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A SIMPLE PROCEDURE FOR A LARGE-SCALE PREPARATION OF TRITIUM-TRIGLYCERIDE-LABELED VERY LOW DENSITY LIPOPROTEIN OF THE RAT

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A simple procedure for the preparation of large quantities of tritium-triglyceride-labeled very low density lipoprotein (VLDL) from rat serum is described. Triton WR-1339 (0.10 ml) was intraperitoneally injected into rats weighing 200–300 g, and 100 μCi of tritium-triolein in corn oil (1 ml) was simultaneously administered orally. VLDL was separated by ultracentrifugation from the serum administration of the above compounds for 2 d. At least 10 mg of protein of VLDL was obtained from each animal, and the radioactivity in VLDL was exclusively found in triglyceride. Isolated radioactive VLDL was injected into normal rats, and the decay of the radioactivity in serum was found to be almost identical with that of triglyceride, but different from that of cholesterol.

Keywords—VLDL; very low density lipoprotein; lipoprotein lipids; Triton WR-1339

Very low density lipoprotein (VLDL) plays a very important role in the transport of serum lipid, but many aspects of the metabolism of its lipid still remain to be elucidated. This is partly because it has been very difficult to prepare large amounts of VLDL, and also to label the core lipid of VLDL with a radioisotope.

In this paper, a simple and large-scale preparation of tritium-labeled VLDL is described.

METHODS

Preparation of ^{3}H-Triglyceride Labeled VLDL—Male Wistar rats (250–300 g body weight) were fasted for 12 h and then given orally 1 ml of corn oil containing 100 μCi of [2-^{3}H]-glycerol triolein (500 mCi/mmol). Subsequently, the rats were given intraperitoneally 0.1 ml of Triton WR-1339. The treated rats were fasted for an additional 12 h, and were then maintained on Clea chow and water. The rats were bled from the inferior vena cava under light ether anesthesia 2 d after administration of the above compounds. VLDL was isolated by using a Hitachi Model 65P ultracentrifuge (Japan) according to the method of Hatchi and Lee.[1)]

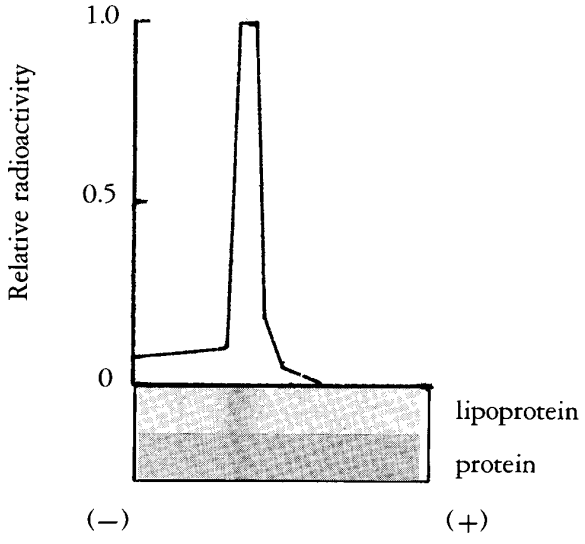

FIG. 1. *The Distribution of Radioactivity of Isolated VLDL*

VLDL was isolated according to the method of Hatch and Lee[1)] from the serum of rats administered with Triton WR-1339.

Cellogel Electrophoresis —Cellogel (Italy) electrophoresis was carried out at 150 V for 45 min. The lipoprotein was stained according to the ozone-Schiff method of Kanno and Tsukamoto,[2] and protein was stained with 0.8% Ponceau 3R in 5% trichloroacetic acid.

Determinations of Cholesterol, Triglyceride and Protein —Cholesterol was assayed by the *o*-phthalaldehyde method,[3] and triglyceride was assayed by the method of Sardesai and Manning.[4] Protein content was determined by the method of Lowry *et al.*[5]

Scintillation Counting —The radioactivity in a sample was determined in toluene-based fluor (general samples) or dioxane-based scintillator (automatic combustion apparatus) using an Aloka LSC 651 scintillation counter (Japan).

RESULTS AND DISCUSSION

It is well known that serum VLDL increases when Triton WR-1339 is administered to rats, as indicated by Fiser *et al.*[6] and Jourdan.[7] The serum after the treatments was centrifuged in a solution with a density of 1.006 g/ml to isolate VLDL. The upper gelatinous layer was separated from the lower one with a tube slicer. Lipid(s) and radioactivity were contained in only the upper layer. The isolated lipid-rich fraction was dissolved in physiological saline, and subjected to Cellogel electrophoresis. The results are shown in

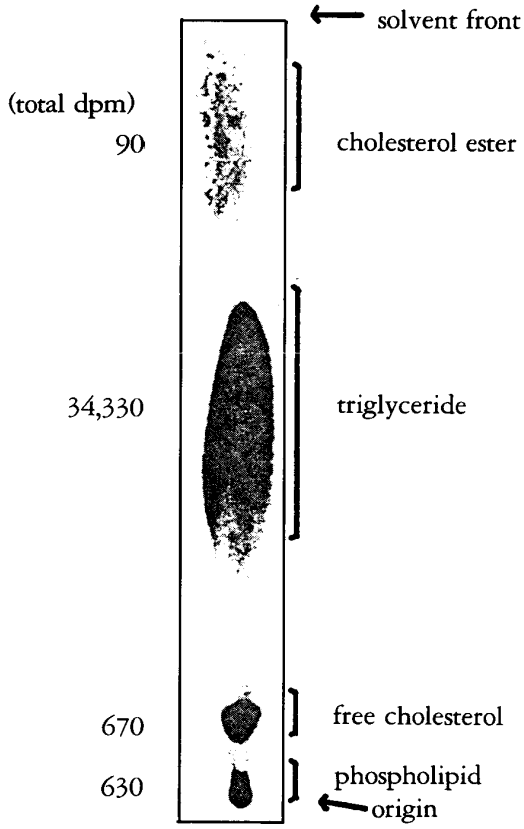

FIG. 2. *Thin Layer Chromatogram of the Lipids in ^{3}H-Labeled VLDL*

The lipids were extracted from the isolated ^{3}H-VLDL with Folch's solvent, and developed with petroleum ether/ether/methanol (90:10:1) on a thin layer of Silica gel G. The gel was stained with 50% H_2SO_4, then sliced into fractions, and the radioactivity of each fraction was assayed.

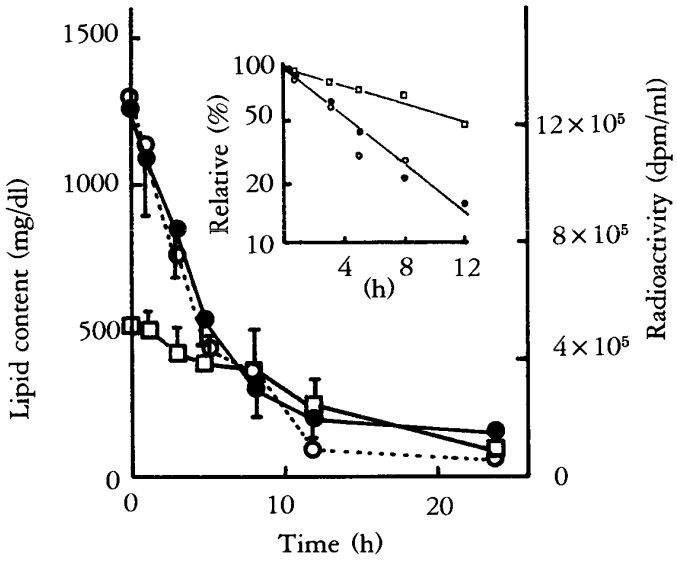

FIG. 3. *Time Course of Serum Radioactivity and Serum Lipids after Administration of ^{3}H-Labeled VLDL*

The ^{3}H-labeled VLDL (containing approximately 5 μCi of radioactivity and 200 mg of triglyceride) was administered intravenously to rats. The radioactivity (○), triglyceride (●) and cholesterol (□) in the blood were determined at various time after administration. Data are means of three experiments. The inset shows the relative contents of radioactivity, triglyceride and cholesterol with respect to the contents immediately after the administration.

Fig. 1. The lipoprotein with the radioactivity was VLDL. Further, the extracted lipid fraction was analyzed by thin layer chromatography (Fig. 2). The radioactivity corresponded mainly to the locations of triolein and tripalmitin. The results indicate that the labeled moiety is triglyceride. When the VLDL obtained by this method was suspended in physiological saline, and the volume was adjusted to the original volume, the protein and lipid contents in the VLDL averaged 2.34 ±0.53 mg protein, 230 ±34 mg triglyceride and 17.2 ±5.45 mg cholesterol per ml, respectively. Five to ten percent of the radioactivity administered to the rats was found in the isolated VLDL. At least 10 mg of VLDL (as protein) was obtained from each rat. The previous method might require more than ten animals in order to obtain an equal amount of isotope-labeled VLDL.[8]

Finally, purified ^{3}H-triglyceride-labeled VLDL was administered intravenously into the tail of normal rats. The plasma decay of radioactivity coincided quite well with that of triglyceride, but not with that of cholesterol (Fig. 3). The half-life of serum triglyceride and radioactivity after the administration was about 4.4 h, and that of cholesterol was 12 h. Eisenberg and Rachmilewitz[9] reported that the disappearance of ^{128}I-labeled VLDL in serum showed two phases; the faster phase had a half-life of less than 30 min, and the slower phase had that of about 4.4 h, which is consistent with our data.

VLDL is one of the most important proteins related to lipid metabolism. The formation and prevention of atherosclerosis are probably associated not only with HDL or LDL but also with VLDL. Nevertheless the metabolism of lipid in VLDL or that of VLDL as a whole is not known in detail. One reason for this is that it has been very difficult to obtain large amounts of VLDL labeled with radioactive lipid. The present labeled VLDL should be very useful for studies on VLDL metabolism and on the formation of atherosclerosis.

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