in the frequency of erythrocytes with micronuclei in rats after exposure to mutagens and in man following treatment with cytostatics; 4) detection of a significant effect of mutagens during analysis of erythrocytes with micronuclei in splenectomized rats and humans.

The results described above thus point to the possibility of using counting micronuclei in peripheral blood erythrocytes as a way of detecting mutagens, but only in experiments on mice; nevertheless, this is of definite value in practical toxicology, for with this intravital method it is possible to observe the cytogenetic effect of the test factors over a period of time.

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COMBINED EFFECT OF DESIALATED AND GLYCOSYLATED LOW-DENSITY LIPOPROTEINS ON LIPID ACCUMULATION IN INTIMAL CELLS OF THE HUMAN AORTA IN VITRO

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KEY WORDS: atherosclerosis; diabetes mellitus; desialated low-density lipoproteins; glycosylated low-density lipoproteins; lipid accumulation.

Diabetes mellitus leads to the earlier onset and more rapid progression of atherosclerosis [4]. The mechanism of enhanced atherogenesis in diabetes has not yet been explained. A characteristic feather of atherosclerosis is massive deposition of lipids in cells of the vascular wall. It has recently been shown that the blood sera of most patients with coronary atherosclerosis and also blood sera of diabetics can induce cholesterol accumulation in cultures of cells taken from the intact intima of the human aorta [1, 11]. The atherogenic effect of sera from patients with atherosclerosis has been shown to be due mainly to low-density lipoproteins (LDL) [12], which differ from healthy human LDL in their low sialic

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TABLE 1. Effect of Native and Modified LDL on Cholesterol Content in Cultured Intimal Cells from Human Aorta

Experimental	Cholesterol content, μg/mg cell protein
Control	24,6±1,1
Native LDL:	
50 μg/ml	27,3±1,1
100 μg/ml	25,5±2,0
Desialated LDL:	
50 μg/ml	34,2±1,7[a]
100 μg/ml	51,3±1,7[a]
Glycosylated LDL:	
50 μg/ml	31,6±1,0[a]
100 μg/ml	41,6±3,0[a]
Glycosylated-desialated LDL:	
50 μg/ml	40,2±2,0[a, c]
100 μg/ml	57,0±2,1[a, c]
Native LDL + glycosylated LDL:	41,9±3,0[a, c]
Native LDL + desialated LDL:	49,4±2,6[a, b]
Native LDL + glycosylated-desialated LDL	52,2±1,9[a, d]
Glycosylated LDL + desialated LDL	83,7±3,4[a, b, c]
Glycosylated LDL + glycosylated-desialated LDL	83,6±7,5[a, c, d]
Desialated LDL + glycosylated-desialated LDL	95,4±5,6[a, b, d]

Legend. a) Significant difference from control, b) from desialated LDL c) from glycosylated LDL, d) from glycosylated-desialated LDL; $p < 0.05$.

acid content [10]. The blood of diabetics also contains modified, actually nonenzymically glycosylated, LDL [2]. These LDL facilitate lipid deposition in cultured macrophages, which are monocyte derivatives [6, 8], and in intimal cells of the human aorta [13].

In the investigation described below we studied the combined effect of desialation and glycosylation of LDL on their ability to induce accumulation of intracellular lipids.

EXPERIMENTAL METHOD

LDL (1.025-1.063 g/ml) were obtained from pooled serum from clinically healthy blood donors by ultracentrifugation [5]. Nonenzymic glycosylation of the native LDL was carried out in the presence of 80 mM glucose at 37°C for 7 days; under these conditions about 5% of the lysine residues was modified [14]. Native and glycosylated LDL were desialated by treatment with agarose-bound neuraminidase (0.01 IU/ml) (Sigma, USA) for 2 h, which led to loss of 70% of sialic acid residues [13].

The subendothelial cells of the intact aortic intima of men dying suddenly from myocardial infarction were isolated with the aid of collagenase and cultured as described previously [9, 12]. On the 7th day of culture the cells were treated with medium 199 containing 10% lipoprotein-deficient serum, obtained from pooled healthy human serum by ultracentrifugation ($p > 1.250$ g/ml), and also with native and modified LDL or various combinations of them, with each type of LDL in a concentration of 50 μg protein/ml (Table 1). After incubation for 24 h the cells were thoroughly washed with phosphate buffer (pH 7.0), the lipids were extracted with a mixture of hexane and isopropanol (3:2 by volume), and the total cholesterol content was determined with the aid of kits from "Boehringer Mannheim" (West Germany) as described previously [13]. The cell protein content was determined by the method in [7].

The significance of differences was estimated by the two-way test, using BDMPIV software [3].

EXPERIMENTAL RESULTS

Native LDL isolated from pooled healthy human blood did not induce lipid accumulation in smooth-muscle cell cultures derived from the intima of the human aorta. Glycosylated LDL in concentrations of 50 and 100 μg/ml increased the intracellular cholesterol content by 1.3 and 1.7 times respectively, whereas desialated LDL in the same concentrations increased it by 1.4 and 2.1 times. Glycosylated LDL treated with neuraminidase (desialated-glycosylated LDL) caused an increase in the intracellular cholesterol content by 1.6 times (50 μg/ml) and 2.3 times (100 μg/ml). The action of desialated-glycosylated LDL increased the effect of glycosylated LDL taken in the same concentrations (Table 1).

Table 1 also gives data on the combined effect of glycosylated, desialated, and desialated-glycosylated LDL on cholesterol accumulation by cells in culture. Cells incubated simultaneously with glycosylated and desialated LDL contained 3.4 times more cholesterol than the control cells. Combined addition of glycosylated and desialated-glycosylated LDL caused the same increase in the intracellular cholesterol content. A combination of desialated and desialated-glycosylated LDL led to a 3.8-fold increase in the cholesterol concentration in the cell cultures. No significant differences were found between the effects of these three combinations. Meanwhile cholesterol accumulation in the cells during culture with LDL taken in any combination was significantly greater than with the use of modified LDL taken separately, in concentrations of 50 or 100 μg/ml (Table 1).

We also studied the effect of combinations of native and modified LDL on cholesterol accumulation by aortic intimal cells. On the addition of glycosylated LDL to native LDL the intracellular cholesterol concentration was increased by 1.7 times, after addition of desialated LDL by 2.0 times, and on the addition of desialated-glycosylated LDL, by 2.1 times compared with the control. Incidentally, the effect of these combinations was significantly greater than the effect of modified LDL taken separately in a concentration of 50 μg/ml.

Table 1 gives representative results of one of three experiments. Similar data were obtained in all cases.

It was shown previously that desialated or nonenzymically glycosylated LDL induce cholesterol accumulation in cells in culture [6, 10, 13]. Incidentally, nonenzymic glycosylation and desialation are the two types of modification of LDL which have so far been found in vivo in human blood plasma [2, 10].

The results of this investigation show that under the simultaneous influence of glycosylated and desialated LDL the cholesterol concentration in cells in culture rises significantly, and this effect is not simple addition of the effects of the glycosylated and desialated LDL taken separately In the case of a combination of these types of modified LDL, synergism evidently takes place.

Since LDL in vivo can be modified in two ways, the possibility cannot be ruled out that some lipoprotein particles may be desialated and glycosylated simultaneously. The results indicate that cultures of human aortic intimal cells accumulate cholesterol more rapidly under the influence of desialated-glycosylated LDL than of simply desialated or simply glycosylated LDL.

It is interesting to note that a combination of modified and native LDL also leads to more rapid cholesterol deposition in cells in culture, In this connection it can be postulated that for an atherogenic effect to appear not all the LDL need be modified, but only a certain part of them.

We have as yet no complete explanation of the mechanism of the synergic effect of modified LDL on intracellular cholesterol accumulation We showed recently that glycosylated and desialated LDL, unlike native LDL, aggregate spontaneously during incubation with cell cultures. It is this aggregation of modified LDL which leads to lipid deposition in the cells in culture [13]. It can accordingly be suggested that after addition of different types of modified LDL to the incubation medium there is a marked increase in LDL aggregation, and this in turn leads to subsequent accumulation of intracellular cholesterol. On the other hand, it is not impossible that modified LDL can induce aggregation of native LDL also; in that case rapid accumulation of cholesterol by cells incubated with native and modified LDL simultaneously can be explained by an increase in the number of lipoprotein aggregates in the medium.

This investigation thus has shown that desialated and glycosylated LDL can induce accumulation of more intracellular cholesterol than purely glycosylated or purely desialated LDL alone. A combination of glycosylated and desialated LDL, like a combination of these with native LDL, leads to even greater cholesterol deposition in cultures of aortic intimal cells. It was observed previously that blood sera from patients with coronary atherosclerosis and concomitant diabetes induce greater accumulation of intracellular cholesterol than blood sera from patients with coronary atherosclerosis, unaccompanied by diabetes [11]. It can be tentatively suggested that blood of such patients contains both desialated and glycosylated LDL. In that case, it is the synergic action of the two types of modified LDL which leads to massive lipid

deposition in the cells. However, the possibility cannot be ruled out that other types of modified LDL (for example, oxidized) may also potentiate the effect of glycosylated and desialated LDL on intracellular lipid accumulation. In our view, data on the synergic effect of the two types of modified LDL, like data on the increase in atherogenic potential of LDL modified by the two methods, may be one explanation of the high ability of sera from patients with coronary heart disease accompanied by diabetes to stimulate intracellular lipid accumulation.

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EFFECT OF CYTOTOXIC DUSTS ON FORMATION OF ACTIVE FORMS OF OXYGEN BY RAT PERITONEAL MACROPHAGES

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There is at present a steady increase in attention paid to the role of free oxygen radicals in the mechanism of injury to cell structures by dust particles and also in the study of free-radical processes during the development of dust diseases of the lungs [1, 2, 4, 8, 11]. Dust particles induce activation of the "respiratory burst" of phagocytic cells (neutrophils, monocytes, alveolar and peritoneal macrophages) [7, 11], during which the uptake of oxygen and glucose by the cells is increased and active forms of oxygen (AFO) are produced, especially the superoxide anion-radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) [6, 14]. Investigations have shown that the cytotoxic activity of different dusts correlates with the quantity and rate of generation of AFO by the cells [4, 7, 11]. Dust particles can induce AFO formation also by other extracellular pathways:

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