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ARTICLE

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Evaluation of rat and rabbit sera lipoproteins in experimentally induced hyperlipidemia by analytical ultracentrifugation

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Abstract Animals of various species are widely used as models with which to study atherosclerosis and the lipoprotein metabolism. The objective of this study was to investigate the lipoprotein profiles in Wistar rats and New Zealand white rabbits with experimentally induced hyperlipidemia by means of ultracentrifugation. The Schlieren curves were utilized to compare suckling and adult rat sera to determine whether aging causes alterations in lipoprotein profiles. A striking feature of the data is the high concentration of low-density lipoproteins (LDL), (> 5.2 mmol/l cholesterol) in the 2-week old rat serum pool which was greatly decreased in the 3-weeks rat serum pool (< 1.3 mmol/l cholesterol). Additional experiments were performed to permit a direct comparison of the amounts of lipoprotein present in rat sera in experimental hyperlipidemia post-Triton WR 1339 administration. Rapid changes in concentrations in very low-density lipoproteins (VLDL), LDL and high-density lipoproteins (HDL) were observed after Triton injection. The administration of Triton WR 1339 to fasted rats resulted in an elevation of serum cholesterol levels. Triton physically alters VLDL, rendering them refractive to the action of lipolytic enzymes in the blood and tissues, preventing or delaying their removal from the blood. Whereas the VLDL concentration was increased markedly, those of LDL and HDL were decreased at 20 h after Triton treatment. Rabbits were fed a diet containing 2% cholesterol for 60 days to develop hyperlipidemia and atheromatous aortic plaques. A combination of preparative and analytical ultracentrifugation was used to investigate of LDL aliquots, to prepare radioactive-labeled lipoproteins and to study induced hyperlipidemia in rabbits. Analytical ultracentrifugation was applied to investigate the LDL flotation peaks before and after cholesterol feeding of rabbits. Modified forms of LDL were detected in the plasma of rabbits with experimentally induced atherosclerosis. ApoB-containing particles, migrating as LDL, intermediate density lipoproteins and VLDL were the most abundant lipoproteins. Gamma camera in vivo scintigraphy on rabbits with radiolabeled lipoproteins revealed visible signals corresponding to atherosclerotic plaques of the aorta and carotid arteries.

Keywords Ultracentrifugation · Technetium labeled LDL · Scintigraphy · Hypercholesterolemia

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Introduction

Atherogenic plasma lipoproteins are of major importance in the development of atherosclerosis, the leading cause of mortality in most countries (Brown and Goldstein 1983; Brown and Goldstein 1986). Since animals of various species are widely used as models to study atherosclerosis and the lipoprotein metabolism, a detailed knowledge of the lipoprotein distribution in such animals is very important. Rats are frequently used for experimental studies in lipoprotein research. There are substantial differences in lipoprotein metabolism between human and rat e.g., the remnant removal pathway and the cholesterol ester transfer reaction. The rat has an especially efficient mechanism for the clearance of chylomicrons and very low-density lipoprotein (VLDL) remnants from the circulation. Relatively, few studies have dealt with the characterization of serum lipoproteins in suckling and adult rat sera (Fernando-Warnakulasuriya et al. 1983). Possible age associated changes in lipoprotein concentration and distribution provide data towards an understanding of these processes. The assessment of Schlieren curves demonstrates substantial differences in low-density lipoproteins (LDL)

concentration levels. The changes have revealed that the lipoprotein metabolism in the suckling rat must occur at a rapid rate. Triton WR gives rise to hyperlipidemia (HLP) and this may be used for study of lipid metabolism (Friedman and Byers 1957). Our Schlieren curves have confirmed the decrease of LDL and the early disappearance of high-density lipoproteins (HDL) after Triton administration. Following heparin-manganese precipitation of LDL the resulting precipitate was dissolved in further aliquots of rat serum. In this way it was possible to obtain a well-defined Schlieren peak of LDL flotation curves from adult rat sera. In response to dietary manipulation the rabbit quickly develops severe HLP leading to premature atherosclerosis (Bocan et al. 1993; Restori et al. 1990). Supplementation of the diet with cholesterol results in rapid marked increase in the production of cholesteryl ester-rich, beta-migrating very low-density lipoproteins (beta-VLDL) by the liver and intestine. The VLDL comprise of the major class of plasma lipoproteins. Subsequent clearance of the beta-VLDL by the liver is reduced due to a downregulation of cell-surface lipoprotein receptors and the saturation of the remaining receptors. The beta-VLDL, including chylomicron remnants, that accumulate in the circulation are highly atherogenic. The plasma levels of cholesterol correlate closely with the extent of lesion development. Human lipoprotein aliquots obtained by preparative centrifugation were used for radiolabeling of lipoproteins with 99 mTc. A combination of preparative and analytical ultracentrifugation methods allows the investigation of lipoprotein aliquots before radioactive labeling with isotopes to identify labeled materials. Radiolabeled LDL offer a promising approach for the identification of the local metabolic fate of these compounds and for the study of LDL accumulation in vascular tissue, because LDL act as trapped ligand in vivo and should be a good tracer for scintigraphic studies on atherosclerosis. (Vallabhajosula and Goldsmith 1990; Lees et al. 1988; Back et al. 1995; Ginsberg et al. 1990).

Materials and methods

Wistar rats (LATI, Hungary) (180–200 g) ($n = 20$) were maintained on a stock powder diet and with water ad libitum. Suckling rats were removed from the mothers on 14, 21 and 28 day during short treatment. New Zealand white rabbits (LATI, Hungary) weighing 3–3.5 kg ($n = 20$) were housed in separate cages at room temperature and submitted to a 12 h light/dark cycle.

Some rabbits ($n = 10$) were fed on a diet of normal rabbit chow (Biofarm, Hungary) enriched with cholesterol (Sigma) to a content of 2% for 60 days. The serum cholesterol levels of the normocholesterolemic (NC) and hypercholesterolemic (HC) rabbits were determined by enzymatic methods during feeding at the beginning of the experiment and after 60 days.

Samples

In each case, sera were derived from pools made up of samples from five animals. The blood samples were taken from 14-day, 21-day and 28-day-old suckling rats after removal from the mother and 3-month-old rats, sera after fasting to 14 h, the animal were bled from a vein under light anesthesia. In the Triton treatment, the rats received an i.p. injection of Triton WR 1339 (a 7% solution in normal saline) at 350 mg/kg. In the Triton treatment group, the rats were then bled from an orbital vein about 20 h after Triton administration. In overnight fasted rabbits sera were obtained from a marginal ear vein. The rabbits were injected in the marginal ear vein with 99 mTc-labeled human LDL (4–0 mCi, 0.5–1.5 mg protein) before scintigraphic examination. Human blood was sampled from healthy subjects after they had fasted overnight. The blood was allowed to clot for 40 min at room temperature. The sera were then separated by centrifugation at 1,200*g*, at 20°C, for 30 min. Sera were stored at 4°C in the presence of 0.01 g/l EDTA salt for a maximum of four days before ultracentrifugation. Aliquots of sera were taken for the estimation of total cholesterol (TC), HDL cholesterol (HDL-C), LDL-cholesterol (LDL-C) by enzymatic methods with the use of the standard Boehringer-Mannheim kits (Mannheim, Germany).

Short run new analytical ultracentrifugal micromethods for the determination of lipoproteins with Schlieren refractometry and ultraviolet absorption optics

We have developed new analytical ultracentrifugal micromethods for the determination of serum lipoproteins directly from ultracentrifugal Schlieren scans (Bozóky et al. 2001). Briefly, we applied band-forming, capillary underlayering type single sector centerpieces furnished with special holes. The 4° band-forming and 12 mm optical path length centerpieces were assembled with negative angle wedge windows (–1°40′) on the bottom of the cell. The flotation of patterns was obtained by underlayering physiological saline solution with serum or isolated lipoprotein fractions raised to a density of 1.3 g/ml in the spinning ultracentrifugation capillary band-forming cell. Samples of 100 µl were transferred with a micro syringe into the holes of the centerpiece from the upper-side-window. Runs were performed at 50,000 rpm. When the rotor was accelerating to 2,000–4,000 rpm, the increasing hydrostatic pressure forced sample with the adjusted density from the holes of centerpiece through capillaries under the physiological saline solution in the sector. In this way a discontinuous gradient was obtained in the analytical cells. The setting of the optical system was selected at a constant Philpot angle of 20°. Besides Schlieren optics, ultraviolet absorption optics was used at 254 nm to record the flotation of the lipoproteins bands in the cell at the same time. Photographs of the Schlieren pattern were taken at

10-min intervals from the time that the gradient curves became well visible i.e., at 60, 70, 80 and 90 min. Via the positions of the lipoproteins in the cell, the Schlieren curves were numerically integrated. Quantitative results were obtained by measuring the Schlieren areas between the sample curves and the reference baseline curve by means of computerized numerical and graphic techniques (Swinkels et al. 1989; Lindgren et al. 1969; Swinkels et al. 1987).

Preparative density gradient ultracentrifugation

The preparative isolation of serum lipoproteins was performed by a method described aerlier (Fülöp and Jánoki 2002). Briefly, 3 ml of serum was adjusted to a density of 1.3 g/ml with solid KBr (0.49 g/ml), placed at the bottom of a cellulose acetate centrifuge tube and overlayered with 8.5 ml of 1.006 g/ml NaCl solution containing 0.01 g/l EDTA salt made up to a volume of 11.5 ml. Tubes were loaded into a P 50 angular 20° rotor for 130 min at 50,000 rpm. At the end of the run the tubes were removed according to the localization of lipoprotein bands: 1.5, 4, 2.5, 1.5 and 2 ml (lipoprotein free serum protein, HDL, LDL, IDL and VLDL, respectively) and from these aliquots analytical ultracentrifugation runs and the radioactive labelling of lipoproteins were performed on.

Other methods

Heparin-manganese precipitation of LDL was carried out by the addition of 0.1 volume of the heparin-manganese reagent solution was added (heparin sodium 2,500 U/ml) to volume of serum (1 ml). This resulted in a final concentration of 4.1 mg/ml for heparin and 0.083 M $MnCl_2$. After 10–15 min at room temperature, the samples were centrifuged for 30 min at 3,000 rpm and 20°C. The supernatant was removed completely and any resultant precipitate was dissolved or suspended in 0.2 ml of another aliquot of the same serum. Aliquots of supernatant were taken for Schlieren analysis after adjustment of the density of the solutions with solid KBr to 1.30 g/l and underlayering with physiological solution, and the lipoprotein distribution was investigated with Schlieren optics. The differences in the Schlieren distribution profiles of the lipoproteins were used to follow the interaction of LDL and the precipitation reagents and in vitro accumulation of LDL in mononuclear cells.

Investigation of the accumulation of LDL in mononuclear cells by analytical ultracentrifugation

Fresh rabbit blood was diluted (3:1) with phosphate-buffered saline (PBS) (150 mM/l NaCl, 8.5 mM/l Na_2HPO_4, 1.5 mM/l NaH_2PO_4, pH 7.4) containing 200 U/ml of heparin. From this solution, 15 ml was applied to 12 ml of Ficoll-Paque density gradient ($d = 1.077$), which was then centrifuged for 45 min at $750g_{av}$ at room temperature. The mononuclear interphase was collected, washed three times with cold PBS/heparin, and resuspended in complete medium. After an overnight incubation at 37°C in the incubator (5% CO_2, 95% air), nonadherent cells were removed by aspiration, followed by washing with RPMI 1640. The LDL uptakes of normal and pathological mononuclear cells were compared by the analysis of the supernatant before and after incubation with LDL fraction.

LDL labeling with 99 mTc

Lipoprotein aliquots obtained by preparative centrifugation were used for radiolabeling of lipoproteins with 99 mTc (Hay et al. 1991; Lupatelli and Virgolini 1991; Virgolini et al. 1991). 1–3 mg LDL in a volume of 1–3 ml was mixed with 10–30 mCi 99 mTc-pertechnetate ($TcO4^-$) and 10 mg sodium dithionite, which was dissolved just prior to use in 0.5 M glycine buffer, pH 9.8, and the mixture was incubated for 30 min. The protein content was determined in an assay of its complex with the dye Coomassie Brilliant Blue.

Applications of labeled LDL

The radiolabeled LDL was tested in an atherosclerosis model. Rabbits fasted overnight were injected in the marginal ear vein with 99 mTc-labeled human LDL (4–10 mCi, 0.5–1.5 mg protein). The rabbits were then subjected to gamma scintillation camera scanning.

Results

We have determined total, VLDL, LDL, HDL cholesterol levels in rat sera pools of 2-, 3-, 4-weeks and 3-months age rat samples. The great differences in the cholesterol values were between 2 and 3 rat sera pools according to total cholesterol and LDL cholesterol levels. Cholesterol levels of rat samples are given in Table 1.

We have compared the Schlieren patterns obtained from sera pools of 2-, 3-, 4-weeks and 3-months age rat samples. The LDL peaks on the Schlieren diagrams were optimum at 80 min after full-speed centrifugation as became clear from the sequential every 10-min photographs between 60 and 100 min. These Schlieren pictures show the difference of the concentration values of LDL between two and three weeks age rat samples (Fig. 1a).

We have digitalized our photos into x/y-coordinate pair of concentration curves in multiple time points/scans at least 3, relatively closely spaced of 10 min from 60 min to 80 min after reaching full speed, where we

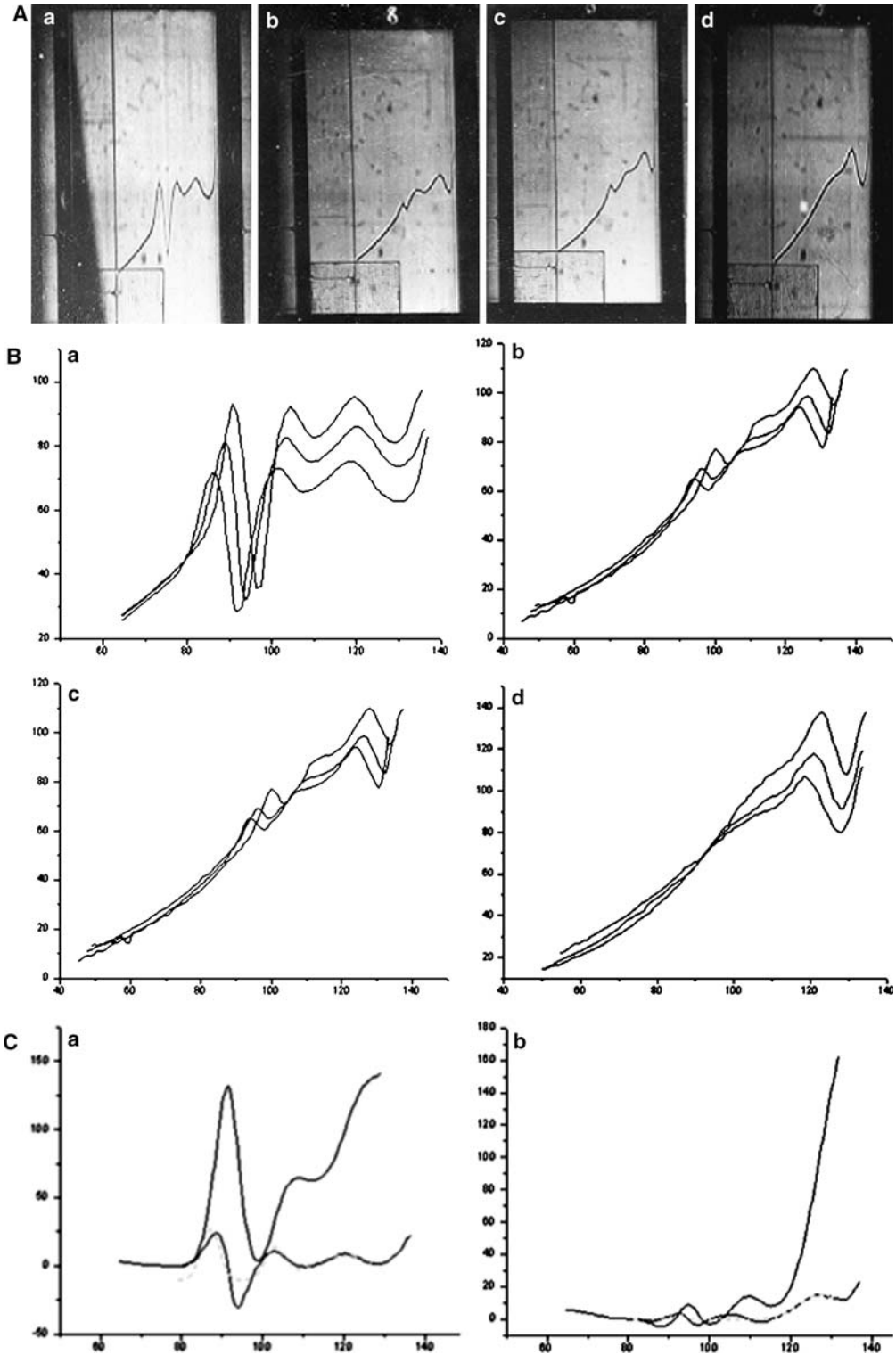

Fig. 1 Schlieren diagrams of 2-, 3- and 4-week and 3-month-old rat serum pools (**a**, **b**, **c**, **d**, respectively). **a**, **b**, **c**, **d** Schlieren diagrams of rat serum pools at 80 min after attainment of full speed (50,000 rpm). **b** Digitalized Schlieren diagrams of 2, 3 and 4-weeks and 3-months-old rat serum pools (**a**, **b**, **c**, **d**, respectively at 60, 70, and 80 min after attainment of full speed). The positions on the photographs were measured by the aid of inner edges of reference holes. Ten-fold magnification was determined: between the inner edges of reference holes was measured and divided by the actual distance (1.6 cm). The radial distance of the boundary peak from the reference edge was read and then the distance of the reference hole from the axis of rotation (5.7 cm) can be added (**c**) Integrals of the Schlieren curves of 2- and 3-week-old rat samples (**a**, **b**)

Table 1 Cholesterol levels of the rat sample pools studied

Rat age	Number of samples	Total serum cholesterol (mmol/l)	VLDL cholesterol (mmol/l)	LDL cholesterol (mmol/l)	HDL cholesterol (mmol/l)
2 weeks	5	7.75	0.99	5.26	1.50
3 weeks	5	3.16	0.47	1.09	1.59
4 weeks	5	2.82	0.27	1.01	1.53
3 months	5	2.22	0.17	0.40	1.64

could fit the boundaries to a sum of gaussians. (Fig. 1b) The peak position of curves would allow to calculate the Svedberg flotation (Sf) values of each species (gradient ultracentrifugation into Svedberg flotation rate). Sf values of rat samples are given in Table 2.

The most characteristic differences in the lipoprotein distribution profiles analyzed on the Gaussian curves were observed between the 2- and 3-week-old rat samples. LDL (> 5.2 mmol/l cholesterol) in 2-week-old rat sera pool have decreased in three weeks rat serum pool (< 1.3 mmol/l cholesterol). Figure 1c show the integrals of the 2- and 3-week-old rat Schlieren samples.The integrals are proportional to the concentrations.

We have used a combination of analytical ultracentrifugation method and lipoproteins precipitation method to obtain LDL concentration level to the detection of Schlieren optics. A combination of analytical ultracetrifugation and lipoprotein precipitation methods allowed the detection of LDL fraction in the 3-month-old rat serum at low concentration levels. Heparin-manganese precipitation of LDL to a volume of serum (1 ml) and any resulting precipitate was followed by the dissolution or suspension in another 0.2 ml. aliquot of the same serum (Fig. 2).

In order to control the method, the Schlieren patterns were registered from various aliquots from 100, 80, 60 µl volumes after reaching full speed. In samples with elevated VLDL concentrations a small peak or shoulder was observed in the gradient curve corresponding to the density value of floating VLDL in the early phase of the run up to 20 min. Afterwards, the LDL peak emerged in the central region of the cell, whereas the VLDL were then compressed against the surface of the sample solution by centrifugation. The integrated Schlieren curves indicated changes of LDL concentration according to the dilution of LDL (Fig. 3).

The effect of Triton on plasma LDL concentration are shown on the basis of Schlieren pictures (Fig. 4). We have detected the decrease of LDL concentration values in all rat sera pools and the increase of LDL heterogeneity. (This figures may be compared with Fig. 1a). In the early phase of the run up to 20 min we have detected increased level of VLDL in all sample pools. The effects of the Triton can be observed on the same age animal samples. The integrated Schlieren curves indicated changes in the LDL concentration. Screening of the LDL by analytical ultracentrifugation demonstrated that the LDL content decreased by more than 50%.

The general physiology of rabbits is similar to humans, and therefore, the rabbit has been used as a model for human diseases with some frequency. The rabbit is an important model for the study of the relationship between plasma cholesterol metabolism and atherosclerosis. The rabbit rapidly develops severe hypercholesterolemia leading to premature atherosclerosis in response to dietary manipulation. Two months after the beginning of cholesterol feeding, the total cholesterol level in the sera of the HC rabbits was approximately 30 times higher than that for the NC rabbits (1.56–1.95 mmol/l).

Schlieren diagrams of LDL are indicated in a normolipidemic rabbit sample cointaining 0.91 mmol/l LDL cholesterol (A), and in an hyperlipidemic rabbit sample containing 4.55 mmol/l LDL cholesterol level (B) (Fig. 5).

Schlieren diagrams of LDL are indicated in an normolipidemic human sample containing 3.38 mmol/l (Fig. 6).

Only at the bottom of the cells can be seen any differences between the two curves.

Schlieren detection of concentration changes of LDL was established by heparin precipitation (Fig. 7).

Table 2 Svedberg flotation (Sf) values of the rat sample pools studied

Rat age	Number of peaks	Sf values of peak1	Sf values of peak 2	Sf values of peak 3
2 weeks	3	5.14E-13	6.79E-13	8.63E-13
3 weeks	3	5.7E-13	7.25E-13-13	9.38E-13
4 weeks	3	5.47E-13	9.08E-13	
3 months	1	8.71E-13		

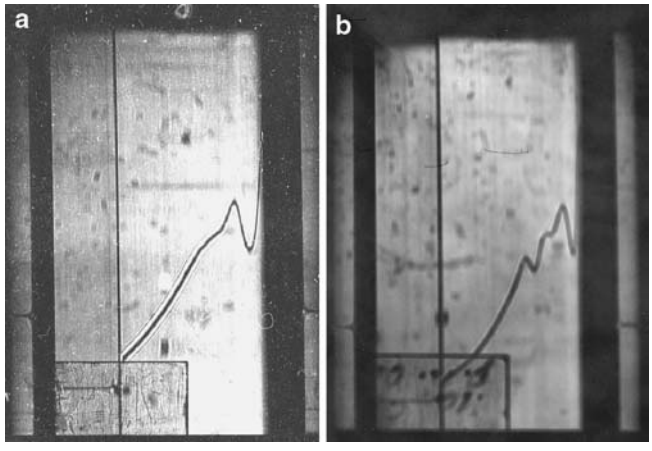

Fig. 2 Schlieren diagrams of 3-month-old rat serum pools without any precipitate (**a**), and with the resultant precipitate resolved (**b**)

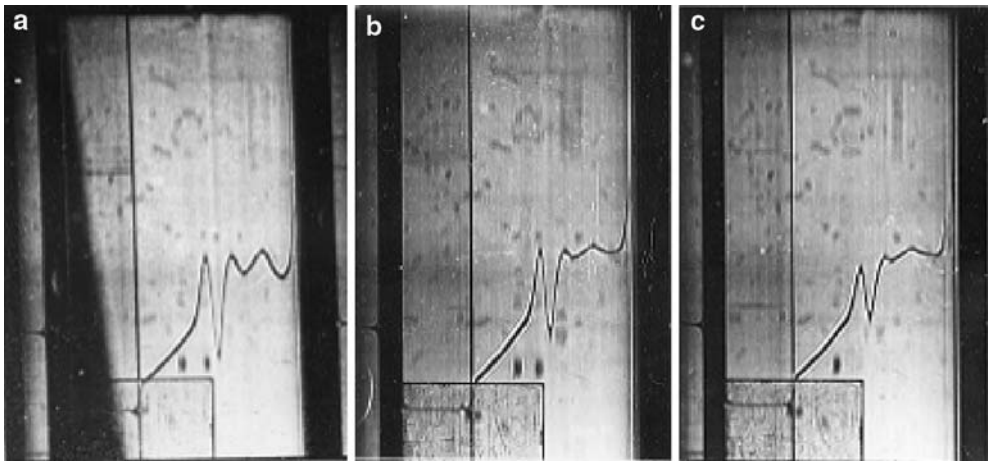

Fig. 3 Schlieren diagrams of 2-week-old rat sera pools of 100, 80 and 50 µl volumes (**a**, **b, c**, **d**, respectively)

Before heparin precipitation, the LDL cholesterol concentration was about 3.38 mmol/l, after heparin precipitation (not full precipitation.) about 0.39 mmol/l remained in the serum aliquots (A), (in two cells run at the same time) and full LDL precipitation with heparin (B). Similarly to heparin, the interaction of LDL with other molecules (e.g. glycosaminoglycan or lipoprotein lipase) can be used before the radioactive labeling of lipoprotein aliquots to increase modified LDL uptake of endothelial cells for scintigraphic detection in animal models or in vitro models.

In the middle of the cell can be seen LDL flotation band by ultraviolet absorption optics (Fig. 8). With the absorption optics, the absolute concentration is available at any point rather, than a concentration difference with respect to a reference point. The LDL cholesterol concentration of this sample was 3.38 mmol/l.

The Schlieren diagrams show LDL cholesterol levels of about 3.38 mmol/l and 2.6 mmol/l. The difference between the two curves (0.78 mmol/l) indicates the LDL uptake by mononuclear cells in the in vitro model.

We have designed in this experimental order to detect LDL receptor or scavenger receptor uptake of LDL (without labeled lipoproteins) in the native state of serum after the incubation of serum with mononuclear cells by Schlieren optics (Fig. 9).

Gamma camera scintigraphy—the detection and quantification of gamma-emitting radionuclides is commonly used to track individual molecules or cells. For diagnostic imaging agents, technetium−99 m is a frequently used radionuclide, because it has optimal nuclide properties (half-life of 6 h and appropriate gamma-energy of 140 keV). In vivo detection of atherosclerotic lesion may be a good means to image atherosclerotic plaques using a receptor specific radio-pharmaceutical of isolated and prepared modified lipoprotein fractions. Dynamic gamma camera imaging revealed a faster plasma clearance of oxidized-LDL than native-LDL. The rapid blood clearance of oxidized-LDL resulted as a consequence of more rapid uptake by macrophage rich tissue (Fig. 10).

Discussion

LDL is implicated in an atherosclerotic process in which the blood flow is restricted by cholesterol-related plaque in the vessels and becomes a major component of atherosclerotic plaque lesions. We have developed rapid and reproducible ultracentrifugation methods to obtain lipoprotein aliquots for radiolabeling and to evaluate lipoprotein labeling techniques which are suitable for the scintigraphic delineation of experimental atherosclerotic lesions. Preparative and analytical ultracentrifugation methods were used for the investigation of lipoprotein aliquots in order to determine concentrations and to

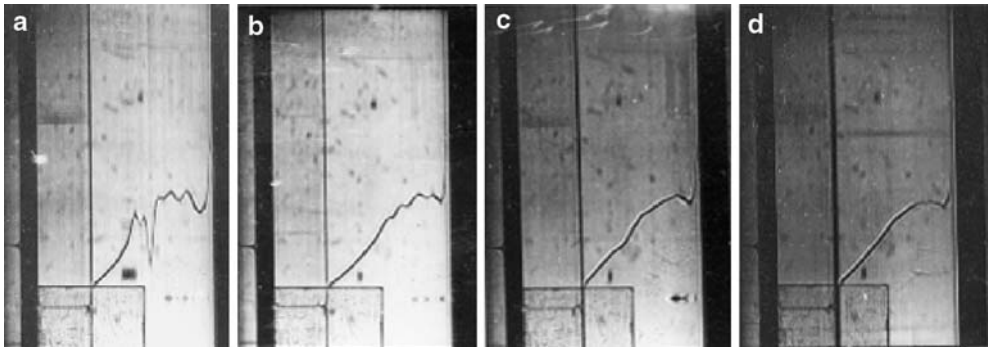

Fig. 4 Schlieren diagrams of 2-, 3- and 4-week and 3-month-old rat serum pools (**a**, **b**, **c, d**, respectively) 20 h after Triton administration. Schlieren diagrams of rat serum pools at 80 min after attainment of full speed (50,000 rpm)

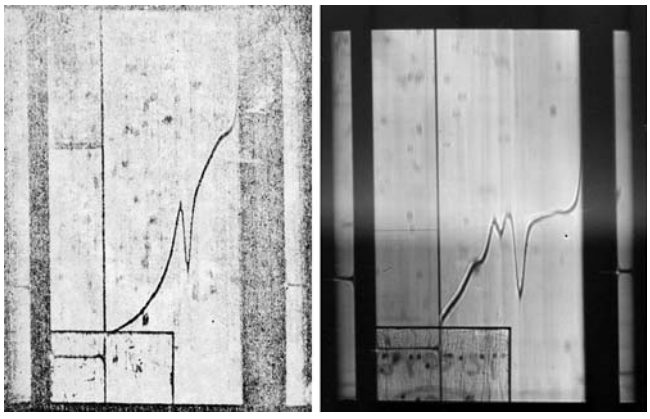

Fig. 5 Schlieren diagrams for normo- and hyperlipidemic rabbits The Schlieren dphotographs were taken at 80 min after attainment of full speed (50,000 rpm). Schlieren diagrams of LDL in a normolipidemic rabbit sample cointaining 35 mg/dl LDL cholesterol (**a**), and in an hyperlipidemic rabbit sample containing 175 mg/dl LDL cholesterol level (**b**)

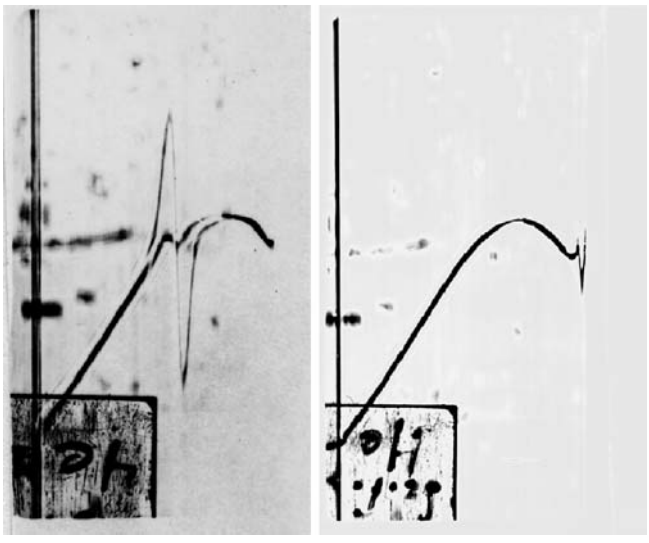

Fig. 7 Low-density lipoproteins (LDL) precipitation with heparin. The photograph of the Schlieren pattern was taken at 80 min after attainment full speed (50,000 rpm)

utilize the lipoprotein Schlieren data in order to gain an insight into the profile, allowing the comparison of serum lipoprotein concentrations.

The Schlieren method provides a simple way to characterize lipoprotein distribution in the whole serum without resort to stepwise separated preparative centrifugation, which has many drawbacks. A striking feature of the rat serum data is the monitor of the high concentration of LDL in 2-week-old rat serum pool, the concentration being greatly decreased in 3-week-rat serum pool. Experiments were performed to determine the changes in the LDL distribution profiles after a simple injection of Triton. The Schlieren curves reveal that there is a decrease in the concentration of LDL. We applied analytical ultracentrifugation to detect the LDL after its isolation by precipitation from the serum and dissolution of the precipitate in another aliquot of the same serum. By a combination of precipitation and analytical ultracentrifugation methods we obtained sufficient quantities of LDL in the 3-months rat serum pool for the analysis of Schlieren curves. From a screening aspect, we regard the procedure as a very promising technique with which to visualize LDL via Schlieren curves for some of the domestic and laboratory animals frequently used for studies on atherosclerois. It is known that diet-induced hyperlipidemia and atherosclerotic lesions develop more easily in some species than in others. For example, the rabbit develops hyperlipidemia and arterial lesions on less extensively modified diets than do rats. In the analysis of serum samples the method appears reproducible and linearity was observed in the analyses of sample quantities. A combination of pre-

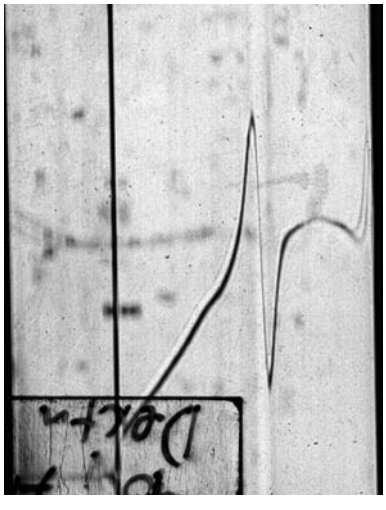

Fig. 6 Reproducibility of the method in an two cells run experiment at the same time to prepare LDL fraction before radioactive labelling of this aliquot

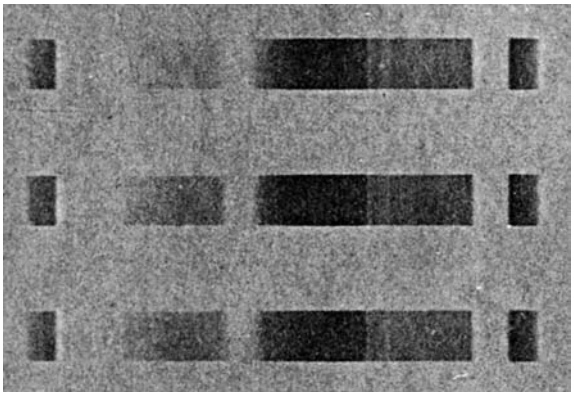

Fig. 8 Ultraviolet absorption optics was used at 254 nm to record the flotation of the LDL band in the cell at the same time, as the recording of Schlieren diagrams. The photographs of ultraviolet absorption patterns of LDL were taken at 60, 70, 80 min after the attainment of full speed (50,000 rpm)

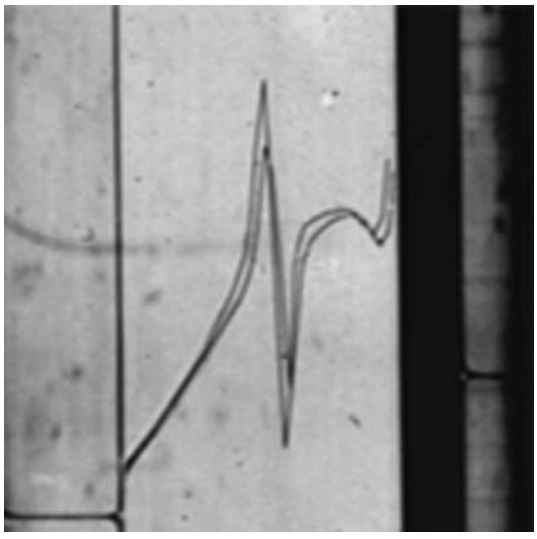

Fig. 9 Schlieren diagrams of supernatants by analytical ultracentrifugation to study the in vitro uptake of LDL by monomolecular cells before and after incubation, The photographs of Schlieren pattern were taken at 80 min after attainment of full speed (50,000 rpm)

parative and analytical ultracentrifugation will be used for the investigation of human LDL aliquots to prepare radioactive-labeled lipoproteins. We think so that the method of integration of Schlieren peaks as an assay for lipoprotein level with Schlieren optical facility can be extended to the Rayleigh interference optical system available on current instrumentation. The interferometer optical system records the refractive index difference between the reference end test solutions which is proportional to the concentration difference between the two solutions. The sensitivity of interferometer optics is one order of magnitude greater that of Schlieren optics.

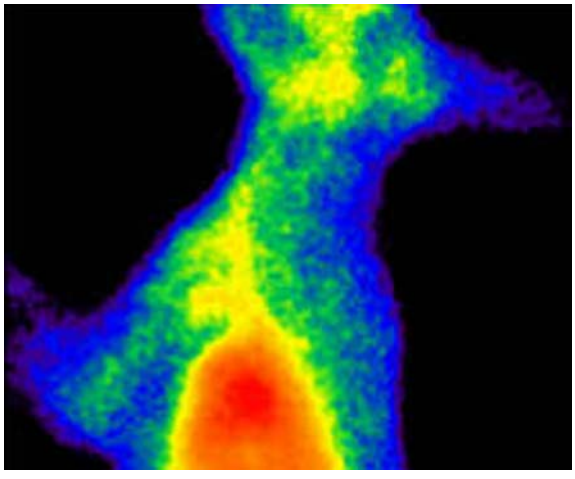

Fig. 10 Gamma camera in vivo scintigraphy of a hypercholesterolemic rabbit 1 h postinjection left lateral view Image of New Zealand White rabbit of 360 MBq 99mTc-labeled oxidized human LDL. LDL was oxidized by copper and by gamma irradiation of five gray

Depending on the height of cell centrepiece, it can be used in a very broad concentration range. A refractive index difference at 12 mm light path gives a discernible fringe shift. Good accuracy and resolution are obtained with solutions of 0.1–0.3 g/l initial concentration. The displacement of fringes can be measured horizontally in a capillary type synthetic boundary cell. If the refractive index increment of the lipoprotein analysed and the optical parameters of the instrument are known, then the concentration difference related to fringe displacement can be determined. The integration of comparable Schlieren and and Rayleigh interference plots may give comparable information concerning the different concentration ranges of lipoproteins. The radiolabeling of LDL fractions was tested in atherosclerosis on the basis of scintigraphic examinations in animal models. A radiolabeled compound the radiopharmaceutical is injected (in most cases intravenously) and the distribution of the radioactivity throughout the body is visualized using a gamma camera. Identifying the radiotracers that localize in large superficial vessels, such as the carotid and aorta arteries, and the feasibility of detecting lipid laden plaques using radiolabeled LDL and external planar gamma camera imaging becomes an major challenge. Our preliminary results showed that gamma camera scintigraphy of rabbits in vivo revealed visible signal corresponding to atherosclerotic plaques of the aorta and the carotid arteries, which were confirmed by pathological examinations.

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