was precipitated (Kane et al., 1975) with 1,1,3,3-tetramethylurea, extracted with chloroform/methanol (1:1, v/v), washed with ether, and dried under N₂. Amino acid analysis of this protein corresponded to that of apolipoprotein B, as did its electrophoretic mobility on urea/polyacrylamide-gel electrophoresis. Its specific radioactivity was obtained by radioactivity counting and protein determination (Lowry et al., 1951), after dissolving in 1.0ml of 0.5m-NaOH. On both diets the protein was cleared from the plasma following first-order kinetics. The slope of the decay curve was therefore a measure of the fractional clearance rate of apolipoprotein B. This value, together with the VLD-apoprotein obtained from a known volume of plasma), was used to calculate the synthetic rate of the protein (the product of the fractional clearance rate and pool size in the steady state).

With respect to diet S, diet P produced a consistent fall in the total plasma concentration of cholesterol, triacylglycerol and VLD apolipoprotein B (Table 1), with significant alteration of the composition of this lipoprotein. The most prominent diet-induced compositional changes were seen in the palmitate, oleate and linoleate content of VLD cholesteryl esters, triacylglycerols and phospholipids. In general the percentages of palmitate and oleate fell in these moieties, whereas that of linoleate increased. These alterations in VLD-lipoprotein fatty acyl content were accompanied by modification of the gross composition of the lipoprotein, resulting in particles of increased triacylglycerol content and decreased in phospholipid.

The kinetic parameters of VLD-apolipoprotein-B decay (Table 1) indicated that the fall in plasma VLD-apolipoprotein-B concentration on diet P resulted not from an increase in the fractional clearance rate of the protein but rather from a 36%(P<0.01) decrease in its rate of synthesis. From these data we conclude that the rate of synthesis of VLD apolipoprotein B in normal subjects on a polyunsaturated-fat diet is decreased, and this is accompanied by a significant change in the composition of the circulating lipoprotein.


Plasma Clearance and Tissue Distribution of Liposomes Containing Different Sterols

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Increasing use is being made of liposomes as vehicles for the entrapment of drugs and proteins before their administration to animals. Several workers have investigated the effect of lipid composition on the distribution of liposomes in the body, with the aim of directing entrapped drugs etc. to specific tissues (Tyrrell et al., 1976; Gregoriadis, 1976). Cholesterol is usually incorporated into liposomes to decrease leakage of their contents but the effects of altering sterol composition on the final distribution of the liposomes has not been studied, although various sterols can be incorporated into them and transferred from them to cells (Bruckdorfer et al., 1968).

Liposomes were prepared by sonicating 20mg of 10μCi of 3H-labelled egg phosphatidylcholine with 10mg of the appropriate sterol in 10ml of phosphate-buffered saline solution, pH 7.4. The dispersions were centrifuged at 100000g for 45min and filtered through a
Adult white mice were injected via the tail vein with liposomes containing [3H]phosphatidylcholine (200 µg/animal) and the appropriate sterol. The animals were killed at various time intervals and the blood was collected for determination of 3H radioactivity. Each point is the mean for three determinations. The liposome preparations contained the following sterols: ○, cholesterol; ●, 3-hydroxycholest-5-en-7-one; □, cholesta-4,6-dien-3-one; ■, 3-hydroxycholest-3-en-2-one; ▲, 1-methyl-10-norcholesta-1,3,5(10)-trien-3-ol.

0.22 µm Millipore filter and then samples (0.2 ml) were injected into the tail veins of adult white mice. After 2.5, 20, 60 or 120 min, the blood was removed by cardiac puncture and livers, kidneys, spleen, lungs and intestines were also removed. Lipid was extracted from the organs (Folch et al., 1957) and its 3H content determined by liquid-scintillation counting. Animals were also injected with 125I-labelled albumin to measure the amount of blood in the tissues. A correction for this has been applied to all tissue-uptake values.

The disappearance of the labelled phospholipid from the bloodstream after injection of the various liposome preparations is shown in Fig. 1. Liposomes containing cholesterol, 3-hydroxycholest-5-en-7-one and cholesta-4,6-dien-3-one left the circulation relatively slowly and at approximately the same rates. Even after 60 min about 40% of the label was still present in the circulation and after 120 min 15–25% remained. That this represents removal of the whole liposome was shown in similar experiments with 3H-labelled phosphatidylcholine and [14C]cholesterol in the liposomes. Both isotopes left the bloodstream at similar rates, although the cholesterol was removed rather more slowly, presumably because of exchange with plasma lipoproteins and blood cells.

Liposomes containing 3-hydroxycholest-3-en-2-one and 1-methyl-10-norcholesta-1,3,5(10)-trien-3-ol gave quite different results with a very rapid initial rate of removal from the circulation. In both cases, more than 50% of the lipid was lost from the bloodstream within 2.5 min. This was followed by a much slower phase of removal since more than one-half of the 3H-labelled phospholipid present at 20 min after injection was still present after a further 100 min.

The uptake of liposomes by the various tissues at 20 min after injection is shown in Table 1. As found by other workers (Tyrrell et al., 1976; Gregoriadis, 1976), the liver is by far the major site of uptake for all the liposome preparations. The uptake of liposomes containing 3-hydroxycholest-3-en-2-one and 1-methyl-10-norcholesta-1,3,5(10)-trien-3-ol by all tissues was much greater than that of the other liposomes.
Table 1. Tissue distribution of \(^{3}H\)phosphatidylcholine at 20 min after the intravenous injection of liposomes

<table>
<thead>
<tr>
<th>Liposomal sterol</th>
<th>Sterol/phospholipid molar ratio in liposomes</th>
<th>(^{3}H) content (% of injected dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>0.98</td>
<td>Liver 12.6  Spleen 6.1  Intestine &lt;0.5  Lung &lt;0.5  Kidney &lt;0.5  Carcass* 21.2</td>
</tr>
<tr>
<td>3-Hydroxycholest-5-en-7-one</td>
<td>0.77</td>
<td>Liver 6.1  Spleen 0.8  Intestine &lt;0.5  Lung &lt;0.5  Kidney &lt;0.5  Carcass* 17.2</td>
</tr>
<tr>
<td>Cholesta-4,6-dien-3-one</td>
<td>0.52</td>
<td>Liver 7.7  Spleen 0.5  Intestine &lt;0.5  Lung &lt;0.5  Kidney &lt;0.5  Carcass* 3.8</td>
</tr>
<tr>
<td>3-Hydroxycholest-3-en-2-one</td>
<td>0.57</td>
<td>Liver 36.2  Spleen 1.5  Intestine 1.9  Lung 1.0  Kidney 1.0  Carcass* 31.4</td>
</tr>
<tr>
<td>1-Methyl-10-norcholesta-1,3,5(10)-trien-3-ol</td>
<td>0.55</td>
<td>Liver 44.9  Spleen 2.3  Intestine 4.8  Lung 0.9  Kidney 1.9  Carcass* 31.4</td>
</tr>
</tbody>
</table>

* Taken as the difference between the amount injected and the amount found in the blood and tissues.

and this was also true at 2.5 min after liposome injection.

The amount of label from the two rapidly removed liposome preparations, present in liver, spleen and lungs, falls with time, whereas label from the other liposomes increases so that at 2 h after injection the distribution of \(^{3}H\)-labelled phosphatidylcholine is similar for all the liposomes.

Experiments in which the polar-lipid content of liposomes has been varied and the clearance and tissue distribution measured have not given consistent results but it has been established that the size of the liposomes has a very marked effect on their fate in vivo (Juliano & Stamp, 1975; Kimelberg, 1976; Sharma et al., 1977). Large liposomes are very rapidly removed and small unilamellar ones are cleared more slowly. Examination of liposome samples by negative staining showed that they all contained structures 25–100 nm in diameter, including many unilamellar vesicles. However, it was noted that the preparations that contained 3-hydroxycholesterol-3-en-2-one and 1-methyl-10-norcholesta-1,3,5(10)-trien-3-ol tended to aggregate more than the others. Thus the reason for the very rapid initial phase of their clearance could be that the liposomes aggregated or fused together in the circulation forming structures larger than 100 nm in diameter that were quickly removed. On the other hand, because this rapid removal is by phagocytic cells, one would have expected a much greater uptake by the spleen and possibly also the lungs (Sharma et al., 1977).

Another possibility is that liposomes containing different sterols bind different amounts of plasma proteins, since the rate of clearance of liposomes is also modified by their association with such proteins (Black & Gregoriadis, 1976).

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