In our opinion the increase in the rate of glucose synthesis from noncarbohydrate compounds in myocardial infarction is due to stress, as a result of which the concentration of hormones with powerful gluconeogenic properties (adrenalin, glucocorticoids, glucagon, somatotropin, and so on) rises. The marked stimulation of gluconeogenesis in myocardial infarction can be regarded as a compensatory-adaptive mechanism, aimed at supplying the additional quantity of glucose in order to maintain homeostasis of the affected organ and of the body as a whole.

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CHEMILUMINESCENCE OF INDIVIDUAL HUMAN BLOOD SERUM FRACTIONS ACTIVATED BY FERROUS IONS

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KEY WORDS: serum lipoproteins; chemiluminescence.

Measurement of the parameters of chemiluminescence (CL) of the blood serum is used as an additional diagnostic test in several diseases [4, 12]. For instance, the state of patients with various surgical diseases can be assessed from changes in Fe^{++}-activated CL of whole serum [3, 9]. However, the causes of these changes in CL have not yet been finally explained [3]. This accounts for the special importance of the study of the contribution to total serum CL made by its individual fractions [2, 7, 11].

The investigation described below showed that when changes in CL of whole blood serum are interpreted not only the quantity of apoB-containing lipoproteins (LP), but also changes in the levels of individual proteins and of high-density LP must be taken into consideration.

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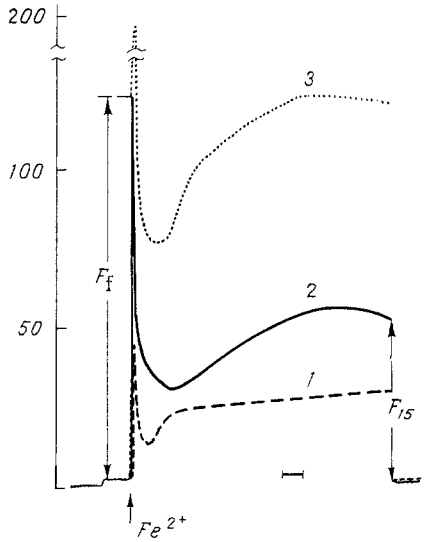

Fig. 1. Chemiluminescence of apoB-containing LP (1), serum (2), and residue with d > 0.163 g/ml (3). F_f and F_{15}) intensity of "fast" and "slow" flashes of CL respectively 15 min after addition of 2.5 mM Fe^{++} (arrow). Cholesterol concentration in serum 164 mg%, in apoB-containing LP and in residue — 74 and 89 mg% respectively. Volume of serum added to cuvette 0.2 ml. Ordinate, intensity of CL (in relative units). Calibration: 1 min.

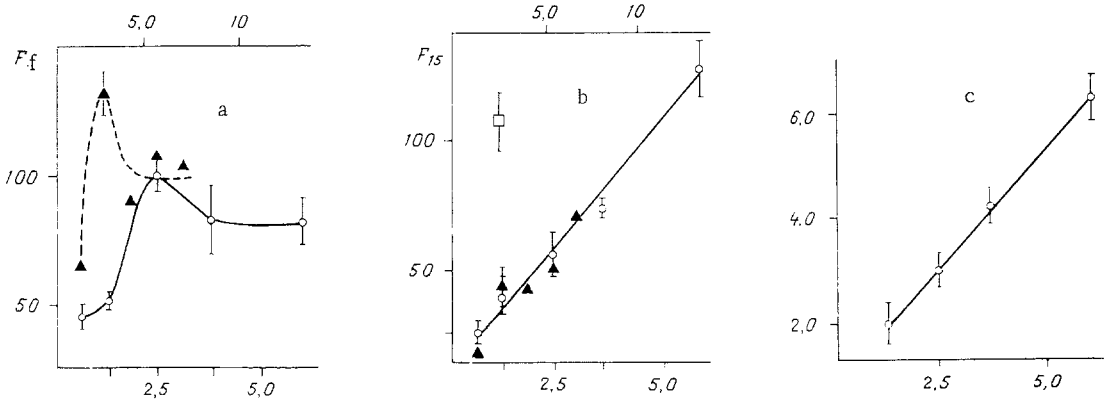

Fig. 2. Changes in intensity of "fast" flash (a) and of "slow" flash (b) of CL and MDA concentration (c) depending on cholesterol level in serum and in fraction of apoB-containing LP. Circles — apoB-containing LP, triangles serum, square — residue (cholesterol concentration 1.61 mg%). Abscissa: top — cholesterol concentration (in mg%) in serum, bottom — level of apoB-containing LP estimated as cholesterol (in mg%); ordinate: a, b) intensity of CL of "fast" and "slow" flashes respectively (in relative units); c) MDA concentration (in μmoles/ml).

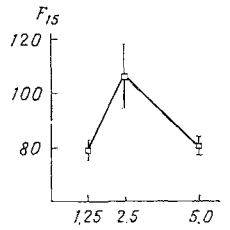

Fig. 3. Effect of Fe^{++} on intensity of "slow" flash of CL in fraction free from apoB-containing LP. Cholesterol concentration in sample 1.61 mg%. Abscissa, Fe^{++} concentration (in mM); ordinate, F_{15} (in relative units).

EXPERIMENTAL METHOD

ApoB-containing LP or low- and very low-density LP (fraction I, d < 1.063 g/ml) were isolated from serum of healthy blood donors by ultracentrifugation [15]. After removal of the bouyant apoB-containing LP, part of the serum containing proteins and high-density LP (fraction II, d > 1.063 g/ml) remained in the centrifuge tube. The isolated fractions were kept at 4°C and used in the first week after obtaining the serum. To study CL, the serum or isolated fractions, diluted 1:50 with phosphate buffer, containing 53.4 g Na_2HPO_4 and 13.3 g KH_2PO_4, pH 7.4 (from Radiometer, Copenhagen), was treated rapidly with $FeSO_4$ solution. CL was measured at 20°C on an apparatus described previously [9]. Cholesterol was determined in the isolated fractions by means of a Technicon AAII automatic analyzer and lipid peroxidation (LPO) activity was estimated by the reaction with 2-thiobarbituric acid [8].

EXPERIMENTAL RESULTS

Typical recordings of chemiluminescence of whole serum and its subfractions in the presence of an equal concentration of activator are shown in Fig. 1; the ratio of Fe^{++} to the individual serum components was the same, moreover, as during investigation of native blood serum. As Fig. 1 shows, the character of the kinetics of CL for serum and its fractions was similar, but the intensity of the "slow" flash for fraction II was about 2.5 times greater than for fraction I or serum. Meanwhile a decrease was observed in the intensity of the "fast" flash (F_f) for apoB-containing LP compared with that for serum, just as was observed previously [7].

The character of the change in the "fast" flash of luminescence depending on the content of serum or fraction I can be judged on the basis of data given in Fig. 2a. In Fig. 2 the change in the parameters of serum CL was compared with the same for LP in concentrations and proportions observed in whole blood. For example, after addition of 2.5 mM Fe^{++} the intensity of the "fast" flash for 0.2 ml of whole serum was 132 ± 9 relative units, whereas for apoB-containing LP isolated from the same volume of plasma, F_f = 51 ± 3 relative units (Fig. 2a). It will be clear from Fig. 2 that the intensity of the "fast" flash was not a linear function of the concentration of oxidation substrate (serum or fraction I), although accumulation of one LPO product, namely malonic dialdehyde (MDA), with an increase in substrate concentration was linear in character (Fig. 2c). This state of affairs, like the wide scatter of the data (poor reproducibility of the results), prevent the intensity of the "fast" flash from being used as a reliable indicator of the intensity of LPO.

The change in the "slow" flash (F_{15}), measured 15 min after addition of Fe^{++}, is far more informative in this respect. The data given above show that with an increase in the content of apoB-containing LP in the reaction mixture F_{15} rose in a straight line, as also

did the MDA concentration (Fig. 2c). The intensity of LPO in LP can thus be judged from the intensity of the "slow" flash of CL.

The fact will be noted that the intensity of the "slow" flash of fraction II, containing high-density LP and free proteins, was about 2.5 times greater than for the same proportions of serum or low-density LP (fraction I, Fig. 2b). The intensity of this flash for fraction II and for a different concentration of activator was greater than for serum of fraction I in the same relative concentrations (Fig. 3). It must be pointed out that 15 min after addition of 2.5 mM Fe^{++} the intensity of CL of the two fractions became identical only if the concentration of fraction I was approximately 5 times greater than that of fraction II. The results are evidence that CL of serum is not the simple sum of the CL of its individual parts — fractions I and II.

It was shown previously that CL of blood serum in the presence of Fe^{++} is due to LPO, mainly of lipoproteins [2, 7]. Among the main groups of human serum LP (very low-, low-, and high-density), it is the low-density LP which have the greatest luminescence [7]. It was observed in the same investigation that CL of whole serum is not the simple sum of the fluorescence of its individual components. Besides low-density LP, high-density LP also possess CL, and in the serum of dogs, rats, and rabbits they are the principal "providers" of quanta of light [1, 2, 10].

It was thus found that when apoB-containing LP are separated from serum proteins and high-density LP ($d > 1.063$ g/ml) CL is activated in the presence of Fe^{++} by several times compared with CL of whole serum or low-density LP. This may be due to the fact that certain lipid-soluble inhibitors of CL, for example, α-tocopherol, most of which is bound with low-density lipid-protein particles, are removed from the apoB-containing LP as a result of ultracentifiguration [13, 14]. The results of the present investigation indicate that changes in chemiluminescence of whole serum under pathological conditions must be interpreted with caution: attention must be paid not only to the content of low-density LP in the serum [5, 6, 11], but also to changes in the levels of individual proteins and subfractions of high-density LP ($d > 1.063$ g/ml).

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MECHANISMS OF THE HARMFUL ACTION OF FLUORESCENT DYES ON THE RETINA

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KEY WORDS: retina; fluorescent dyes; singlet oxygen; lipid peroxidation; oligomerization of rhodopsin; thermostability of rhodopsin; antioxidants.

The method of fluorescence angiography is widely used in ophthalmologic practice [14, 2]. It has recently been shown that angiographic investigations with fluorescent dyes may be associated with injury to the retina, especially if high-intensity light sources are used [3, 5].

To overcome the limitations imposed on the method of fluorescence angiography by these side effects, their mechanisms must be elucidated. It can be tentatively suggested that the photic injury to the retina produced by fluorescent dyes is based on a phenomenon of photosensitized generation of singlet oxygen ($*O_2$) and subsequent accumulation of lipid peroxidation (LPO) products, as has been demonstrated recently in the membranous structures of the retina [7, 4], in accordance with the scheme:

$$S + h\nu \longrightarrow S^*$$
$$S^* + O_2 \longrightarrow S + *O_2$$
$$*O_2 + RH \longrightarrow ROOH,$$

where S and S* denote the basic and excited state respectively of the dyes, $*O_2$ the singlet-excited state of O_2, and RH and ROOH represent molecules of exogenous substrates and their peroxidation products respectively.

In connection with the facts described above an investigation was undertaken to study mechanisms of the light-induced injurious action of a fluorescent dye (methylene blue — MB) — on the retina and its membranous structures in different model systems: in a suspension of the outer segments of the rods (OSR) of the frog's retina and on preparations of the isolated frog retina, and also *in vivo*, on the retina of chinchilla rabbits.

EXPERIMENTAL METHOD

The OSR fraction was obtained from the retinas of dark-adapted frogs (*Rana temporaria*) by centrifugation in a sucrose density gradient by methods described previously [1, 10]. Criteria of purity of the OSR suspension varied from 2.1 to 2.4 ($A_{280/500}$). Thermal denaturation of rhodopsin was determined and the thermodynamic parameters calculated by the method in [9]. Proteins of OSR were separated by gel-electrophoresis in Na-SDS-polyacrylamide gel [11]. Protein was determined by Lowry's method [12]. Lipids were isolated by the method in [8]. The level of LPO products was determined spectrophotometrically by measuring absorption at 232 nm, characteristic of diene conjugates [6]. The content of carbonyl compounds — secondary LPO products — was determined by the reaction with 2-thiobarbituric acid [14]. The isolated frogs' retinas and rabbits' eyes were illuminated by means of the illuminating system of the MPS50 L spectrophotometer, with a high-pressure xenon lamp. MB was excited by a photic flux with $\lambda \geqslant 620$ nm (0.3 mW/cm^2) and retinal by a photic flux with $360 \leqslant \lambda \leqslant 380$ nm, the spectral regions being isolated by cutoff filters. Preliminary bleaching of rhodopsin in the

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