The membrane activity of serum from patients with CRF, revealed by these experiments, evidently lies at the basis of its lytic action on erythrocytes. In their effect these toxins are similar to the widely distributed protein cytotoxic factors from microorganisms [2] and also from snake venoms [3].

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LIPID METABOLISM IN CELLS OF THE ATHEROSCLEROTIC HUMAN AORTA. EXPERIMENTS IN PRIMARY CULTURE

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The accumulation of lipids, mainly cholesterol esters, in the aortic wall is a characteristic feature of atherosclerosis [4, 6]. It is nowadays considered that the chief source of the accumulating lipids is the low-density lipoproteins circulating in the blood plasma [3, 7]. Meanwhile other possible mechanisms of the increased lipid content in the atherosclerotic aorta cannot be ruled out, including a disturbance of intracellular lipid metabolism in the affected regions.

This paper describes an attempt to compare lipid metabolism in cells of both unaffected and atherosclerotic regions of the human aorta. The investigations were carried out in primary culture, in which the cells preserve their similarity with cells in vivo in many properties. The fact that all cells in culture are under identical conditions allows the intensity of the lipid metabolism of cells from unaffected regions of the aorta and from lipid patches and atherosclerotic plaques to be compared.

EXPERIMENTAL METHOD

Cells of the intima and media of the aorta were isolated separately by dispersion with collagenase and elastase as described in [1]. The isolated cells were transferred to plastic Petri dishes (Falcon, USA), 60 mm in diameter, in a density of 0.5×10^6 to 1×10^6 cells per dish. The cells were cultured in 5 ml of medium 199 containing embryonic calf serum and 2.5 μg/ml fungizone, 100 μg/ml kanamycin, and 2 mM glutamine (all reagents from Gibco, USA) at 37°C in an atmosphere of 5% CO_2 and 95% air, saturated with water. On the 7th day the medium was changed and 40 μCi/ml of the sodium salt of [^{3}H]-acetic acid (from the Radiochemical Centre, Amersham, England) was added; the specific radioactivity of the preparation was 300 mCi/mmole. Incubation with labeled acetate continued to 6 h. At the end of incubation the medium was poured off and the dishes were washed three times with isotonic Dulbecco phosphate buffer, pH 7.4 (from Gibco, USA). The cells were suspended with 0.25% trypsin (from the same firm) and the suspension was centrifuged (200g, 10 min) and resuspended in isotonic phosphate buffer. Lipids were extracted from the cells by the method in [2]. The principal

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TABLE 1. Incorporation of [^{3}H]-Acetate into Intracellular Lipids of Intima and Media of Unaffected Areas of the Human Aorta (M ± m)

Type of cell	Incorporation of [^{3}H]-acetate, pmoles/10^5 cells				
	PL	FS	FFA	TG	ChE
Of intima	5,3±1,1 (26)	3,2±0,7 (16)	9,3±1,9 (46)	1,3±0,4 (7)	0,8±0,2 (4)
Of media	13,4±1,3* (47)	2,7±0,2 (10)	8,9±1,3 (32)	2,2±0,8 (8)	1,0±0,2 (4)

Legend. PL) Phospholipids, FFA) free fatty acids, FS) free sterols, ChE) cholesterol esters, TG) triglycerides. Mean data of five experiments conducted on cultures obtained from five aortas. *P < 0.05 compared with intima; incorporation of [^{3}H]-acetate (in % of total radioactivity of lipid fraction) shown in parentheses.

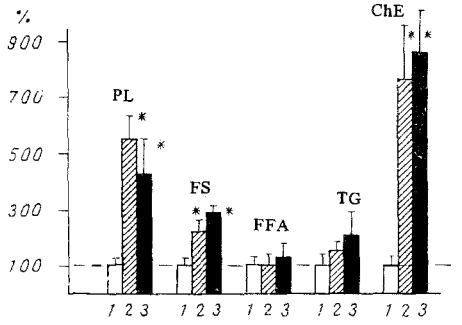

Fig. 1. Incorporation of [^{3}H]-acetate into lipids of intimal cells from unaffected areas of aorta (1), lipid patches (2), and atherosclerotic plaques (3). Here and in Fig. 2: abscissa, fractions of lipids; ordinate, incorporation of [^{3}H]-acetate (in % of control). *P < 0.05 compared with control. Remainder of legend in text.

classes of lipids were separated by thin-layer chromatography on plates with an adherent layer of "Silufol" silica-gel (from Kavalier, Czechoslovakia), in a system of solvents of N-hexane – diethyl ether – acetic acid (73:25:2 v/v). As reference substances 5 μg each of cholesterol oleate, trioleate, oleic acid, cholesterol, and phosphatidylethanolamine were applied to the plate (all reagents from Sigma, USA). Lipids were located by staining with phosphomolybdic acid. Areas of the plate containing the main classes of lipids were cut out and placed in standard flasks for scintillation counting, into which 10 ml of toluene scintillator was poured (4 g PPO, 0.1 g POPOP in 1 liter toluene). Radioactivity was determined on a Mark III liquid scintillation counter (Nuclear Chicago, USA). The significance of differences was estimated by Student's t test and the Wilcoxon–Mann–Whitney nonparametric test.

EXPERIMENTAL RESULTS

The fact that acetate is a precursor of all the main classes of lipids enables the intensity of lipid metabolism in toto of cells isolated from unaffected and atherosclerotic regions of the aorta to be compared on the basis of incorporation of labeled acetate into the lipid fraction. In the intimal cells obtained from unaffected regions of the human aorta 90% of the total radioactivity of the lipids was concentrated in fractions of phospholipids (PL), free fatty acids (FFA), and free sterols (FS) (Table 1). Cells of the intima isolated from atherosclerotic lesions such as lipid patches and atherosclerotic plaques incorporated 2.5-3 times more labeled ace-

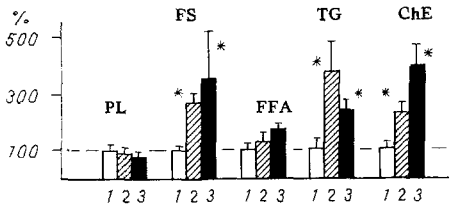

Fig. 2. Incorporation of [^{3}H]-acetate into intracellular lipids of media lying beneath unaffected areas of aorta (1), lipid patches (2), and atherosclerotic plaques (3).

tate into the lipid fraction. The increase in incorporation took place mainly on account of the PL fraction and cholesterol esters (ChE; Fig. 1). These results are in agreement with the increase observed in lipid synthesis in the aortic wall of laboratory animals with experimental atherosclerosis [5, 8]. Both in investigations on the animal aorta in vivo and in the present experiments in vitro in primary culture, the greatest increase in incorporation of [^{3}H]-acetate was found in the ChE fraction.

Cells from the media of unaffected regions of the aorta incorporated 1.5-2 times more [^{3}H]-acetate into the lipid fraction than intimal cells. Cells from the two layers of the aorta also differed in the distribution of label among the main lipid classes. For instance, cells of the media accumulated labeled acetate in their PL fraction 2.5 times more effectively than cells from the intimal layer of the aorta (Table 1).

Lipid metabolism in cells of the intima and media of the affected areas also differed. As was pointed out above, incorporation of [^{3}H]-acetate into fractions PL and ChE was sharply increased in intimal cells from atherosclerotic lesions, whereas incorporation of the label into the PL fraction was no greater than normally in cells of the media lying beneath the lipid patches and atherosclerotic plaques (Fig. 2). Compared with intimal cells, the level of incorporation of the labeled precursor into the ChE fraction was reduced in cells of the media from affected regions, and accumulation of [^{3}H]-acetate in the triglyceride fraction (TG; Figs. 1 and 2) was increased.

The results of this investigation thus show that in cells both of the intima and of the media in atherosclerotic lesions in the human aorta lipid metabolism shows changes compared with that in cells from unaffected areas of the aorta. In cells of the intima isolated from lipid patches and atherosclerotic plaques incorporation of labeled precursor into the fractions of phospholipids, free sterols, and cholesterol esters – i.e., lipids whose content is increased in the wall of the atherosclerotic aorta [3, 6] – is increased. These findings, in our view, indicate that changes in intracellular lipid metabolism may be one cause of the accumulation of lipids in the atherosclerotic human aorta.

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