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ELECTRON-MICROSCOPIC INVESTIGATION OF UPTAKE OF LOW-DENSITY LIPOPROTEINS BY PERICARDIAL MACROPHAGES FROM PATIENTS WITH ATHEROSCLEROSIS

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In atherosclerosis in man and experimental atherosclerosis in animals cells of the mononuclear phagocyte system take up lipids and are converted into "foam cells." These cells are found in organs of the reticuloendothelial system and also actually in the atherosclerotic focus in the vessel wall [1, 2, 10]. Experiments *in vitro* on albino rat macrophages have shown that foam cells can be formed by accumulation of products of lysosomal degradation of low-density lipoproteins (LDLP) [6]. The existence of a similar mechanism in man has also been postulated.

This paper describes an electron-microscopic study of LDLP uptake by pericardial macrophages of patients with atherosclerosis based on cell culture in medium containing homologous lipoprotein particles.

EXPERIMENTAL METHOD

Macrophages obtained from the pericardial fluid of seven men aged from 36 to 57 years, undergoing open heart operations for coronary atherosclerosis in the Clinic of the Surgical Faculty of the First Leningrad Medical Institute were used.

LDLP were isolated in the Department of Biochemistry (Head, Academician of the Academy of Medical Sciences of the USSR A. N. Klimov), Research Institute of Experimental Medicine, from blood donors' plasma within the density range 1.019-1.063 by the method of Havel et al. [9].

The cells were cultured immediately after removal from the patient directly in pericardial fluid at 37°C, with the addition of LDLP in a final concentration of between 0.5 and 3.6 mg/ml, which is within the limits of variation of the LDLP level in human lymph and blood plasma [7, 11]. In the control series of experiments macrophages of three patients were cultured without the addition of LDLP. After culture for 5 and 10 min the pericardial fluid was mixed with an equal volume of aldehyde fixative (2% paraformaldehyde and 2.5% glutaraldehyde), made up in 0.1 M cacodylate buffer (pH 7.2). The cells were fixed for 1 h at 4°C and then sedimented in a centrifuge. Electron-microscopic detection of LDLP in the macrophages was based on the method [14] which, as has been shown in the case of the developing hen's egg [12], gives a clear picture of intracellular lipoprotein particles. In the present investigation the material was processed as follows: After sedimentation in a centrifuge the cell clot was washed in 0.1 M cacodylate buffer for 24 h at 4°C, postfixed in a 2% solution of OsO_4 in 0.1 M cadoylate buffer for 1.5 h at 4°C, etched in 2% tannin solution for 2 h at 4°C, and washed for 10 min in a 1% solution of sodium sulfate at room temperature. After dehydration in alcohols of increasing strength the cells were embedded in Araldite. Sections were cut on the LKB-III Ultrotome, stained with lead citrate, and examined in the JEM-7A electron microscope.

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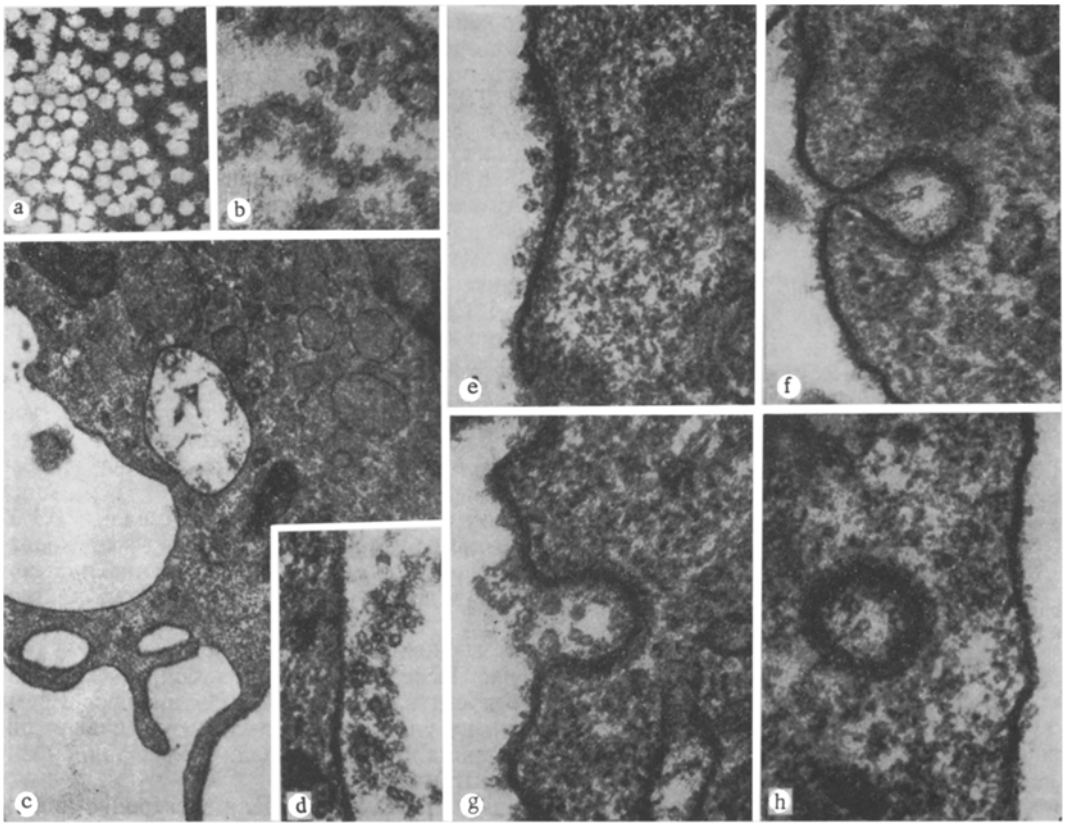

Fig. 1. Ultrastructural characteristics of LDLP and their uptake: a) LDLP on carbon film. Negative staining with phosphotungstic acid; b) ultrathin section of LDLP mounted in gelatin; c, d) LDLP inside a pinocytotic vacuole in a pericardial macrophage; e-h) consecutive stages of pinocytosis of LDLP by means of coated vesicles. a, b, d-h) 128,000 ×; c) 30,000 ×.

The LDLP fractions were studied electron-microscopically by negative staining with phosphotungstic acid on carbon films. The LDLP also were studied in ultrathin sections. For this purpose the lipoprotein particles were mounted in 15% gelatin, fixed, and treated by the method described above for cells.

EXPERIMENTAL RESULTS

On carbon films the LDLP were circular in shape and measured from 19 to 27 nm in diameter (Fig. 1a), in agreement with data of other workers [8, 11]. On mounting in gelatin the LDLP were smaller — from 14 to 23 nm; instead of being circular, often the particles were oval or irregular in shape. The central part of the lipoprotein particles had lower electron density than the peripheral zone, so that some particles were annular in appearance (Fig. 1b).

Particles morphologically identical with LDLP mounted in gelatin were found on the surface of the cells 5 and 10 min after addition of LDLP to the macrophage cultures; most of them were concentrated in the region of invaginations coated on their cytoplasmic surface — so-called coated pits (CP) [3, 4]. These pits, together with adsorbed lipoprotein particles, gradually moved deeper and gave rise to microvesicles of coated type. Consecutive stages of LDLP pinocytosis by means of coated vesicles (CV) are illustrated in Fig. 1e-h. The number of lipoprotein particles detected in CP and CV increased with an increase in the concentration of LDLP introduced into the pericardial fluid. During culture of the macrophages without addition of LDLP solitary particles of LDLP type were found only in individual CP and CV.

Lipoprotein particles were detected not only in CV, but also inside pinocytotic vacuoles. Such vacuoles in human pericardial macrophages are evidently formed through fusion of outgrowths of the cell surface, i.e., by a mechanism characteristic of the serous macrophages of other mammals [4, 13]. After culture of the macrophages for 5 min in medium with the addi-

tion of homologous LDLP, vacuoles with lipoprotein particles were located close to the plasmalemma (Fig. 1c, d). If the period of culture was increased to 10 min the vacuoles moved into deeper layers of the cytoplasm and decreased in size; the lipoprotein particles agglutinated into large conglomerates, which sometimes filled the greater part of the bulk of the vacuoles.

The use of tannin as an etching agent thus enabled LDLP to be detected both on the surface and in the interior of human macrophages. However, the possibility cannot be ruled out that this technique makes structures of the glycocalyx stand out in greater contrast. This could give rise to difficulties in the differentiation of LDLP. It must be pointed out, however, that an increase in the number of particles detected on the surface and also in the endocytotic microvesicles and vacuoles of pericardial macrophages accompanying an increase in the LDLP concentration in the extracellular medium demonstrates that the technique is definitely specific and can be used as an adjunct to the known methods of LDLP demonstration, namely autoradiographic, immunohistochemical, and tagging the lipoprotein particles with ferritin [5, 15].

It was shown previously that pinocytosis of LDLP is the initial stage in the transformation of peritoneal macrophages into typical foam cells in albino rats [6]. The results of the present investigation, showing that macrophages of patients with atherosclerosis can take up LDLP as whole particles indicate that the mechanism of foam cell formation in man may be similar to that in experimental animals. Elucidation of the subsequent changes in liproprotein particles taken up by pericardial macrophages in the course of pinocytosis will be a topic for future research.

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