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# KINETICS OF LIPID PEROXIDATION IN THE ISOLATED SURVIVING RAT LIVER

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The kinetics of accumulation of lipid peroxidation products (primary products, hydroperoxides; secondary products, malonic dialdehyde and "fluorescent pigments") in the isolated unperfused and previously perfused liver was investigated during aerobic incubation. During survival intensive accumulation of primary, secondary, and end products of lipid peroxidation was shown to take place, and its kinetics is extremal in character. The velocity of this process in the unperfused liver is much higher than in the previously perfused liver.

KEY WORDS: survival of the liver; lipid peroxides; hydroperoxides; diene conjugation; malonic dialdehyde.

One of the most important factors in the destruction of biological cell structures is free-radical oxidation of unsaturated fatty acids of the phospholipids of their biomembranes [1, 2]. In vivo, in normally functioning organisms, this process is always taking place but at a low level and under steady-state conditions. This is achieved through the presence of a complex multicomponent system in the tissues: biological antioxidants, enzymic protective systems, quenchers of singlet-excited oxygen, and systems regulating the supply of oxygen to membrane phospholipids [3, 8].

In isolated tissues during perfusion favorable conditions are created for lipid peroxidation (LPO) as a result of accumulation of $H^+$, a shift of the redox equilibrium toward reduction, disturbance of the bond between protein and lipid, accumulation of nonhemin iron, and a decrease in the content of biological antioxidants. This process may perhaps lie at the basis of the pathogenesis of ischemic lesions [2-5], for products of LPO can cause an increase in membrane permeability, oxidation of reduced thiols, inactivation of membrane-bound enzymes, polymerization of certain amino acids and proteins, and disintegration of cell metabolism [9, 13]. A detailed investigation of LPO in isolated tissues during survival could provide information on the mechanism of irreversible tissue damage after death and for the study of optimal methods of conservation of organs and tissues.

The object of this investigation was to study the kinetics of accumulation of primary molecular products (hydroperoxides and diene conjugates) and of secondary products of LPO (malonic dialdehyde, MDA) and of intermolecular cross-linkages between them and aminophosphatides ("fluorescent pigments") in the isolated unperfused and perfused rat liver during survival at 37°C for 24 h.

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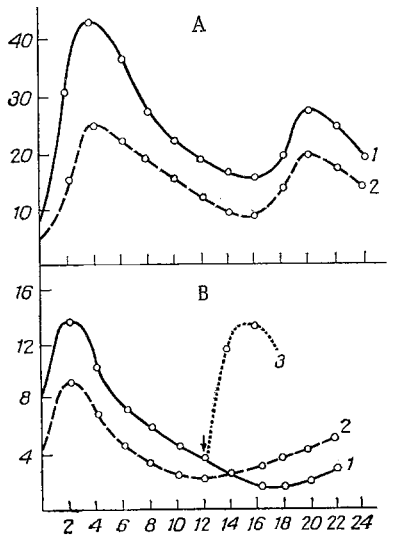


Fig. 1. Kinetics of accumulation of hydroperoxides (A) and diene conjugates (B) in isolated liver during incubation at 37°C. Abscissa, incubation time (in h); ordinate, total hydroperoxides and diene conjugates, in nmoles/mg lipids. 1) Unperfused liver; 2) perfused liver; 3) after addition of $FeSO_4$ and ascorbate followed by bubbling oxygen through for 20 min (arrow indicates time of addition).

## METHODS

Experiments were carried out on the liver of Wistar albino rats. The animals were killed by decapitation and the liver removed. In the experiments without perfusion the liver was placed in a Petri dish in Ringer's solution and incubated at 37°C for 24 h. In the experiments with perfusion, after dissection the liver was perfused for 1.5 h with physiological saline at 15°C [11] and then incubated under the above conditions. Dissection, perfusion, and incubation were carried out with sterile precautions. Lipids were removed from the liver by the method in [12]. The content of total hydroperoxides was determined on the ON-104 square-wave polarograph. The concentration of diene conjugates was assessed spectrophotometrically from absorption in the 232 nm region [15]. The MDA content was determined by the method of Zalkind and Tappel with certain modifications [4]. Accumulation of fluorescent products was estimated from the intensity of fluorescence with emission maximum in the 435-450 nm region [10].

## RESULTS

During incubation of the isolated unperfused liver at 37°C accumulation of primary, secondary, and end products of LPO took place. The kinetics of accumulation of LPO products was extremal in character.

The content of total hydroperoxides increased appreciably during the first 4 h of survival to reach the first maximum. Next followed a decrease in the hydroperoxide content, until the 16th hour of survival. Accumulation of hydroperoxides then increased once again, to reach a second maximum after 18 h, although it was only two-thirds as high as the first maximum (Fig. 1A). The content of conjugated dienes, by contrast with total hydroperoxides, increased only during the first 2 h of survival, then decreased throughout the rest of the experiment (Fig. 1B).

The change in the MDA content during postmortem survival obeyed the same relationship as the content of total hydroperoxide; a sharp increase in MDA during the first 6 h of sur-

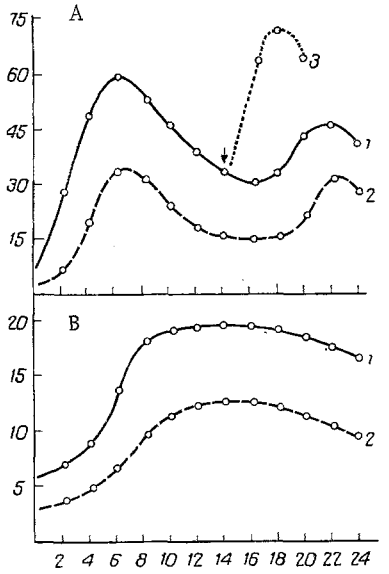


Fig. 2. Kinetics of accumulation of malonic dialdehyde (A) and fluorescent pigments (B) in isolated liver during incubation at 37°C. Ordinate, nmoles MDA/g tissue (A), intensity of fluorescence in relative units (B). Remainder of legend as in Fig. 1.

vival, followed by a decrease in the MDA content until 16 h, and a fresh increase in MDA accumulation until 20 h of survival (Fig. 2A).

The kinetics of accumulation of fluorescent products formed by interaction between MDA and aminophosphatides differed from that found for total hydroperoxides and MDA. The intensity of fluorescence increased slowly during the first 4 h of the experiment during postmortem survival. After this period there was a sharp increase in the concentration of fluorescent products and the intensity of fluorescence came out on a plateau by the 8th hour of survival. During the next 10 h the intensity of fluorescence showed little change, and not until 18 h of survival did a gradual decrease begin (Fig. 2B).

The kinetics of accumulation of LPO products in the perfused liver was in general similar to the kinetics in the unperfused liver. In this case, two maxima also were observed on the curves of accumulation of total hydroperoxides and MDA, and one maximum on the curve of diene accumulation; the intensity of fluorescence rose slowly and came out on a plateau 10 h after the beginning of the experiment, and thereafter showed only a negligible change until the end of the experiment. However, a characteristic feature of the perfused liver was that during the first few hours of survival the increase in the content of LPO products was small, and the overall velocity of lipid peroxidation was much lower for the experiment as a whole than in the case of the unperfused liver. This can evidently be explained on the grounds that during perfusion primary LPO products and also certain compounds with a prooxidative action (hemin compounds, metals of variable valency) were rinsed out of the liver.

Analysis of the kinetics of accumulation of LPO products in the isolated liver depending on the time of incubation showed a decrease in the velocity of LPO after the first few hours of survival. The concrete mechanism of this phenomenon is not yet clear. Nevertheless it can definitely be stated that slowing of the intensity of LPO in the late stage of survival (6-16 h) was not due to substrate exhaustion. Experiments to study changes in the fatty acid composition of the lipids depending on the time of incubation showed that over the period of 18 h of survival of the liver at 37°C, of the polyunsaturated fatty acids, the content of arachidonic and docosahexaenoic acids decreased by only 14 and 23% respectively. Addition of $FeSO_4$ and ascorbate, followed by aeration with $O_2$ again led to a marked increase in the intensity of LPO (Figs. 1B and 2A).

According to the available data, the fall in the MDA level was due to a decrease in polyunsaturated lipids, an increase in the intensity of decomposition of MDA, interaction between them and amino groups of amino acids, proteins, and aminophospholipids, and finally, a condensation reaction [9, 13]. Considering that after the first few hours of survival the overall velocity of LPO was reduced, and that the decrease in the MDA content coincided with an increase in the intensity of fluorescence, it can be postulated that the decrease in MDA was due to intensification of its interaction with aminophospholipids and proteins.

Investigation of the kinetics of LPO thus shows that intensive accumulation of primary, secondary, and end products of LPO takes place in the isolated liver during aerobic incubation in the early period of survival. The velocity of LPO in the isolated perfused liver during incubation was much lower than in the unperfused liver. This must be taken into account when steps are taken to prevent ischemic damage to isolated organs and tissues.

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