THE EFFECT OF LOW MOLECULAR WEIGHT
DEXTRAN INFUSIONS ON PLASMA LIPIDS AND
LIPOPROTEINS

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SUMMARY

1. Infusions of Dextran 40 induced a pronounced fall in the plasma concentration of cholesterol, in the absence of marked changes in plasma volume. Similar falls occurred in the plasma concentrations of phospholipids, triglyceride and low density lipoprotein. It was further observed that the return to normal was slow, and at 15 days was still incomplete.

2. Lipoprotein turnover studies failed to demonstrate altered rates of catabolism or evidence for altered synthetic rates. They were better interpreted in terms of redistribution from the plasma to the rest of the extracellular fluid, although there was no indication as to its exact site.

Dextran infusions have been widely used in clinical practice for expansion of plasma volume, reduction in blood viscosity, and increase in capillary blood flow. Whilst attempting to induce a diuresis in nephrotic children, Mollison & Rennie (1954) observed falls in serum cholesterol concentration below levels anticipated from simple consideration of plasma volume expansion. This observation has been confirmed in further human and animal studies (Heymann et al., 1958; Allen, Baxter & Goodman, 1961; Lusztig et al., 1961; Flotte & Buxton, 1963) and has been shown also to affect other plasma lipids (Rothschild et al., 1960; Allen et al., 1961; Lusztig et al., 1961), and possibly also serum proteins (Carbone, Uzman & Plough, 1955).

Although the most marked effects have followed infusion of dextran, albumin has induced a similar effect in normal and nephrotic subjects (Soothill & Kark, 1956; Rosenman, Friedman & Byers, 1956).

As Flotte & Buxton (1963) suggested that the fall in concentration was not immediately reversed, it appeared important to define carefully the time course of this phenomenon and to examine the extent to which it involved all plasma lipids. Apart from the demonstration by

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Rothschild et al. (1961) of an impairment in the rate of synthesis of one serum protein, albumin, and the link with plasma heparin and histamine concentration by Lusztig et al. (1962) and Jozsa et al. (1962), there is little evidence available to provide a reasonable explanation for these findings. As it was found that low density lipoprotein (LDL) and cholesterol concentrations fell in parallel, measurements were also made of the rates of turnover and distribution of $^{131}$I-labelled LDL.

**MATERIALS AND METHODS**

*Subjects studied*

The preliminary observations were made on a number of patients being treated with infusions of low molecular weight dextran for rest pain resulting from arterial insufficiency of the legs. They were otherwise in satisfactory health. In the major part of this work, four elderly subjects of both sexes (1-4) were studied in detail, all suffering from the effects of atherosclerosis, with either myocardial or cerebral ischaemia. Any acute thrombotic episode had occurred not less than 3 weeks prior to the study. Two others (5 and 6) have subsequently been studied and the results on catabolic rate are included in Fig. 4.

*Procedure for dextran infusions and lipid measurements*

Intravenous infusions of 500 ml of 10% Dextran 40 in 5% glucose solution were given each morning for 4 days. Samples of venous blood were taken in the fasting state on the morning of the day before the first infusion and on each morning during the period of infusions. Similar samples were taken at frequent intervals thereafter for a total period of 15 days. The sample of blood was collected in a dry tube, allowed to clot at room temperature, and the serum obtained by centrifugation. A 2.0 ml sample of serum was extracted with chloroform-methanol according to the method of Folch, Lees & Sloane-Stanley (1957). The lipid classes were then separated by thin-layer chromatography on silicic acid. Two separate separations were carried out, one for neutral lipids and the other for phospholipids. The neutral lipid separation and determinations were as described by Gloster & Fletcher (1966). The phospholipids were separated on silicic acid plates as described by Skipski, Peterson & Barclay (1964), except that phosphatidyl serine and phosphatidyl inositol were not clearly separated. After scraping the lipid-containing regions of silicic acid from the plate, the phosphorus content was determined directly without elution (Parker & Peterson, 1965). Serum low-density lipoproteins were measured in the Spinco Model L ultracentrifuge by a saline density gradient procedure (Walton, Campbell & Tonks, 1965a).

In one subject (patient 3), the lipid content of the red cells was measured before infusion and also on the seventh day. In this case, the blood was transferred to a tube containing sequestrene as anticoagulant. After centrifugation, the plasma was removed, and the red cells washed three times with normal saline. The red cells were recentrifuged and the packed cell volume measured before extracting and analysing the lipid content as described for serum.

*Preparation and administration of $^{131}$I-labelled LDL*

In all studies the lipoprotein used was isolated from the patient's own serum. Two days prior to the study period, 40 ml of blood were withdrawn and defibrinated by slow stirring with wooden sticks. The lipoprotein was separated in a pure state by ultracentrifugation (Walton...
Lipid lowering effect of dextran and labelled by the iodine monochloride method (McFarlane, 1956; Walton et al., 1965b). Free iodide was removed by passage of the labelled material down a column of Sephadex G-25 and the resulting solution was shown by precipitation with trichloroacetic acid to have less than 5% of the radioactivity unattached to protein. Immunodiffusion studies revealed a pure LDL preparation, and previous examination after labelling showed that no radioactivity could be detected in the albumin region (Walton et al., 1965b).

Following the oral ingestion of 100 mg potassium iodide, less than 50 μCi of labelled protein solution was slowly injected intravenously. A daily oral dose of 100 mg potassium iodide was maintained throughout the study. Blood samples were taken without venous constriction into dipotassium ethylene diamine tetra-acetic acid (EDTA), and in patients 2, 3, 5 and 6 24-hr collections of urine were made. Creatinine measurements were made on the urine samples as a check on completeness of collections.

Radioactivity measurements and analysis of data

Plasma and urine samples were counted in a well-type sodium iodide crystal scintillation counter. Whole body radioactivity was measured in a whole body counter (Nuclear Enterprises Ltd) by four sodium iodide crystals (4 in. deep, 4½ in. diameter) arranged two above and two below the supine patient to give satisfactorily uniform geometry, crystals and patient being enclosed in a room made of battleship steel 15 cm thick and lined by 3 mm lead. The impulses from the four 5-in. photomultiplier tubes were fed into a 512-channel spectrometer. Final values for whole body radioactivity were obtained by summing all pulses from the 0.36 MeV and 0.64 MeV photo-peaks.

Plasma and whole-body radioactivity measurements were expressed as percentages of the values 10 min after injection and were plotted semilogarithmically. From the curves drawn by eye through these points, values were obtained for the mean plasma and whole body radioactivities for each urinary collection period, and these values were used to calculate the following ratios:

1) Fractional (plasma) catabolic rate (FCR) =

\[
\text{24-hr urine radioactivity} \quad \times 100
\]

\[
\text{mean plasma radioactivity}
\]

2) Fractional (whole body) catabolic rate (FWBCR) =

\[
\frac{\text{24-hr urine radioactivity}}{\text{mean whole body radioactivity}} \times 100
\]

RESULTS

Infusion of Dextran 40 into a number of patients suffering from rest pain resulted in appreciable falls in concentration of serum cholesterol, unaccompanied by comparable changes in haematocrit or total plasma proteins. Results for such a case are illustrated in Fig. 1, where falls of approximately 60% were observed in serum cholesterol and LDL concentration, in the presence of a sustained fall in total protein of only 20%. In other patients, similar changes

\( F \)
were induced in cholesterol, with the same or lesser changes in total protein concentration. Hence a detailed study was planned of all plasma lipids in a further group of patients (1-4).

![Graph showing plasma concentrations of total protein, cholesterol, and low density lipoprotein over time.]

**FIG. 1.** The effect of daily infusions of dextran on plasma concentrations of total protein, cholesterol and low density lipoprotein (arrows indicate dextran infusions). ×, Protein 100% = 5.1 g/100 ml; ○, cholesterol 100% = 280 mg/100 ml; □, LDL 100% = 590 mg/100 ml.

**Analysis of plasma lipids**

Subsequent detailed measurements of sub-fractions of plasma lipids are summarized in Table 1. The initial levels of the lipid classes before infusion are shown together with the levels found on days 5, 10 and 15. Day 1 has been taken as the day before the first infusion was given, so that Day 5 samples were taken after three infusions of dextran and Day 10 samples 5 days after the last infusion. Fig. 2 shows in greater detail the observations in patient 4.

In all subjects a decrease occurred in the total lipid concentration and, to a variable degree, the various lipid classes during and following the infusion. The maximum decrease occurred between Day 5 and Day 10 after which the concentration began to rise again; in most instances, recovery was still incomplete by Day 15.

The results of lipid red cell measurements are given in Table 2. The lipid composition of the red cells was different from that of the plasma, and, although there was some increase in the red cell cholesterol and phosphatidylethanolamine, the major changes in the plasma of this patient occurred in other lipid components, cholesterol ester, phosphatidylcholine and sphingomyelin. These changes could not be accounted for by an increased concentration of these substances in the red cells.

Since the infusions of dextran also contained glucose, the effects of pure glucose infusions were studied in one patient on a separate occasion (patient 1). The total lipid concentration in the pre-glucose serum was 527 mg/100 ml and at their lowest level the total lipids fell to 469 mg/100 ml, a decrease of only 11% compared with a decrease of 44% observed at the same
<table>
<thead>
<tr>
<th>Lipid</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol ester</td>
<td>198</td>
<td>270</td>
<td>208</td>
<td>334</td>
<td>208</td>
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<tr>
<td>Cholesterol</td>
<td>56</td>
<td>30</td>
<td>25</td>
<td>37</td>
<td>55</td>
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<tr>
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<td>89</td>
<td>80</td>
<td>86</td>
<td>76</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>9</td>
<td>12</td>
<td>29</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>Phosphatidyserine</td>
<td>9</td>
<td>18</td>
<td>11</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>124</td>
<td>95</td>
<td>76</td>
<td>106</td>
<td>220</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>35</td>
<td>33</td>
<td>35</td>
<td>28</td>
<td>54</td>
</tr>
<tr>
<td>Lysophosphatidylcholine</td>
<td>15</td>
<td>20</td>
<td>11</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Total lipid</td>
<td>576</td>
<td>426</td>
<td>376</td>
<td>434</td>
<td>812</td>
</tr>
</tbody>
</table>

Table 1. Changes in plasma lipid concentrations following infusions of Dextran 40
FIG. 2. Changing plasma concentrations of cholesterol ester (CE), phosphatidyl choline (PC), triglyceride (TG), free cholesterol (C) and sphyngomyelin (Sp) resulting from infusions in patient 4.

Table 2. Red blood cell and plasma lipid concentrations of patient 3, before and after infusion of Dextran 40

<table>
<thead>
<tr>
<th></th>
<th>mg lipid/100 ml red cells</th>
<th>mg lipid/100 ml plasma</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before Day 7</td>
<td></td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>9 7</td>
<td>197 154</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>98 114</td>
<td>51 42</td>
</tr>
<tr>
<td>Triglyceride</td>
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<td>50 45</td>
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<tr>
<td>Phosphatidylethanolamine</td>
<td>70 91</td>
<td>10 15</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>46 42</td>
<td>12 17</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>94 94</td>
<td>153 118</td>
</tr>
<tr>
<td>Sphyngomyelin</td>
<td>76 79</td>
<td>52 35</td>
</tr>
<tr>
<td>Lysosphatidylcholine</td>
<td>11 5</td>
<td>17 17</td>
</tr>
<tr>
<td>Total lipid</td>
<td>404 432</td>
<td>542 444</td>
</tr>
</tbody>
</table>

time interval after dextran. The greatest changes induced by glucose infusions were in cholesterol ester which fell from 200 to 163 mg/100 ml, free cholesterol which decreased from 50 to 39 mg/100 ml and a slighter fall in triglyceride from 104 to 91 mg/100 ml; the concentration of the phospholipids were not affected. Two days after this slight fall the resting lipid values were again regained.
Lipid lowering effect of dextran

Turnover of $^{131}$I-LDL

In three of the patients whose lipid responses to dextran are reported above, the metabolism of isologous LDL was studied, and the results illustrated in Fig. 3. Results from patients 1, 2 and 3 are illustrated from left to right, and whole body radioactivity relating to the plasma curves is represented in the upper half of the figure. In two of these patients (1 and 3), two studies of LDL turnover were carried out consecutively, the second commencing immediately after the conclusion of the first. A separate batch of protein was prepared and labelled for each study, and in only one study of each pair was dextran infused. For ease of comparison, the separate studies on each patient are included on the same graph, and the period of infusions is indicated. No more than 3% of plasma radioactivity was associated with free iodide at any time, and the ratio of iodide to protein bound radioactivity did not change after dextran infusions.

Curves of whole body radioactivity appear to have been uninfluenced by the infusions, suggesting that the absolute rate of catabolism remained unaltered. The curves of plasma radioactivity, however, were markedly altered, and dextran appeared to induce in each instance a fall in radioactivity roughly commensurate with the fall in concentration of plasma lipids. The anticipated direction of the curve after the infusion period is indicated by dotted lines.
Fig. 4. Fractional whole body catabolic rate (U/WB%) and fractional catabolic rate (U/F%) for patients 2, 3, 5 and 6.
drawn parallel to the whole body curve. In patient 3, total plasma protein concentration and haematocrit were measured simultaneously with radioactivity. Although each dextran infusion resulted in a marked fall in both indices of volume 4 hr later, they returned to their basal value before the next infusion. Hence there was no evidence for sustained changes in blood volume.

Examination of unwashed blood cells revealed no rise in radioactivity after dextran, and saline-washed red cells and buffy coat taken 4 hr after an infusion did not contain any measurable radioactivity.

Because of intermittent incontinence, urine collections on patient 1 had to be abandoned, but in patients 2 and 3 and in two others subsequently studied (5 and 6), collection was satisfactory, daily creatinine output being within 10% of a mean value in all. Plasma and whole-body radioactivity curves on the two additional patients were essentially similar to those shown in Fig. 3. In all four patients, total urinary radioactivity continued to fall exponentially with time at a rate identical with the fall in whole body radioactivity, even during the period of dextran infusions. Hence fractional whole body catabolic rate (FWBCR) remained unchanged during the period of dextran infusions, whereas the fall induced in plasma radioactivity was reflected, in at least two of these four patients, by rising values for FCR (Fig. 4).

**DISCUSSION**

Infusion of dextran induces blood volume expansion (Gelin, 1956), the duration of which depends upon the molecular size of the preparation used. High molecular weight dextrans have been designed for prolonged expansion in shock (Takaori & Safar, 1966), whereas the low molecular weight substances have been used mainly for their effects on blood viscosity and flow, volume expansion being of relatively short duration (Long et al., 1961). This was confirmed in the present studies in which haematocrit values fell just after the completion of an infusion but returned to pre-infusion values within 24 hr. Measurements of total protein gave similar results, and the prolonged decrease in concentration was never greater than 20%. Although some degree of dilution might have contributed to the results obtained, enlargement of plasma volume cannot provide the entire explanation for falls in lipid concentration of up to 50%.

The lipid lowering effect was clearly not limited to cholesterol, but in this study affected all the major lipid components of plasma (cholesterol, cholesterol ester, triglyceride, phosphatidylcholine and sphingomyelin) to a similar degree. It did not appear to affect the concentrations of the minor lipid constituents, phosphatidylethanolamine, phosphatidylserine and lysophosphatidylcholine. These concentrations were, however, very low compared to those of the major lipids, and as fluctuations were relatively more intense it is doubtful whether definite conclusions can be drawn on these responses. In no patient did the combined concentrations of these substances reach more than 8% of the total lipid concentration. After reaching minimum values between the fifth and tenth days after the commencement of infusions, concentrations rose slowly towards normal, and most of the reductions were eliminated by the fifteenth day. Later measurements indicated that the pre-infusion values were reached within 3 weeks, rather earlier than observed by Flotte & Buxton (1963). It has recently been demonstrated (Seaman et al., 1965) that dextran induces a temporary shift of lipid to the red cell, but this must be a short-lived phenomenon, as changes in red cell lipids in the present study were
unimpressive. Furthermore, no radioactivity could be detected in the cellular elements of the blood after dextran infusions. It seems, therefore, that there was a real and sustained fall in the various lipid fractions of the plasma, and that this was not due to their adsorption on to the blood cells.

The turnover of $^{131}$I-LDL has previously been shown to be a reasonable measurement of lipoprotein metabolism (Walton et al., 1965b). The use of isologous material eliminates the possibility of immunological intolerance, and duplicate studies provided a useful comparison in individual patients. It is clear that plasma radioactivity and the major plasma lipid levels were reduced to a similar degree, as was lipoprotein in the patient in whom this was measured. These changes were not, however, accompanied by a comparable alteration in whole body activity, making it unlikely that lipoprotein had been eliminated from the body. As the urinary and whole body radioactivities fell in parallel, thus producing a constant ratio between the two (Fig. 4), it may be concluded that the absolute rate of catabolism of lipoprotein did not alter, assuming that urinary elimination of the label was rapid. If the lowered serum concentration did not result from an alteration in catabolic rate, it must have been due either to depressed rates of synthesis, or to a change in distribution. Rothschild et al. (1961) have shown that albumin synthesis is depressed by infusion of dextran, but on its own such a mechanism would result in a fall in the lipoprotein concentration, and would not accentuate the rate of decline in plasma radioactivity. The alteration in the plasma radioactivity was similar to the reduction in lipoprotein concentration, hence maintaining the same steady rate of decline in protein specific activity. Hence depression of lipoprotein synthesis cannot be the sole explanation for the present findings. Redistribution appears the most likely cause for the depression of serum lipoprotein and probably also of plasma lipid concentrations. The geometrical relationship between plasma and whole-body radioactivity curves is generally interpreted as reflecting protein distribution between intravascular and extravascular sites during such studies, and it is clear that a marked alteration occurred in this relationship following administration of dextran. Again, it appears that an extravascular shift of radioactivity has occurred, and as there was at this time no increase in the ratio of free to protein-bound iodine, it is probable that this shift affects the labelled lipoprotein. Thus the evidence of these turnover studies favours redistribution of the lipoprotein to an extravascular site.

Although there was no change in the absolute catabolic rate, there did appear to be some increase in the fractional catabolic rate. Whether this implies a double effect of the infusions is not clear, and it cannot be denied that in addition to redistribution, some lesser change may have occurred in the processes of catabolism. If catabolic rate for LDL is related to total exchangeable protein mass rather than to that present in the plasma alone, then catabolism has not been altered in this study. Catabolic rate for albumin is directly related to the protein content of plasma (McFarlane, 1963), but this has not yet been demonstrated for lipoprotein, and, if anything, the present results point in the opposite direction. This question must remain open until more information is available.

The mechanism for and site of redistribution of the lipoprotein has not been revealed. Dextran has been shown to affect the electrophoretic mobility of albumin (Ponder & Ponder, 1960) and of $\beta$-lipoprotein (Keler-Baka & Pučar, 1966), although this latter is much less pronounced after the addition of low molecular weight preparations such as Dextran 40. The concentrations used by Keler-Bačoka & Pučar were never lower than 1.7%, and it is unlikely that dextran concentrations in our patients rose above 0.8% in the general circulation.
Laurent (1963) has proposed a different mechanism by which proteins could be precipitated from solution; steric exclusion of the protein from the volume of fluid contained within the dextran molecule. Hence the effective concentration of the protein would be raised above its solubility, resulting in precipitation or shift of the molecule to another compartment. In further studies, Laurent was unable to demonstrate precipitation with dextran concentrations below 12% (Iverius & Laurent, 1967), but this value could be much lower if more sensitive techniques were available for measurement. It is also apparent that dextran concentrations of up to 5% may have occurred in the vein receiving the infusion, and so these physico-chemical differences may be important in explaining the present findings. Any possible explanation must account for the surprisingly long duration of the reduction in LDL, which virtually rules out a simple extravascular shift to areas drained by peripheral lymphatics. Further studies are in progress in an attempt to elucidate the problem.

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REFERENCES


