<td>Quantity (%)</td>
</tr>
</tbody>
</table>

— = absence of activity

obtained suggest that neither appreciable hydrogenation or dehydrogenation of fatty acids nor intermixture with endogenous fatty acids takes place in the intestinal wall and appear to indicate that triolein and tripalmitin are formed predominantly following oral administration of oleic acid and palmitic acid respectively. It is generally believed that dietary fatty acids (as well as dietary triglycerides) are transported in all instances in the form of triglycerides to the fat depots, viz. one as chylomicrons and the other low-density lipoproteins from
the liver.9-11 And it would be certain that, even if these triglycerides liberate their constituent fatty acids immediately preceding their gaining entrance to the fat depots, depot fat triglycerides are reconstituted biosynthetically from the liberated fatty acids.

It might follow from these facts that dietary fatty acids possessing the same constituent fatty acid radices as the depot fat are more predominantly transported direct to the fat depots whereas those of which constituent fatty acid radices differ from those of the depot fat are conveyed to the fat depots only after having been transformed in the liver.

This hypothesis has been verified by the observation of rapid storage of orally administered $\alpha$, $\beta$-dioleyl-$\alpha'$-palmitin and rat depot fat (Figs. 1(f) and 1(h)).

However, as evident from Figure 1(d), dietary triolein was noted to be cleared from the circulating plasma into the fat depots rather slowly as compared with its storage in the liver. Triolein content in the rat depot fat is high,7 and this may probably be because a portion of dietary triolein is converted to saturated fatty acids in the liver before it enters the fat depots. The same may apply to other fats as well.

It was reported by Gordon et al12 that lymphatic chylomicrons injected into albino rats receiving carbohydrates by oral route simultaneously are deposited in the adipose tissue earlier than in the liver. In the study herein described, nevertheless, all kinds of fats except for two fatty acids administered orally along with carbohydrates were noted to be deposited rather slowly in the adipose tissue as compared with intrahepatic deposition. A separate experiment where triolein or rat depot fat was administered intragastrically to rats maintained on a commercial diet (carbohydrate content: approximately 70%) yielded the results practically consistent with the said (description omitted).

SUMMARY

Various fats tagged with radioactive carbon isotope, C14, were administered orally along with carbohydrates to 48-hour-starved albino rats, of which the total fat contents in the liver and fat depot (adipose tissue) were measured consecutively to investigate pathways of dietary fatty acids to the fat depots. Rat depot fat and $\alpha$, $\beta$-dioleyl-$\alpha'$-palmitin were found to be conveyed direct to the fat depots predominantly as compared with other dietary fats, probably because their constituent fatty acid radices are identical with those of depot fats.
REFERENCES


