# Investigation of Serum Lipoprotein Metabolism in Man with $I^{131}$- Labeled Triolein

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THE present investigation was undertaken to study the role of the several lipoprotein components of human serum in the transport of dietary fat. Labeled fatty acids in the form of $I^{131}$ triolein were fed to normal subjects and patients with various blood lipid abnormalities in order to study the nature of these deviations from normal and their effects on lipid transport.

## METHODS

Following an overnight fast, 30 ml. of venous blood was obtained from each subject and was permitted to clot. One gm. of sodium iodide was then administered intravenously to prevent thyroid uptake of $I^{131}$ and conversion to protein-bound iodine. The subject was then fed a standard breakfast containing 60 gm. of carbohydrate, 10 gm. of protein and 20 gm. of fat including $I^{131}$ triolein (Raolein,® Abbott). The labeled lipid was previously emulsified with a phosphatide-glycerol (1:10 w/w) mixture (30 ml. mixture plus 1 to 2 ml. Raolein plus 300 ml. water in Waring Blendor) and 50 $\mu$c. aliquots added to milk. At intervals of two, four, seven and twenty-four hours, 30 ml. samples of venous blood were drawn, permitted to clot at 4°C. and the sera separated by centrifugation at 500 × G for twenty minutes.

Lipoprotein fractions were separated by a density gradient ultracentrifugation procedure.[1] Five ml. portions of the serum were placed in lusteroid centrifuge tubes of 13.5 ml. capacity, overlayed with 4 ml. of 0.15 M sodium chloride and centrifuged for thirty minutes at 9,300 × G in the Spinco Model L ultracentrifuge. The chylomicra formed a turbid band at the top of the saline layer and were separated from the infranatant serum with a tube cutter. Dark-field examination indicated essentially complete removal of visible particles of chylomicron from the serum. This serum was then layered over 5.5 ml. of 2 M sodium chloride in another lusteroid tube, overlayed with 0.15 M sodium chloride to fill the tube, and centrifuged at 100,000 × G for eighteen hours. The $S_f$ 10–400 lipoproteins were concentrated in a turbid band at the top of the tube, the $S_f$ 3–9 lipoproteins formed a clear orange-yellow band in the center, while the high density lipoproteins plus the remaining serum proteins sedimented to the bottom of the tube. The lipoprotein fractions were separated with the tube cutter. The fractions from the zero-hour specimen were transferred to volumetric flasks and the lipids extracted with ethanol-ether mixture (3:1 v/v) for cholesterol and phospholipid analysis. The two, four, seven and twenty-four-hour specimen fractions were quantitatively transferred to test tubes with minimal amounts of 0.15 M saline and the final volume measured. Two ml. aliquots of the fractions as well as of the original sera were then treated to remove inorganic $I^{131}$ by adding

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TABLE I

Per Cent of Total Dose of $I^{131}$ Administered per Liter Serum at Various Intervals after $I^{131}$-Triolein Test Meal in Normal, Hypercholesterolemic (Elevated $S_f$ 3–9) and Hyperlipemic (Elevated $S_f$ 10–400) Subjects

| Subject | Total Serum $I^{131}$ Per Cent Dose/Liter | | | | Serum Lipid $I^{131}$ Per Cent Dose/Liter | | | | Lipid $I^{131}$/Total $I^{131}$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 hr. | 4 hr. | 7 hr. | 24 hr. | 2 hr. | 4 hr. | 7 hr. | 24 hr. | 2 hr. | 4 hr. | 7 hr. | 24 hr. |
| *Normal* | | | | | | | | | | | | |
| D. M. | 1.53 | 4.60 | 3.28 | 0.88 | 0.81 | 3.40 | 1.70 | 0.15 | 0.53 | 0.74 | 0.52 | 0.17 |
| R. K. | 2.31 | 4.35 | 2.52 | 0.50 | 0.81 | 2.96 | 1.16 | 0.09 | 0.35 | 0.68 | 0.46 | 0.18 |
| M. S. | 5.71 | 6.48 | 4.30 | 0.78 | 3.37 | 3.43 | 0.73 | 0.07 | 0.59 | 0.53 | 0.17 | 0.09 |
| L. P. | 3.75 | 4.96 | 4.15 | 1.04 | 1.35 | 1.94 | 1.37 | 0.05 | 0.36 | 0.39 | 0.33 | 0.05 |
| R. F. | 3.21 | 3.55 | 2.53 | 0.90 | 0.38 | 0.90 | 0.32 | 0.04 | 0.12 | 0.25 | 0.13 | 0.04 |
| *Elevated $S_f$ 3–9* | | | | | | | | | | | | |
| G. G. (Idiopathic hypercholesterolemia) | 4.86 | 6.55 | 2.85 | 0.60 | 3.20 | 4.45 | 0.57 | 0.11 | 0.66 | 0.68 | 0.20 | 0.18 |
| C. K. (Nephrosis) | 3.07 | 5.95 | 3.91 | 0.74 | 1.78 | 4.23 | 1.72 | 0.06 | 0.58 | 0.71 | 0.44 | 0.08 |
| P. S. (Myxedema) | 1.18 | 2.70 | 1.82 | 0.67 | 0.53 | 1.70 | 0.84 | 0.12 | 0.45 | 0.63 | 0.46 | 0.18 |
| W. S. (Myxedema) | 2.67 | 7.20 | 8.40 | 2.39 | 1.14 | 4.78 | 6.36 | 0.58 | 0.43 | 0.67 | 0.76 | 0.24 |
| W. H. (Biliary cirrhosis) | 3.40 | 5.04 | 5.36 | 1.71 | 1.98 | 3.18 | 3.46 | 0.27 | 0.58 | 0.63 | 0.64 | 0.16 |
| *Elevated $S_f$ 10–400* | | | | | | | | | | | | |
| H. A. (Xanthoma tuberosum) | 1.74 | 5.65 | 6.06 | 5.14 | 0.92 | 4.63 | 5.22 | 4.21 | 0.53 | 0.82 | 0.86 | 0.82 |
| H. P. (Hyperlipemia) | 1.02 | 2.63 | 3.48 | 6.74 | 0.40 | 2.27 | 2.86 | 6.20 | 0.39 | 0.86 | 0.82 | 0.92 |
| W. S. (Diabetes; hyperlipemia) | 4.75 | 5.74 | 5.12 | 2.19 | 3.60 | 4.31 | 3.72 | 1.41 | 0.76 | 0.75 | 0.73 | 0.65 |
| L. S. (Xanthoma tuberosum) | 0.74 | 4.40 | 8.24 | 2.38 | 0.18 | 1.46 | 4.11 | 1.25 | 0.24 | 0.33 | 0.50 | 0.53 |

0.1 gm. of Dowex® 2 × 10 anion exchange resin in the chloride form, agitating gently for several minutes to ensure complete removal and spinning at low speed in a clinical centrifuge to separate the resin. One ml. aliquots of the supernatant were then assayed for radioactivity as were 1 ml. samples of the original sera. The results were expressed as per cent of dose per liter for total and lipid radioactivity in whole serum and lipid radioactivity in the chylomicron, $S_f$ 10–400, $S_f$ 3–9 and high density lipoprotein fractions.

## RESULTS

Some representative values obtained from normal subjects and patients with abnormal blood lipid patterns are given in Table I. It may be seen that in the normal subjects peak levels of lipid radioactivity as well as total radioactivity always occurs before seven hours. In addition the lipid radioactivity at the end of twenty-four hours is negligible even though the total radioactivity may be appreciable. This is expressed another way in the last column which shows that the lipid fraction of the total radioactivity after twenty-four hours is always less than 20 per cent and usually considerably less than this. Figure 1 shows a typical distribution of the lipid radioactivity among the various lipoprotein fractions at different times. The labeled lipid appears mainly in the $S_f$ 10–400 and chylomicron fractions.

The second group in Table I consists of patients with elevated $S_f$ 3–9 lipoprotein levels (the hypercholesterolemic states). As a group they do not appear to handle the labeled lipid much differently than the normal subjects,

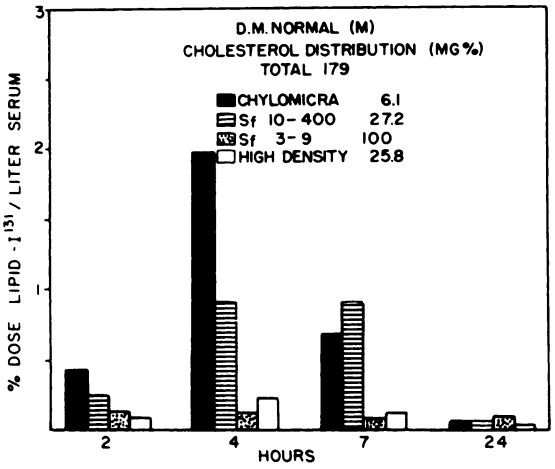


FIG. 1. Distribution of $I^{131}$-labeled lipid in serum lipoproteins after $I^{131}$-triolein meal in a normal subject.

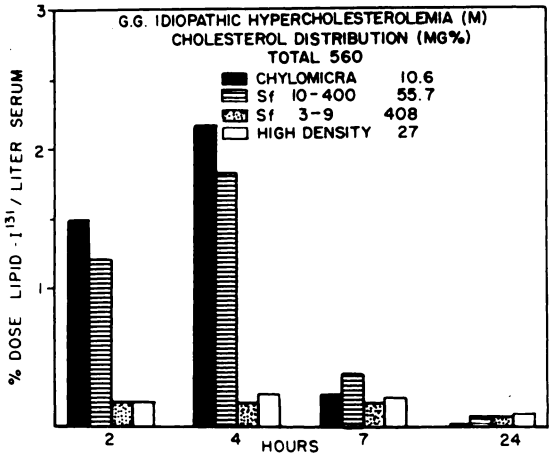


FIG. 2. Distribution of $I^{131}$-labeled lipid in serum lipoproteins after $I^{131}$-triolein meal in a patient with hypercholesterolemia.

although the patients with myxedema show a tendency to delayed removal of the labeled lipid. This is particularly evident in patient W. S. who shows a delayed peak (seven hours) as well as a somewhat higher than normal lipid $I^{131}$ fraction after twenty-four hours (24 per cent). Figure 2 shows the transport pattern of a patient (G. G.) with atherosclerosis (post-myocardial infarction) and hypercholesterolemia. In spite of a total serum cholesterol of 560 mg. per 100 ml. and markedly elevated $S_f$ 3–9 fraction, his handling of dietary lipid as measured by this test appears normal. Figure 3 illustrates the pattern of one of the patients with myxedema (P. S.). Aside from the suggestion of a slight delay in clearing, the over-all pattern appears normal.

The last group in Table I contains representative examples of patients with elevated $S_f$ 10–400 lipoprotein levels (the hyperlipemic states). The transport patterns here are all grossly abnormal. Peak labeled lipid levels are usually beyond the four-hour period. Even more striking is the high percentage of the total radioactivity which is still in the lipid fraction after twenty-four hours. This is always over 50 per cent and frequently approaches 90 per cent. Figure 4 shows the pattern obtained from a thirty-seven year old white man (H. A.) with lipemic serum and xanthoma tuberosum. His serum lipid $I^{131}$ at twenty-four hours was still 81 per cent of the peak value and the lipid portion of the total radioactivity at twenty-four hours was 82 per cent of the total. Figure 5 illustrates an even more extreme defect in clearing ability. This patient (H. P.) had a total serum cholesterol of 1212 mg. per 100 ml., 705 mg. of which was found in the $S_f$ 10–400 fraction and 346 mg. in the chylomicron fraction. The amounts in the $S_f$ 3–9 (108 mg. per 100 ml.) and high density (26 mg. per 100 ml.) were within normal limits. The total lipid $I^{131}$ at twenty-four hours was more than twice that at seven hours, and this represented ninety-two per cent of the total $I^{131}$ present in the serum.

## COMMENTS

Previous studies employing lipoproteins labeled in the protein moiety have indicated that $S_f$ 10–400 and $S_f$ 2–9 lipoproteins are related to each other as precursor and product.[2] In addition, these studies demonstrated that in patients with nephrosis and hyperlipemia the elevation in $S_f$ 10–400 lipoproteins could be explained at least in part as resulting from a delay in the transformation of this class to the $S_f$ 3–9 class. Immunochemical studies[3] also indicate a close relationship between the two classes of lipoproteins. The fate of the chylomicrons and their relationship to other serum lipoproteins have been more difficult to elucidate, but it appears to be established that the protein moieties of the chylomicra and the high density lipoproteins are similar if not identical.[4]

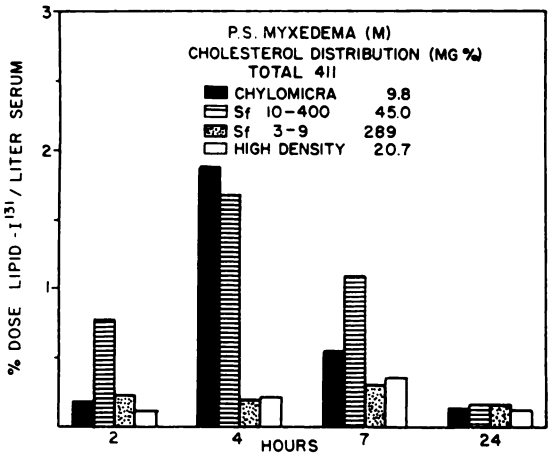


FIG. 3. Distribution of $I^{131}$-labeled lipid in serum lipoproteins after $I^{131}$-triolein meal in a patient with thyroid deficiency and hypercholesterolemia.

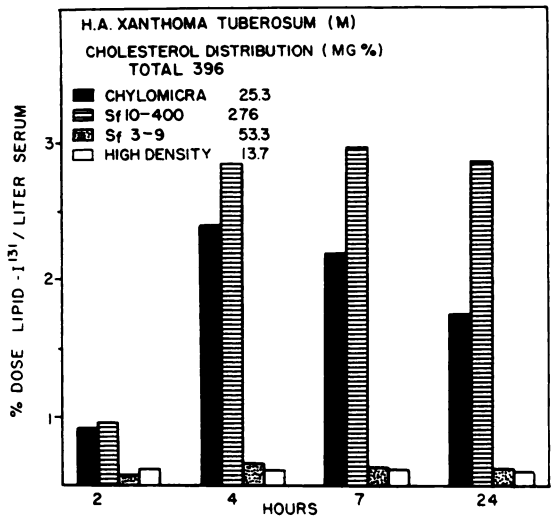


FIG. 4. Distribution of $I^{131}$-labeled lipid in serum lipoproteins after $I^{131}$-triolein meal in a patient with hyperlipemia and xanthoma tuberosum.

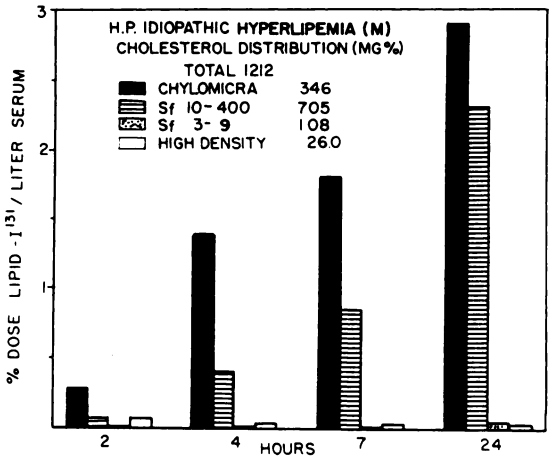


FIG. 5. Distribution of $I^{131}$-labeled lipid in serum lipoproteins after $I^{131}$-triolein meal in a patient with idiopathic hyperlipemia.

We have observed[5] that the intravenous infusion of fat emulsions results in a decrease in high density lipoprotein cholesterol corresponding to an increase in the "chylomicron" fraction. The disappearance of these "chylomicrons" coincides with an increase in the $S_f$ 10–400 lipoprotein fraction. It would appear that the chylomicrons and high density lipoproteins constitute one system and the $S_f$ 10–400 and $S_f$ 3–9 lipoproteins a distinct but parallel system for transporting triglycerides in the blood.

We can picture the chylomicrons as the initial transport vehicle for the transport of exogenous fat in the blood. These are rapidly cleared from the blood, presumably in the reticuloendothelial system, and triglyceride reincorporated into newly synthesized $S_f$ 10–400 molecules in the liver.[6] The latter molecules can be considered as secondary transport vehicles for exogenous fat and in addition as vehicles for the transport of endogenous triglyceride. The $S_f$ 3–9 molecules appear to be synthesized directly by the liver in the absence of a lipid load, as well as being formed from an unloading of $S_f$ 10–400 molecules. In the presence of an exogenous lipid load resulting from the clearing of chylomicrons after a fat meal or under conditions where endogenous non-esterified fatty acids are elevated as in diabetes mellitus, excess fatty acid is incorporated as triglyceride to form $S_f$ 10–400 molecules rather than $S_f$ 3–9 molecules. These relationships are shown schematically in Figure 6.

The use of $I^{131}$-labeled fatty acids to study mechanisms of lipid transport is subject to criticism. Van Handel and Zilversmit[7] for example, have shown in experiments with dogs that the specific activity of plasma triglycerides after oral administration of mixtures of $I^{131}$ triolein with various fats and oils was always less than that of the fed triglyceride mixture. The same was true of triglycerides of the thoracic

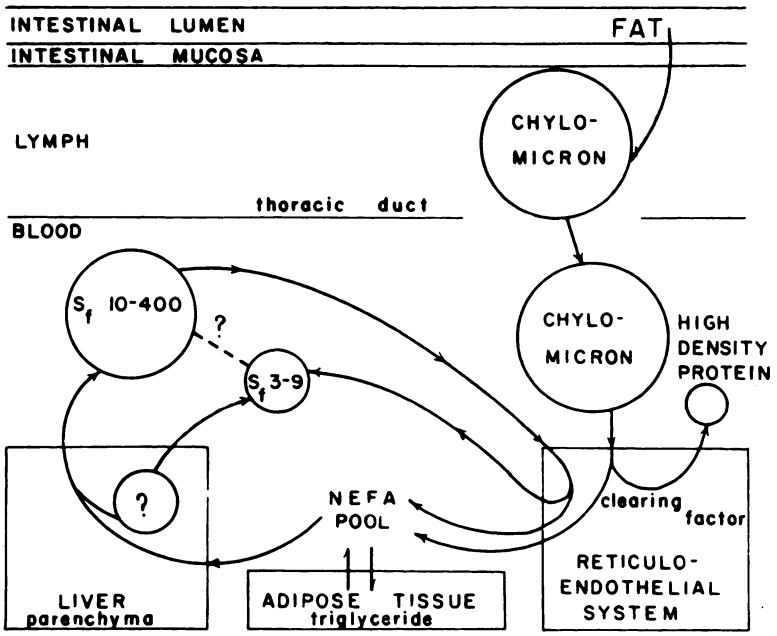


FIG. 6. Hypothetical scheme showing transport of dietary triglyceride via the blood lipoprotein system.

duct lymph in the rat. That this was not a dilution effect due to endogenous triglyceride was shown by the fact that the specific activity of $C^{14}$-labeled fatty acid containing triglycerides was the same in the duct lymph as in the meal. Thus, there is a difference in the appearance in lymph and blood of $I^{131}$-labeled and unlabeled triglycerides. This difference might reflect a difference in rate of absorption or might indicate a preferential catabolism of the $I^{131}$ lipid somewhere between the intestinal lumen and the thoracic duct.

On the other hand, these same authors showed that intravenously administered $I^{131}$-labeled fat emulsions in dogs maintained a constant specific activity throughout a two-hour period during which most of the infused lipid disappeared from the circulation. It would thus appear that the rate of removal of $I^{131}$-labeled triglyceride from the circulation is a valid measure of the rate of removal of unlabeled triglyceride. It must be pointed out however, that since the level of blood lipids at any given time is the resultant of the processes of absorption and clearing, the actual values reported should not be interpreted as indicating those which would be found with unlabeled or $C^{14}$-labeled lipid. On the other hand, this label, apart from ease in handling, offers certain advantages over chemical analysis for triglyceride since the twenty-four–hour period following a given meal can be studied without interfering with or interference from the normal spacing or content of meals throughout the day. It also has the advantage over $C^{14}$-labeling in that once metabolized there is no reincorporation of the label in either lipid or other substances.

With these considerations in mind it appears from the results presented that $I^{131}$-labeled triglyceride is a useful tool in the study of fat transport in abnormal as compared with normal blood lipid states. The dynamics of $I^{131}$-lipid transport reflects the defects in triglyceride clearing in hyperlipemic states and also the essentially normal transport of triglyceride in hypercholesterolemic states.

It should be pointed out that approximately 90 per cent of the labeled serum lipid following the ingestion of $I^{131}$-labeled triolein is present as triglyceride, and the remainder mostly as diglyceride. No label is found in the phospholipid and cholesterol ester fractions as separated on silicic acid columns.[8] It is also of interest

to note that in contrast to phospholipid[9] and cholesterol,[10] which exchange rapidly *in vitro* between the various lipoprotein species, $I^{131}$-labeled triglyceride, in addition to being associated primarily with chylomicron and $S_f$ 10–400 species, does not exchange appreciably between these two fractions *in vitro*.[11]


## SUMMARY

$I^{131}$-labeled triolein was fed to subjects with normal and abnormal blood lipid patterns in order to study the role of the various serum lipoproteins in lipid transport. In all subjects labeled lipid appeared almost exclusively in the chylomicron and $S_f$ 10–400 lipoprotein fractions. In the normal and hypercholesterolemic (elevated $S_f$ 3–9) subjects the labeled lipid peaked early and was essentially cleared from the blood within twenty-four hours. Patients with hyperlipemia (elevated $S_f$ 10–400) exhibited a delayed peaking of the $I^{131}$-lipid level which was still considerably elevated after twenty-four hours. The results are interpreted in terms of a hypothetical scheme for lipid transport. The validity of results obtained with $I^{131}$-labeled lipid is discussed.


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