# CHEMILUMINESCENCE OF APO-B-CONTAINING LIPOPROTEINS IN RABBITS WITH EXPERIMENTAL HYPERCHOLESTEREMIA

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KEY WORDS: chemiluminescence; hypercholesteremia; lipid peroxidation; mammalian blood.

The problem of the early diagnosis of hypercholesteremia (HCE) is currently in the forefront in the fight against atherosclerosis and its complications [3]. Intensification of lipid peroxidation (LPO) in the blood serum has been found in patients with atherosclerosis [5]. The simplest and most informative method of investigation of LPO in biological objects is chemiluminescence [1, 9]. Great importance in the development of atherosclerosis is attached to the low-density lipoproteins of the blood serum [4], which are the main constituents of the apo-B-containing lipoproteins (apo-B-LP) [6].

The object of this investigation was to study changes in chemiluminescence of apo-B-LP induced by bivalent iron in the blood serum of rabbits with experimental HCE.

## EXPERIMENTAL METHOD

Experiments were carried out on 41 rabbits weighing 3-4 kg. The animals were divided into three groups: 31 rabbits of group 1 were fed with cholesterol in a dose of 0.25 g/kg body weight daily for 20 days (the cholesterol was rubbed into the cabbage leaves on which the animals were fed); group 2 consisted of five rabbits fed with cholesterol in a dose of 0.25 g/kg daily for 12 weeks, then transferred to a cholesterol-free diet; group 3 was the control (five rabbits). Every 4 weeks samples of blood were taken from the marginal vein of the animals' ear for biochemical and biophysical investigation.

Chemiluminescence was studied on an apparatus for recording very weak luminescence [8], in conjunction with the FEU-39A photoelectronic multiplier. To measure the chemiluminescence of apo-B-LP it was isolated as follows. To 0.2 ml blood serum 2 ml of a 0.28% solution of $CaCl_2$ and 0.2 ml of a 1% solution of crystalline heparin were added. After incubation for 4 min at 20° C the resulting suspension was centrifuged for 5 min at 3000g. The residue was diluted in 1 ml 0.85% NaCl and transferred to the cuvette of the instrument, where 8 ml of phosphate buffer, pH 7.4 (100 mM KCl, 20 mM $KH_2PO_4$) was added. A flash of chemiluminescence appeared and its intensity was estimated relative to the light sum of the slow flash, which was calculates as the area beneath the curve from the beginning of the slow flash until chemiluminescence of maximal intensity was reached.

Total cholesterol [12], apo-B-LP [10], and cholesterol in apo-B-LP [11] were determined in the blood serum. The relative proportions of the lipoprotein fractions were determined by disc electrophoresis in polyacrylamide gel.

## EXPERIMENTAL RESULTS

As Table 1 shows, during the development of experimental atherosclerosis in rabbits HCE and dissociation between changes in the lipoprotein fractions were observed: a decrease in the relative proportion of high-density lipoproteins in the blood serum and an increase in the relative proportion of low-density lipoproteins and also of apo-B-LP, which is characteristic of the development of atherosclerosis [3. 4]. At the same time there was an increase in the

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TABLE 1. Changes in Chemiluminescence of Apo-B-LP and Some Parameters of Lipid Metabolism in Experimental HCE (M ± m)

| Experimental conditions | Number of animals | Chemiluminescence, relative units | Apo-B-LP, mg/100 ml | Cholesterol in apo-B-LP, mg/mg | Content of lipoproteins, % | |
|---|---|---|---|---|---|---|
| | | | | | low density | high density |
| Initial state | 41 | 54±11 | 435±57 | 0,037±0,006 | 22,5±2,03 | 31,8±3,17 |
| ACE | | | | | | |
| 4 weeks | | 152±22[a] | 656±88 | 0,161±0,015[a] | 26,9±3,07 | 15,2±1,63[a] |
| 8 " | | 195±15[a] | 835±65[a] | 0,173±0,010[a] | 21,3±3,30 | 7,1±1,02[a] |
| 12 " | | 206±27[a] | 884±93[a] | 0,216±0,012[a] | 32,7±3,56 | 3,7±0,54[a] |
| 16 " | | 201±24[a, b] | 714±71 b | 0,203±0,012[a, b] | 35,6±1,10[a, b] | 4,8±0,84[a] |
| 20 " | | 216±13[a, b] | 1017±91[a, b] | 0,188±0,019[a, b] | 55,2±10,3[a] | 5,1±0,20[a, b] |
| After change of diet (4 weeks) | | 181±33[a, b] | 595±71 b | 0,190±0,051[a, b] | 24,5±2,28 | 6,1±0,63[a] |
| Control: | | | | | | |
| 16 weeks | | 48±2 | 236±75 | 0,065±0,004 | 19,9±2,64 | 7,0±1,10[a] |
| 20 " | | 37±10 | 424±61 | 0,066±0,005 | 44,0±6,67[a] | 14,3±2,34[a] |

Legend. n = 41 for initial level, n = 31 for HCE, n = 5 for control level, n = 5 for animals with HCE of 12 weeks' duration followed by change of diet for 4 weeks. a) $P < 0.05$ compared with initial level, b) $P < 0.05$ compared with corresponding time of keeping of control animals; for group of rabbits with change of diet, comparison with control animals at the 16th week of keeping.

concentration of cholesterol in apo-B-LP. During cholesterol feeding of the rabbits **significant changes** took place in their lipid metabolism with the onset of HCE, evidence that the animals developed experimental atherosclerosis.

A change in the intensity of chemiluminescence of apo-B-LP took place parallel to the changes in lipid metabolism. Chemiluminescence increased statistically significantly in the 4th week of development of atherosclerosis, and like the disturbance of lipid metabolism, it reached its maximal intensity in the 8th week, after which it remained high **without any significant changes.** Chemiluminescence of apo-B-LP in rabbits with HCE thus increased, reflecting the development of experimental atherosclerosis.

Changes in the chemiluminescence of apo-B-LP were also evidence of elevation of the LPO level in apo-B-LP during the development of experimental atherosclerosis, for chemiluminescence initiated by bivalent iron reflects the state of LPO in apo-B-LP but, at the same time, it directly depends on the concentration of low-density lipoproteins in the cuvette [2, 7]. Accordingly it could be expected that with an increase in the apo-B-LP content in the test sample the chemiluminescence of apo-B-LP was also increased in the corresponding proportion. However, with an increase in the apo-B-LP concentration in the blood serum by 1.51 times in the 4th week of HCE, chemiluminescence increased by 2.77 times. This indicates that the increase in chemiluminescence of apo-B-LP was due not to a simple increase in the apo-B-LP concentration in the blood serum, but to an increase in the intensity of LPO in them. The possibility cannot be ruled out that it is the intensification of LPO in apo-B-LP which plays the leading role in the development of atherosclerosis and contributes to the more rapid penetration of cholesterol into cell membranes in the intima of blood vessels.

During the development of experimental atherosclerosis there is thus an increase in the intensity of chemiluminescence of apo-B-LP initiated by bivalent iron, reflecting the **intensification of** LPO in apo-B-LP in atherosclerosis. The increase in chemiluminescence in the early stages of experimental atherosclerosis can be used for the early diagnosis of atherosclerosis under both experimental and clinical conditions.

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# CHANGES IN THE NEPHRON AND NEUROENDOCRINE APPARATUS OF THE KIDNEYS AFTER INJECTION OF SALMONELLA ENDOTOXIN

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One of the most important factors in the pathogenesis of alimentary toxic infections is endotoxemia. Many aspects of the action of salmonella endotoxin that lead to disturbance of homeostatis have already been established. For instance, endotoxin is known to damage the endothelium, which leads to the release of tissue thromboplastin [6], activated prothrombin, and causes intravascular blood clotting and also stimulates the production of prostaglandin E [5]. Besides the hemodynamic disturbances connected with intravascular coagulation, it also leads to the development of trophic changes, edema, and cellular infiltration of organs [1, 3, 6]. The most marked changes arise in the kidneys. Previous investigations have not been aimed at the discovery of the intimate mechanisms of action of salmonella endotoxin and have been mainly descriptive in character. In particular, no morphological evidence has been obtained in support of the fact that endotoxin stimulates the synthesis of prostaglandin E, which is known to be produced by the interstitial cells (IC) of the renal medulla.

The object of this investigation was an ultrastructural analysis of the effect of salmonella endotoxin on different parts of the nephron and on the neuroendocrine apparatus of the kidneys.

## EXPERIMENTAL METHOD

Experiments were carried out on 12 rabbits weighing 2.5-3 kg, into which the endotoxin of *Salmonella typhimurium*, purified by Boivin's method, was injected intravenously in a dose of 2 mg/kg. The animals were killed (six at a time) 3 and 24 h later by injection of air into the auricular vein. The body weight of the rabbits 24 h later had fallen on average by 500 g because of diarrhea. The control group consisted of six rabbits. Pieces of renal cortex and medulla were fixed in 1% $OsO_4$ solution, dehydrated in alcohols of increasing strength, and embedded in Araldite. Light-optical investigation of the material and counting of the lipid granules in IC (50 cells from each animal) were carried out on semithin Araldite sections stained with methylene blue—azure II—fuchsin. To characterize activity of the juxtaglomerular apparatus (JGA) a quantitative method of assessment of granule formation on electron micrographs was used; our previous investigations [2] showed that this method objectively reflects the degree of activity of the JGA. Guided by results obtained by other workers [7], granules of three types and also intermediate forms, depending on the density of the sub-

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