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# MODIFICATION OF PLASMA LIPOPROTEINS BY HEXANAL, A LIPID PEROXIDATION PRODUCT

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Damage to low-density lipoproteins (LDL) by malonic dialdehyde leads to loss of their ability to interact with the B,E-receptor of liver cells, fibroblasts, and smooth-muscle cells, but it enhances their uptake by macrophages, inducing conversion of the latter into lipid-loaded cells [6]. Meanwhile the principle products of lipid peroxidation (LPO) in a composition of peroxidized LDL are not dialdehydes, but monoaldehydes, including hexanal and 4-hydroxynonenal [5].

The aim of this investigation was accordingly to study the action of hexanal on the functional properties of lipoproteins: the rate of elimination of LDL from the bloodstream and the rate of cholesterol acceptance by high-density lipoproteins (HDL).

## EXPERIMENTAL METHOD

Hexanal (caproic aldehyde) was obtained by interaction between allylmagnesium iodide and triethylorthoformate, and subsequent hydrolysis of the sulfuric acid acetal thus formed. The physical constants of the substance obtained agreed with data in the literature [3]. LDL and HDL were isolated from blood plasma from a random donor by ultracentrifugation within a density gradient of 1.02-1.05 and 1.063-1.21 g/ml, respectively (Beckman L2-65B ultracentrifuge, 50 Ti rotor, 42,000 rpm, 15°C, 22 h. After dialysis against 0.15 M NaCl containing 0.05 M sodium-phosphate buffer, pH 7.4, the LDL were labeled with $^{125}I$ by the chloramine method. To remove unbound radioactive iodine the samples were dialyzed against 0.15 M NaCl. Acetylation of labeled LDL was carried out with acetic anhydride. Protein was determined by Lowry's method, using bovine serum albumin as the standard.

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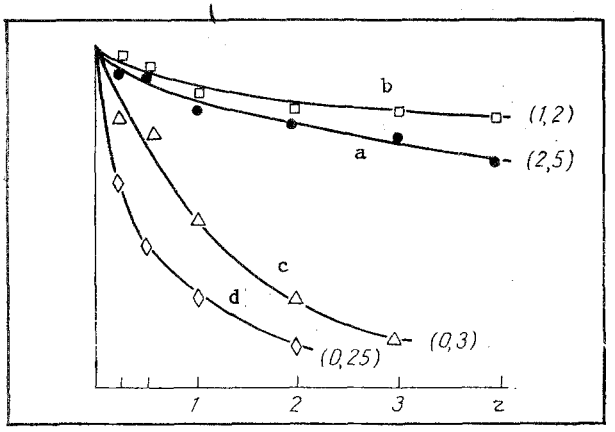


Fig. 1. Elimination of LDL from rabbit blood plasma. a) Initial LDL; b) LDL treated with 10 μmoles hexanal/mg protein; c) LDL treated with 1 μmole hexanal/mg protein; d) acetylated LDL. Each point is the mean of two independent experiments. Values in parentheses are $T_{1/2}$ – half-eliminaton time of injected lipoproteins (in h).

Experiments to study elimination of LDL from the bloodstream were carried out on rabbits weighing 2.5-2.8 kg, and the lipoproteins were injected into the auricular vein in a dose of $2 \cdot 10^6$ cpm (0.4 mg protein). Blood was taken 1 min later from the opposite auricular vein and the radioactivity of plasma obtained from this portion of blood was taken as 100%.

Lipoproteins were modified by hexanal as follows. A solution of hexanal in ethanol was added to the lipoproteins at the rate of 2-100 μl of a 1 M solution (2-100 μmoles to 1 mg of lipoprotein, expressed as protein). After 5 h the solution was dialyzed against 0.15 M NaCl, pH 7.4. Uptake of LDL by mouse peritoneal macrophages was estimated by esterification of $^{14}$C-oleic acid with cholesterol [4].

Acceptance of $^{14}$C-cholesterol, adsorbed on Celite-545, and HDL were determined by the method described in [2]. Radioactivity was measured on an LKB RackBeta spectrometer (Sweden).

## EXPERIMENTAL RESULTS

Aldehydes, including hexanal, interact readily with amino groups of proteins with the formation of Schiff's bases. Curves of elimination of labeled LDL from the bloodstream are illustrated in Fig. 1. The effective half-elimination time ($T_{1/2}$) was 2.6, 12.0, 0.3, and 0.25 h for initial LDL, LDL modified by 1 and 100 μmoles hexanal, and acetylated LDL, respectively.

Treatment of LDL with hexanal in low concentrations thus delays their elimination from the bloodstream, but in high concentrations, increases it. In the latter case $T_{1/2}$ was close to that for acetylated LDL, after blocking of about 60% of the amino groups (determined as fluorescence of fluorene). This effect can be explained on the grounds that after modification of fewer than 20% of amino groups interaction of LDL with cells through the B,E-receptor is reduced, but after blocking of more than 20% of the amino groups it is increased through elimination of these lipoproteins by cells of the reticuloendothelial system [7, 9], i.e., treatment of LDL with hexanal, just as by 4-hydroxynonenal [8], disturbs catabolism of this class of lipoproteins.

Meanwhile the results suggest that LDL circulating in the bloodstream cannot be strongly modified by LPO products because of their rapid elimination. However, accumulation of weakly modified LDL is most likely because of disturbance of the interaction of these lipoproteins with B,E-receptors and absence of interaction with the scavenger-receptors of the macrophages. For instance, on incubation of LDL, treated with 4 μmoles hexanal (per milligram protein) with peritoneal macrophages, incorporation of $^{14}$C-oleic acid into cholesterol esters amounted to $54.9 \pm 12.1 \cdot 10^3$ and $51.0 \pm 9.3 \cdot 10^3$ cpm/mg cell protein for modified and original lipoproteins.

Data on acceptance of labeled cholesterol from Celite by HDL after their treatment with hexanal are given in Fig. 2. Clearly, reduction of the cholesterol-accepting capacity was directly connected with the amount of modifier added. In other words, modification of HDL by LPO leads to loss of the ability of this class of lipoproteins to incorporate cholesterol, i.e., to depression of their cholesterol-accepting function. Similar results were obtained after initiation of formation of LPO products in the composition of HDL by ions of bivalent metals [1].

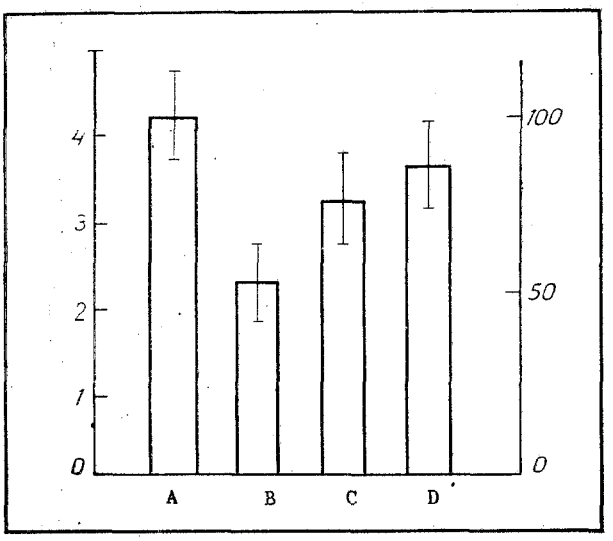


Fig. 2. Adsorption of $^{14}$C-cholesterol from Celite by HDL. A) Initial HDL; B) HDL treated with 100 μmoles hexanal/mg protein; C) HDL treated with 20 μmoles hexanal/mg protein; D) HDL treated with 2 μmoles hexanal/mg protein. Mean results of three measurements shown. Ordinate: on left – radioactivity, $10^3$ (in cpm/ml); on right – the same (in %).

The results of this investigation thus show that monoaldehydes produced by endothelial cells [5] are capable of disturbing the functional properties of both LDL and HDL. If this process takes place in the wall of the aorta, the former become high-affinity ligands for macrophages, whereas the latter lose their ability to accept cholesterol from the vessel walls.

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