CHANGES IN PLASMA LIPOPROTEIN LIPIDS IN HYPERCHOLESTEROLAEMIC PATIENTS TREATED WITH THE BILE ACID-SEQUESTRING RESIN, COLESTIPOL

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SUMMARY

1. Seven patients with type II hyperlipoproteinaemia were treated with the bile acid-sequestering resin, colestipol (5 g three times daily), after a prolonged period of taking placebo.

2. After 8–9 weeks of treatment, the plasma concentration of the non-esterified cholesterol of very-low-density lipoprotein (VLDL) had risen by a mean of 0.09 mmol/l (43% increase, \( P < 0.001 \)), that of the esterified cholesterol of VLDL had risen by a mean of 0.11 mmol/l (38% increase, \( P < 0.01 \)), and that of the triglyceride of VLDL had risen by a mean of 0.40 mmol/l (53% increase, \( P < 0.001 \)). During the same period, the plasma concentration of the non-esterified cholesterol of low-density lipoprotein (LDL) decreased by a mean of 0.44 mmol/l (26% decrease, \( P < 0.01 \)), that of the esterified cholesterol of LDL decreased by a mean of 1.28 mmol/l (30% decrease, \( P < 0.001 \)), and that of the triglyceride of LDL decreased by a mean of 0.04 mmol/l (8% decrease, \( P < 0.01 \)). No significant changes occurred in the plasma concentration of either the cholesterol or triglyceride of high-density lipoprotein (HDL) during treatment.

3. During the early period of treatment with colestipol, changes took place in the specific radioactivity of plasma cholesterol (labelled by intravenous injection of \( [\text{\textsuperscript{3}}\text{H}] \)cholesterol), which, together with the changes in the mass of cholesterol within the individual plasma lipoproteins, were consistent with an increased influx into plasma of non-esterified cholesterol within VLDL, and an increased efflux of cholesterol from plasma within LDL.

Key words: bile acid, colestipol, hyperlipoproteinaemia, hypercholesterolaemia, lipoprotein.

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Biliary diversion in rats increases both bile acid secretion and hepatic cholesterol synthesis (Myant & Eder, 1961). In man, treatment with bile acid-sequestering resins, such as cholestyramine or colestipol, increases the synthesis of bile acids (Garbutt & Kenney, 1972) and the faecal excretion of bile acids (Carey & Williams, 1961; Moutafis & Myant, 1969; Miettinen, 1970; Grundy, Ahrens & Salen, 1971; Nazir, Horlick, Kudchodkar & Sodhi, 1972; Miller, Clifton-Bligh & Nestel, 1973a), while simultaneously increasing cholesterol synthesis in the liver (Miettinen, 1970) and cholesterol turnover (Goodman & Noble, 1968; Moutafis & Myant, 1969; Miller et al., 1973a).

The synthesis of bile acids may also be associated with the metabolism of triglycerides. Gallo, Harkins, Sheffner, Sarrett & Cox (1966) found that cholestyramine treatment increased the hepatic synthesis of both triglycerides and cholesterol in rats. The serum triglyceride concentration may rise during cholestyramine treatment in man (Weizel, Estrich, Splitter, Pomeroy & Kinsell, 1969; Grundy et al., 1971), although this rise may be of short duration (Levy et al., 1973).

A sustained rise in serum triglyceride concentration has been noted in hypercholesterolaemic patients treated with colestipol (Miller, Clifton-Bligh, Nestel & Whyte, 1973b). Patients with endogenous hypertriglyceridaemia may have increased bile acid production rates (Kottke, 1969; Einarsson & Hellström, 1972; Wollenweber & Stiehl, 1972), suggesting that bile acid synthesis in the liver may be related to the formation and secretion of triglyceride-rich lipoproteins.

Jones & Dobrilovic (1970) have observed increases in plasma very-low-density lipoprotein (VLDL) cholesterol and triglyceride concentrations during the treatment of patients with cholestyramine, especially in those who were previously hypertriglyceridaemic, suggesting that increased hepatic synthesis of cholesterol and triglyceride during bile acid sequestration may lead to an increased release of triglyceride-rich VLDL into the circulation.

The present study was undertaken to examine the effects of a new bile acid-sequestering resin, colestipol (U-26597A, a high-molecular-weight copolymer of tetraethylenepentamine and epichlorohydrin), on the distribution of cholesterol and triglyceride amongst the plasma lipoproteins in patients with type II hyperlipoproteinaemia (Fredrickson, 1971), for whom this drug might ordinarily be prescribed. The changes induced in the specific radioactivity of 3H-prelabelled plasma lipoprotein cholesterol were also studied in an attempt to gain insights into the nature of the alterations produced in cholesterol transport through the plasma. The metabolism of non-esterified and esterified cholesterol were examined separately.

**METHODS**

**Patients**

Seven patients with type II hyperlipoproteinaemia were studied. Clinical details of the subjects have been published by Miller et al. (1973b) and are given in Table 1. Lipoprotein typing was based on the relative proportions of cholesterol and triglyceride in individual lipoproteins. Five patients (A.L., D.S., E.A., R.B. and M.W.) had elevated low-density lipoprotein (LDL) cholesterol alone (type IIa, Fredrickson, 1971) and two (A.T. and A.H.) had a combined elevation of LDL and VLDL cholesterol (type IIb). Before the study, two patients, E.A. and D.S., had already been eating a diet low in cholesterol and high in polyunsaturated fatty acids for more than 12 months, with resultant lowering of plasma cholesterol levels by 2.0 mmol/l
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(77 mg/100 ml) and 2.6 mmol/l respectively. Patient E.A. was taking propranolol, 460 μmol (120 mg) daily, for angina, and patient A.T. was taking ethinyloestradiol, 70 nmol (0.02 mg) daily. Both substances were continued throughout the study unchanged. Each patient was instructed to continue his regular diet throughout the period of study. The composition of the diet of each patient was checked in detail before entry and at intervals during the study. All patients continued their normal daily activities outside the hospital. Body weights remained essentially constant. A thorough explanation of the purpose of the study was given to each patient whose consent was obtained before its commencement.

**TABLE 1. Clinical details of patients studied**

Lipoprotein phenotypes are those described by Fredrickson (1971).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Mean body weight (kg)</th>
<th>Lipoprotein phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Placebo period</td>
<td>Colestipol period</td>
</tr>
<tr>
<td>A.L.</td>
<td>M</td>
<td>45</td>
<td>71.0</td>
<td>72.3</td>
</tr>
<tr>
<td>A.T.</td>
<td>F</td>
<td>48</td>
<td>59.8</td>
<td>59.5</td>
</tr>
<tr>
<td>D.S.</td>
<td>M</td>
<td>35</td>
<td>62.0</td>
<td>62.9</td>
</tr>
<tr>
<td>A.H.</td>
<td>F</td>
<td>47</td>
<td>64.5</td>
<td>64.8</td>
</tr>
<tr>
<td>E.A.</td>
<td>M</td>
<td>45</td>
<td>77.4</td>
<td>78.3</td>
</tr>
<tr>
<td>R.B.</td>
<td>M</td>
<td>45</td>
<td>69.8</td>
<td>68.5</td>
</tr>
<tr>
<td>M.W.</td>
<td>M</td>
<td>35</td>
<td>80.0</td>
<td>79.7</td>
</tr>
</tbody>
</table>

**Clinical procedures**

All patients were treated on a common regime beginning with a placebo followed by colestipol. Five patients were studied in greater detail during the change from placebo to colestipol. During the initial control period a placebo of 5 g of microcrystalline cellulose was taken three times daily with each meal, and this regime was continued for an average of 105 days (range 70–221 days) before giving colestipol. About 2 weeks before the end of placebo treatment, four samples of blood were taken from each subject (five samples from A.H.) over an 8 day period, for determination of the lipid composition of plasma lipoproteins. The samples were taken after an overnight fast of 12–14 h.

During treatment 5 g of colestipol was given three times daily with each meal. After 8–9 weeks of colestipol treatment four samples of blood were again taken from each patient (two from A.H.) for the analysis of lipoprotein lipids.

In order to define the immediate effects of colestipol treatment on cholesterol turnover, five patients (E.A., R.B., A.H., A.L. and A.T.) were given an intravenous infusion of tritium-labelled cholesterol at the beginning of the placebo period. Blood was taken at least weekly intervals for the determination of the specific radioactivity of plasma cholesterol, and this was continued at weekly intervals after the commencement of colestipol. In three patients (E.A., R.B. and A.H.) the specific radioactivity of non-esterified and esterified cholesterol was measured in each lipoprotein fraction immediately before and for several days after the beginning of colestipol therapy.
Analytical methods

The blood samples were taken, after an overnight fast of 12–14 h, into polypropylene tubes containing sodium EDTA in a final concentration of 2·5 mmol/l and p-chloromercuriphenylsulphonate (PCMPS) in a final concentration of 2 mmol/l (Glomset, Norum & King, 1970), the latter to inhibit the activity of the plasma enzyme lecithin–cholesterol acyltransferase (EC 2.3.1.43). VLDL was isolated by the ultracentrifugal technique of Fredrickson, Levy & Lindgren (1968). The floating VLDL (d < 1·006 g/ml) was removed by a tube-slicing technique. After careful resuspension of the remainder, the LDL contained in it was precipitated by adding the recommended quantity of manganese chloride and heparin (Burstein, Scholnick & Morfin, 1970). The LDL was sedimented by low-speed centrifugation and the supernatant containing the high-density lipoprotein (HDL) removed by aspiration. Each lipoprotein fraction was extracted in an appropriate volume of Dole’s solution (Dole & Meinertz, 1960). The lipoprotein lipid was separated into non-esterified cholesterol, esterified cholesterol and triglyceride fractions by thin-layer chromatography on silica gel G, by a method similar to that of Marzo, Ghirardi, Sardini & Meroni (1971), with the solvent system hexane–diethyl ether–methanol–acetic acid (180:40:6:4, by vol.).

Cholesterol and triglyceride determinations were made by the Autoanalyzer II method of Technicon Instruments Corporation, and all samples from each patient for the placebo period and the colestipol treatment period were assayed in the same run in duplicate. Triolein (mol.wt. 885) was used as the triglyceride standard. In three patients, E.A., R.B. and A.H., lipoprotein-lipid analysis was made as above except that the mass of cholesterol was measured in duplicate for each sample by the ferric chloride method of Zlatkis, Zak & Boyle (1953).

Percentage recovery of cholesterol and triglyceride from the lipoproteins averaged 95·3 ± 6·0 (mean ± sd) and 93·2 ± 7·8 respectively. It was established that separation of lipoproteins by the procedure described was unaffected by the presence of PCMPS at the concentration employed, and that it gave values for lipoprotein lipids similar to those obtained by sequential ultracentrifugation. Chylomicrons were separated by ultracentrifugal analysis (Hatch & Lees, 1968).

For intravenous administration, about 100 μCi of [1α,2α-3H]cholesterol (specific radioactivity 500 Ci/mol) was dissolved in the patients’ serum and incubated for 1 h at 37°C, or added in a small volume of ethanol to 200 ml of sterile NaCl (155 mmol/l). The purity of the cholesterol, as checked by thin-layer chromatography, was greater than 97%. The radioactivity of plasma samples and fractions was measured in a Packard Tri-Carb liquid scintillation counter (model 574) with 2,5-diphenyloxazole–1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (PPO–dimethyl POPOP) in toluene as the scintillator. Counting efficiency for radioactivity was monitored by automatic external standardization and averaged 28%. Radioactivity counting rates were at least four times background.

Materials

Colestipol was obtained from Upjohn (Kalamazoo, Michigan, U.S.A.). Labelled cholesterol was obtained from The Radiochemical Centre (Amersham, Bucks).

RESULTS

Early effects of colestipol

Three patients were studied intensively during the transition from the placebo to the colesti-
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The triglyceride and cholesterol concentrations of plasma VLDL and LDL fractions, before and at daily intervals after administration of colestipol, are given in Table 2. Cholesterol and triglyceride of HDL did not change. The specific radioactivities of non-esterified and esterified cholesterol in the different lipoprotein fractions are given in Table 3 as a percentage of the last measurement of specific radioactivity made during the control period. The more prolonged effects on the specific radioactivities of esterified and non-esterified cholesterol of whole plasma are shown in Fig. 1 as a mean of the values for five patients. For comparison, the decline of the specific radioactivity of whole plasma cholesterol during the placebo period has been extrapolated into the colestipol period.

**Long-term effects of colestipol**

The amount of triglyceride and of non-esterified and esterified cholesterol in VLDL, LDL and HDL before and after 8–9 weeks of colestipol treatment were determined in all seven patients. Details of the changes observed in the plasma lipoproteins of individual patients have been deposited with the Librarian, the Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE, as Clinical Science and Molecular Medicine Table 74/10; copies may be obtained

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**Table 2. Lipid composition of lipoprotein fractions of three patients during the first 5 days of colestipol treatment**

Figures given are plasma concentrations (mmol/l) and percentages. Colestipol dosage was commenced on day 0. TC = total cholesterol concentration; FC(%) = percentage of total cholesterol that was non-esterified; TG = triglyceride concentration. 5 mmol/l cholesterol = 193 mg/100 ml; 1 mmol/l triglyceride = 88 mg/100 ml (as triolein).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Lipoprotein fraction</th>
<th>Lipid component</th>
<th>Composition late in placebo period</th>
<th>Composition during colestipol treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day of treatment</td>
<td>1</td>
</tr>
<tr>
<td>E.A.</td>
<td>VLDL</td>
<td>TC</td>
<td>0.16</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FC(%)</td>
<td>38</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TG</td>
<td>0.31</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>TC</td>
<td>7.6</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FC(%)</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>R.B.</td>
<td>VLDL</td>
<td>TC</td>
<td>0.29</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FC(%)</td>
<td>44</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TG</td>
<td>0.51</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>TC</td>
<td>5.0</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FC(%)</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>A.H.</td>
<td>VLDL</td>
<td>TC</td>
<td>0.83</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FC(%)</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TG</td>
<td>1.59</td>
<td>2.73</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>TC</td>
<td>4.5</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FC(%)</td>
<td>26</td>
<td>25</td>
</tr>
</tbody>
</table>
Table 3. Specific radioactivity of cholesterol fractions during the first 4 days of colestipol therapy

Colestipol dosage was commenced on day 0. Each result is expressed as a percentage of the last control value obtained before treatment was commenced. These were obtained as follows: E.A., day -1; R.B., day -13; A.H., day -3. Statistical comparisons by Wilcoxon matched-pairs signed-rank test (Siegel, 1956): VLDL FC less than VLDL EC, \( P < 0.01 \); LDL FC less than LDL EC, \( P < 0.01 \); HDL FC less than HDL EC, \( P < 0.01 \); VLDL FC less than LDL FC, \( P < 0.01 \); VLDL FC less than HDL FC, \( P < 0.02 \); LDL FC versus HDL FC, not significant; VLDL EC less than LDL EC, \( P < 0.01 \); VLDL EC less than HDL EC, \( P < 0.05 \); LDL EC versus HDL EC, not significant (FC = non-esterified cholesterol; EC = esterified cholesterol).

<table>
<thead>
<tr>
<th></th>
<th>E.A.</th>
<th>R.B.</th>
<th>A.H.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
</tr>
<tr>
<td>Non-esterified cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>86</td>
<td>62</td>
<td>57</td>
</tr>
<tr>
<td>LDL</td>
<td>98</td>
<td>64</td>
<td>57</td>
</tr>
<tr>
<td>HDL</td>
<td>93</td>
<td>81</td>
<td>74</td>
</tr>
<tr>
<td>Esterified cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>97</td>
<td>74</td>
<td>72</td>
</tr>
<tr>
<td>LDL</td>
<td>98</td>
<td>93</td>
<td>87</td>
</tr>
<tr>
<td>HDL</td>
<td>98</td>
<td>78</td>
<td>74</td>
</tr>
</tbody>
</table>

Fig. 1. Mean specific radioactivities of plasma cholesterol fractions of five patients. The ordinate is on a logarithmic scale. Colestipol dosage was begun on day 0. The differences between the values for non-esterified (●) and esterified (▲) cholesterol were statistically significant \( (P < 0.03) \) by the Sign test (Siegel, 1956) on days 3, 7 and 14 of treatment.
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from the Librarian on request. Comparison of the concentrations before and during treatment was made by the paired t-test.

The mean plasma concentration of VLDL non-esterified cholesterol increased from 0.21 to 0.30 mmol/l (43% rise, \( P < 0.001 \)), that of VLDL esterified cholesterol increased from 0.29 to 0.40 mmol/l (38% rise, \( P < 0.01 \)) and that of VLDL triglyceride increased from 0.75 to 1.15 mmol/l (53% rise, \( P < 0.001 \)). In contrast, the concentrations of all three measured lipids within LDL showed a significant fall: the mean concentration of LDL non-esterified cholesterol decreased from 1.68 to 1.24 mmol/l (26% fall, \( P < 0.01 \)), that of LDL esterified cholesterol decreased from 4.32 to 3.04 mmol/l (30%, \( P < 0.01 \)) and that of LDL triglyceride fell from 0.49 to 0.45 mmol/l (8%, \( P < 0.01 \)). HDL showed only small changes in cholesterol and triglyceride, which were not statistically significant.

The percentage of total cholesterol that was non-esterified did not change significantly in VLDL (placebo 41.7 ± 1.8, mean ± SEM; colestipol 44.0 ± 2.1) or LDL (placebo 28.0 ± 0.6; colestipol 28.9 ± 0.7). The ratio of triglyceride to total cholesterol was also unchanged in VLDL (placebo 1.66 ± 0.12; colestipol 1.80 ± 0.17) and LDL (placebo 0.08 ± 0.01; colestipol 0.10 ± 0.02); similar results were found with HDL. However, there was a very significant positive correlation between the concentration of VLDL triglyceride and the ratio of triglyceride to total cholesterol in HDL in the six patients for whom these values were available. The coefficient of linear correlation for the twelve values (six from placebo and six from colestipol periods) was +0.82 (\( P = 0.001 \)).

During colestipol treatment there was no increase in chylomicrons in the five patients studied.

**DISCUSSION**

This work was prompted by the observation (Miller et al., 1973b) that colestipol, like cholestyramine, may produce a potentially undesirable rise in plasma triglyceride during treatment of hypercholesterolaemia. The new findings are from the same type IIa and IIb patients previously studied. Examination of non-esterified and esterified cholesterol and triglyceride after several weeks' treatment with colestipol showed large and significant rises in VLDL, a fall in LDL, but no change in HDL.

Colestipol produced its effects on cholesterol and triglyceride concentrations within 1 day (Table 2) in the three patients who were studied during the transition. One of the three patients (E.A.) had an immediate increase in the percentage of non-esterified cholesterol in VLDL. LDL cholesterol fell progressively from the second day.

Changes in the turnover of plasma cholesterol were studied with the aid of \(^{3}H\)-labelled cholesterol. As shown by Chobanian, Burrows & Hollander (1962), Lewis & Myant (1967), Goodman & Noble (1968) and Nestel, Whyte & Goodman (1969), the slope of the plasma specific radioactivity–time curve becomes apparently exponential after about 40 days, and our studies during the placebo period support this. The effect of colestipol on the specific radioactivities of the cholesterol fractions (Table 3) was apparent within 2–3 days. Within each lipoprotein the specific radioactivity of non-esterified cholesterol fell more rapidly than that of esterified cholesterol. In five patients the mean specific radioactivities of the cholesterol fractions of whole plasma showed the same differences (Fig. 1). In addition, the specific radioactivities of VLDL non-esterified and esterified cholesterol decreased more rapidly than those
of the corresponding fractions in LDL and HDL (Table 3). As treatment continued there was a gradual convergence of the specific radioactivity values of total esterified and non-esterified cholesterol (Fig. 1).

The more rapid fall in the specific radioactivity of VLDL cholesterol relative to that of LDL and HDL cholesterol (Table 3), together with the rapid increase that occurred in LDL cholesterol mass (Table 2), suggested that an increased influx of VLDL cholesterol into plasma was taking place during colestipol therapy. The greater fall in the specific radioactivity of non-esterified cholesterol relative to that of esterified cholesterol further suggested that the influx of cholesterol within VLDL was occurring predominantly in the non-esterified form, although the ratio of non-esterified to esterified cholesterol in VLDL increased in only one (E.A.) of the three patients studied. The later convergence of the specific radioactivities of non-esterified and esterified cholesterol during continued treatment may have resulted from the increase in plasma lecithin–cholesterol acyltransferase activity, which has been shown to occur during prolonged colestipol therapy (Clifton-Bligh, Miller & Nestel, 1974).

In contrast to these changes in VLDL, the accelerated fall in LDL cholesterol specific radioactivity was associated with a decrease in LDL cholesterol mass in all three patients, suggesting that the rate of removal of LDL cholesterol from plasma had been increased.

VLDL apoproteins are transferred to LDL during VLDL metabolism (Gitlin, Cornwell, Nakasato, Oncley, Hughes & Janeway, 1958; Bilheimer, Eisenberg & Levy, 1972), and if VLDL cholesterol is transferred to LDL in a similar way then some of the increased amount of VLDL cholesterol entering plasma may ultimately become associated with and be removed from the circulation within LDL. This does not necessarily mean that all LDL cholesterol arises from VLDL cholesterol. Non-esterified cholesterol is known to equilibrate rapidly amongst all lipoproteins in vivo (Goodman, 1964), and an increased influx of non-esterified cholesterol in VLDL would be expected to redistribute amongst the other lipoproteins and result in an accelerated fall in the specific radioactivity of their non-esterified cholesterol also. This was observed during the first few days of treatment. The net effect of the changes in plasma lipoprotein cholesterol metabolism induced by colestipol was a marked increase in cholesterol turnover (Miller et al., 1973a).

The site of origin of the increased amount of VLDL cholesterol and triglyceride entering plasma during early treatment with colestipol seems likely to be the liver, since in animals treated with cholestyramine the formation of VLDL in the intestinal mucosa (Jones & Ockner, 1971) and the amount of cholesterol (Wilson & Reinke, 1968) and VLDL delivered into thoracic duct lymph (Ockner, Hughes & Isselbacher, 1969) are reduced. Hepatic synthesis of cholesterol increases during cholestyramine therapy in man (Miettinen, 1970) and it is probable that the newly synthesized hepatic cholesterol is transported out of the liver both as lipoprotein cholesterol and as bile acids, although lipoprotein efflux from the liver has not been measured in cross-liver studies after bile acid sequestration.

Under conditions of increased hepatic synthesis and release of VLDL in rats, the increase in the secretion of VLDL non-esterified cholesterol was proportionately greater than that of VLDL esterified cholesterol (Heimberg, Weinstein, Dishmon & Fried, 1965). In the present study, the increase in the ratio of non-esterified cholesterol to esterified cholesterol in VLDL observed in one of the three patients studied in the early treatment period (Table 2) was compatible with a similar phenomenon taking place. Nestel & Couzens (1966) have noted that cholesterol of hepatic origin entering plasma is relatively richer in non-esterified cholesterol
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than cholesterol already present in plasma. Increased synthesis of hepatic cholesterol produced by bile acid sequestration may accentuate this process.

The early increase in plasma VLDL cholesterol and triglyceride concentration and fall in LDL cholesterol concentration (Table 2) were still present 8–9 weeks after treatment was commenced. Similar falls in plasma LDL cholesterol concentration have been observed by others with colestipol (Glueck, Ford, Scheel & Steiner, 1972) and cholestyramine (Jones & Dobrilovic, 1970; Levy et al., 1973). In the studies of Jones & Dobrilovic (1970) VLDL cholesterol concentrations were also increased during long-term cholestyramine treatment but mainly in individuals already hypertriglyceridaemic. Bile acid-sequestering agents are used to lower elevated plasma cholesterol concentrations and thereby, hopefully, to retard the development of atherosclerosis. An increased risk of ischaemic heart disease with elevated serum triglyceride concentrations has been reported (Carlson & Böttiger, 1972) so that the rise in triglyceride-rich VLDL concentrations after cholestyramine and colestipol administration needs to be considered in this light.

The high degree of correlation between VLDL triglyceride concentration and the HDL triglyceride/cholesterol ratio supports other evidence that the metabolism of these lipoproteins may be closely linked. VLDL apoproteins are transferred within plasma to HDL (Eisenberg, Bilheimer & Levy, 1972) as well as to LDL, and the transfer to HDL correlates with the plasma triglyceride concentration. In vitro, triglyceride can be transferred from VLDL to HDL (Nichols & Smith, 1965). It is not known whether transfer of the VLDL components to HDL is a prerequisite for the subsequent metabolism of either lipoprotein in vivo.

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