of fetal and adult liver, adult kidney, fetal and adult lung, spleen, intestine, ovary, pituitary, or adult stomach, or in samples of donor, pregnant women's or fetal blood serum, pregnant women's and donor urine, breast milk, or saliva.

For location of the site of PSBG synthesis in the male seminal duct, extracts of the semen-producting and semen-ejaculating organs were examined, as were spermatozoa isolated from the seminal plasma. PSBG was detected only in testicular extract but not in extracts of the other male reproductive organs (Table 4).

The data suggest that a protein characterized by β_2-globulin mobility and a molecular weight of 20 kD is present in early placental, fetal renal, and fetal and adult testicular tissue and is secreted into the amniotic fluid, seminal plasma, and cerebrospinal fluid. Placental cells appear to secrete it into the amniotic fluid, and the placenta seems to be responsible for its presence in the fetal stomach, where this protein might enter with the amniotic fluid. Testicular cells may secrete PSBG into the seminal plasma. The presence of this protein in the cerebrospinal fluid merits more detailed study.

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Effect of Lipoproteins Modified by Lipid Peroxidation on Platelet Aggregation

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Platelets play an important role in the injury to the vascular wall during the development of atherosclerosis. In a number of studies the platelets of patients with hypercholesterolemia have been shown to be hyperactive [3]. It has been suggested that lipoproteins (LP) markedly contribute to the changes in platelet aggregation. Low-density LP (LDL) from the blood of patients with atherosclerosis have been found to enhance the collagen-induced platelet aggregation to a greater degree then LDL from the plasma of healthy donors [10]. Similar results have been obtained for LP from the blood of patients with hypercholesterolemia and hyperglycerolemia. *In vitro* experiments have demonstrated that LDL enhance the platelet response to various aggregation inducers [2,8], as well as being able by themselves to stimulate platelet aggregation [8].

The development of atherosclerosis is attended by the activation of lipid peroxidation (LPO). Oxidized LP have been found in the vascular wall and in the blood of patients with atherosclerosis

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[13]. In addition, oxidized LDL play an important role in the formation of foam cells during atherosclerosis [9]. In this connection the question arises as to the effect of oxidized LP on platelet activity. It has been shown that not only is ADP-induced platelet aggregation enhanced by concentrations of oxidized LDL lower than those of unoxidized LDL, but oxidized LDL per se (in concentrations >0.2 mg/ml) may cause platelet aggregation. Similar results were obtained by Bruckdorfer [6], although the minimal concentration of oxidized LDL required for the inducement of aggregation was determined in his study to be 0.5 mg/ml. In both cases the concentration of LPO products in LDL was markedly higher than that observed *in vivo*.

The aim of the present research was to study the effect of LP on platelet aggregation under conditions of "mild" LDL oxidation, when the degree of oxidation of LDL is the same as that observed *in vivo*. Another goal of the experiments was to compare the effects of native and oxidized LDL on the cells.

MATERIALS AND METHODS

LDL (1.019-1.063 g/cm^3) were isolated from the serum of healthy donors by preparative ultracentrifugation [12]. LDL were dialysed for 18-20 h at 4°C against a 1000-fold volume of 10 mM Tris-HCl (pH 7.4) containing 140 mM NaCl and 7.6 mM KCl. The LDL obtained were stored at 4°C and used within two days.

The LDL concentration was characterized by the phospholipid content [15]. Autooxidation of LP was performed by incubating them at 37°C under aerobic conditions for 4-6 h (with continuous stirring). The degree of oxidation of LDL was assessed according to the accumulation of 2-thiobarbituric acid-reactive products (in μmol malonic dialdehyde (MDA) per gram of phospholipids) [14].

For the isolation of platelets we used venous blood of healthy donors stabilized in a 6:1 ratio with anticoagulant of the following composition (mM): sodium citrate 85, citric acid 71, and glucose 111. Platelet-enriched plasma was obtained by centrifugation of the blood for 15 min at 100 g. Washed platelets were obtained by centrifuging the platelet-enriched plasma for 10 min at 350 g and resuspending the pellet in a buffer of the following composition (mM): NaCl 138, KCl 3, $MgCl_2$ 1, glucose 10, HEPES 10, and $NaH_2PO_4 \times H_2O$ 0.37 (pH 7.4). The following reagents were used in the study: ADP, fibrinogen, and HEPES (Sigma, USA); the rest of the reagents were Russian-manufactured.

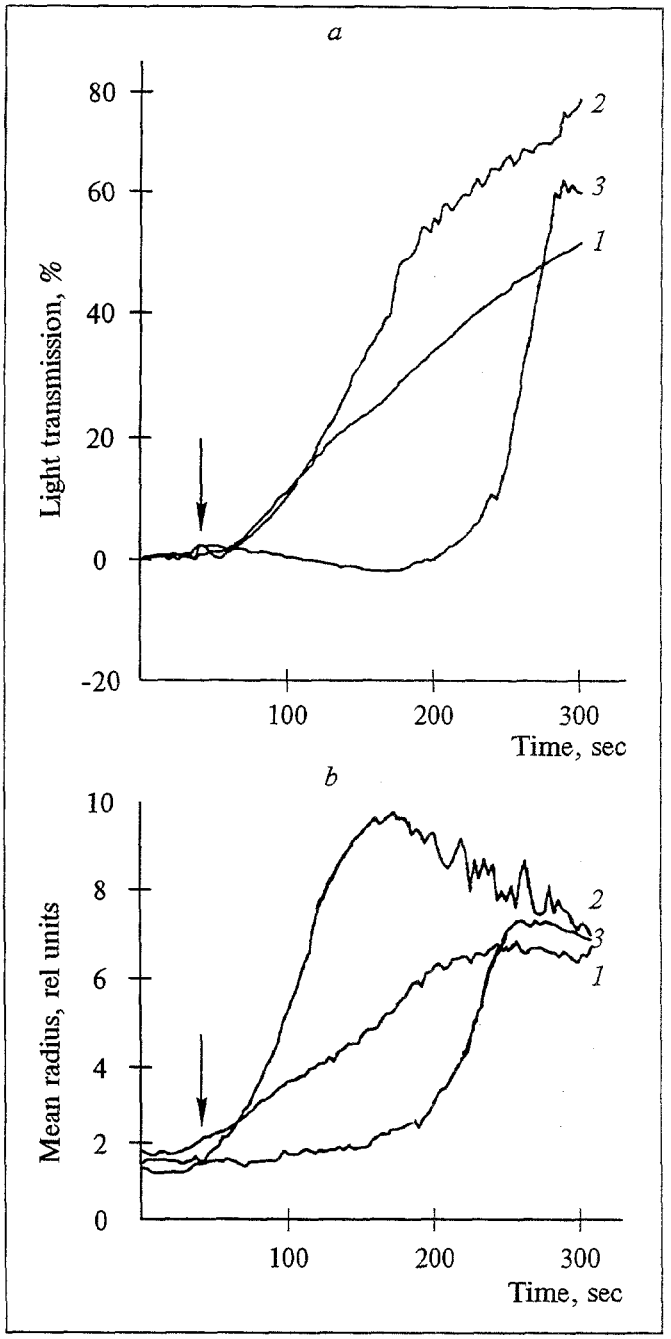

Fig. 1. Light transmission (*a*) and mean radius of aggregates (*b*) in platelet suspension as a function of time of sample incubation. An arrow shows the moment of addition of Ca^{2+}, fibrinogen, and ADP (for curve *1*) in final concentrations of 1 mM, 0.4 mg/ml, and 1 μM, respectively. Experimental conditions: TIRODE buffer, pH 7.36; 3×10^8 cells/ml, 33°C. *1*) 1 μM ADP; *2*) 0.35 mg/ml native LDL, [MDA] = 0.5 μmol/g phospholipids; *3*) 0.35 mg/ml oxidized LDL, [MDA] = 8.3 μmol/g phospholipids.

The platelet concentration in the solution was 3×10^8 cells/ml. The measurements were performed on an automatic analyzer of platelet aggregation (Biola, Moscow). The data were processed with the aid of an IBM PC AT-386. Measurements were performed under the following conditions: 33°C;

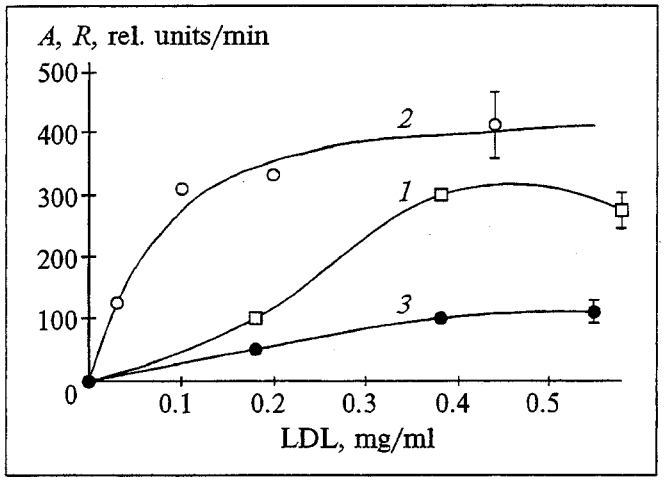

Fig. 2. Maximal rate of change of light transmission (A, *1*, *2*) and maximal rate of change of mean radius of aggregates (R, *3*) in suspension of isolated platelets as a function of concentration of native (*1* and *3*; [MDA] = 0.46 μmol/g phospholipids) and oxidized (*2*, [MDA] = 9.4 μmol/g phospholipids) LDL. Here and in Fig. 3: the experimental conditions are the same as in Fig. 1.

stirring velocity 800 rpm. The platelet suspension (0.3 ml) was placed in an aggregometer cell; 1 mM $CaCl_2$ and 0.4 mg/ml fibrinogen were then added to the sample, and aggregation was induced by the addition of 1 μM ADP in the absence of LDL. Preliminary incubation of LP with the cells lasted 5-10 min.

Each curve in the figures shows the results of one of the experiments. However, similar relationships were obtained for no less than 5 independent experiments. The mean error of the measurements of the aggregation rate does not exceed 10% of the absolute value.

RESULTS

In studies of the effect of LDL on the platelets we analyzed the light transmission of cell suspensions as a function of varied concentrations and degrees of oxidation of LDL. The use of this new platelet aggregation assay provides for simultaneous measurements of the light transmission and of the mean radius of aggregates, which, in a number of cases, yields additional information on cell behavior in the solution [7]. Examples of aggregatograms are presented in Fig. 1: Fig. 1, *a* shows the changes of light transmission in the platelet-containing solution, while in Fig. 1, *b* the corresponding changes of the mean radius of aggregates are presented.

In the absence of inducer the light transmission and the size of aggregates are unchanged. The addition of 1 mM ADP to the solution results in cell aggregation. Moreover, cell aggregation may be induced by LDL. However, the characteristics of the aggregation curves for native and oxidized LDL differ one from another. In the analysis of the aggregation curves we used the following characteristic parameters: 1) the aggregation time, determined as the interval between the initiation of aggregation and the attainment of the maximum light transmission; 2) the aggregation rate as the rate of change of the parameter measured at a specified point (this rate being estimated as the slope of the tangent to the curve). In the following, by "aggregation rate" we mean the maximum rate of change of light transmission for a given curve.

Since the regularities in Fig. 1, *a* are also typical of Fig. 1, *b*, and the relative rate of change of the light transmission is higher than that of the mean radius of aggregates (Fig. 2), the changes of light transmission in the platelet suspension will be principally discussed in the present study.

The parameters of platelet aggregation induced by LPO-modified LDL differ markedly from those of aggregation induced by native LDL. The aggregation times differ most noticeably: in the presence of oxidized LDL no cell aggregation occurs immediately after Ca^{2+} and fibrinogen are added. The changes of light transmission attest to an initial increase in the density of the solution (within 1.5-3 min, this time varying in different experiments), which is associated with the formation of cell microaggregates (since the mean radius of aggregates increases 3-4-fold during this period) (Fig. 1). Longer aggregation times are, as a rule, evidence of a weak effect of the inducer on the cells. At the same time, in comparison with native LDL, oxidized LDL are more potent inducers of aggregation, since the rate of change of the light transmission in the platelet suspension containing oxi-

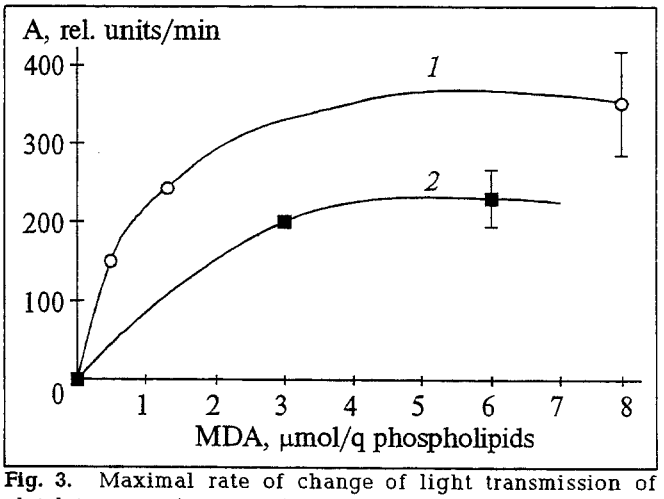

Fig. 3. Maximal rate of change of light transmission of platelet suspension as a function of MDA concentration in LP. *1*) [LDL] = 0.35 mg/ml; *2*) [LDL] = 0.55 mg/ml.

dized LDL is 2-4 times as high as that in the case of native LDL (for equal concentrations of LDL in the solution) (Fig. 2). The long aggregation time which is induced by oxidized LDL may be explained by the specificities of interaction between the LP receptors on the platelet surface and oxidized LDL, this delaying the initiation of aggregation. In addition, platelet aggregation may be partially inhibited by MDA, an LPO product in LDL [4].

The dependence of the maximal rates of change of the light transmission (A) and of the mean radius of aggregates (R) on the LDL concentration is presented in Fig. 2. In our experiments, as well as in those reported by Hassal *et al.* [8], platelet aggregation was found to be induced by native LP. However, the minimal LDL concentration needed to cause aggregation is markedly lower and does not exceed 0.2 mg/ml. An increase in the aggregation rate is observed when the concentration of native LDL is changed in a range of 0-0.4 mg/ml (in different experiments this value does not exceed 0.6 mg/ml). A similar dependence is observed for oxidized LDL, the maximum rate, as a rule, being attained at lower LDL concentrations (0.2-0.4 mg/ml). This is a regular phenomenon, because at equal concentrations of native and oxidized LDL, a higher rate of induced platelet aggregation is characteristic of the latter.

The dependence of the platelet aggregation rate on the MDA concentration in an LDL-containing solution is shown in Fig. 3. The results of two experiments are presented. These findings lead to the conclusion that the maximum rate of change of the light transmission occurs in the platelet-containing samples incubated with weakly oxidized LDL ([MDA] $\leqslant 2$ μmol/mg phospholipids). A further increase in the oxidation products does not lead to any enhancement of the effect (ΔA) observed in the solution. Moreover, we revealed an inhibitory effect of LDL containing [MDA] $\geqslant 20$ μmol/g phospholipids on ADP-induced platelet aggregation. This finding is consistent with other data on the inhibitory effect of oxidized LDL on ADP- and collagen-induced platelet aggregation [5], as well as with data on the inhibitory effect of LPO of LP on collagen-induced platelet aggregation [4].

Our experiments allow us to assert that both native and oxidized LDL are able, even in low concentrations ($\leqslant 0.2$ mg/ml), to induce platelet aggregation. A higher aggregation rate and a long aggregation time are typical of the effect of oxidized LDL on the cells. The increased rate characterizes oxidized LDL as a potent aggregation inducer; an increase of aggregation time may be due either to the presence of LPO products or to specificity of the interaction between oxidized LDL and the cells. A linear dependence between the aggregation rate and the degree of oxidation of LDL is observed to an MDA concentration of [MDA]=3-4 μmol/g phospholipids. Further LP oxidation just leads to a slight change in the rate of induced aggregation.

Thus, oxidized LDL are more effective inducers of platelet aggregation than native LDL, this possibly playing an important role in thrombus formation during atherosclerosis.

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