# INTENSIFICATION OF PEROXIDATION AND CHANGES IN COMPOSITION OF LIPIDS IN THE HOMOGENATE AND SUBCELLULAR FRACTIONS OF ISCHEMIZED LIVER

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Circulatory arrest in the kidney, heart, limbs, and brain is accompanied by the accumulation of lipid peroxidation (LP) products [2, 5, 7-9, 14] and by a decrease in their antioxidative activity (AOA) [1, 9]. The mitochondria are the structures most sensitive to ischemic injury. The endoplasmic reticulum and lysosomes are injured to a lesser degree, and the cell nucleus is least sensitive to ischemia [4]. Since intracellular organelles, which differ in their lipid composition, contain different quantities of unsaturated fatty acids (UFA), LP processess in their membranes may take place at different intensities, and the composition of the lipids must undergo considerable changes in the course of peroxidation.

The object of this investigation was to study LP processes in the homogenate and mitochondrial, microsomal, and nuclear fractions of rat liver after different periods of warm ischemia, and also to examine correlation between the intensity of LP reactions and lipid composition.

## EXPERIMENTAL METHOD

Experiments were carried out on 242 August rats. Ischemia was induced under intraperitoneal hexobarbital anesthesia by clamping the vascular bundle of a hepatic lobule for 15, 30, 60, and 120 min, after which the lobule was excised and immersed in cold physiological saline. Subcellular fractions were isolated by differential centrifugation [11, 13]. Lipids were extracted by Folch's method [10]. The intensity of peroxidation was judged from the value of AOA of the lipids, determined on a methyl oleate model [3], and the peroxide concentration, determined iodometrically [6]. Lipid composition was studied by two-dimensional thin-layer chromatography on silica-gel. The various phospholipid fractions were determined as inorganic phosphate [15]. The protein concentration was measured by the biuret reaction.

## EXPERIMENTAL RESULTS

The initial concentration of lipid peroxides in the homogenate and mitochondrial and microsomal fractions of intact rat liver was very low (Fig. 1). After ischemia for 15 min the level of lipid peroxides in the homogenate was increased by 13 times, in the mitochondrial fraction by 10 times, and in the microsomal fraction by 7 times, but later their concentration fell somewhat, so that after ischemia for 2 h it was 6 times higher than initially in the homogenate, 4 times higher in the mitochondrial fraction, and 5 times higher in the microsomal fractions. This effect may be due both to a decrease in the rate of formation of lipid peroxides and to their decomposition or their participation in processes of further oxidation.

In the course of ischemia of the liver, AOA of lipids of the homogenate and nuclear and microsomal fractions fell steadily. In the mitochondrial fraction, after ischemia

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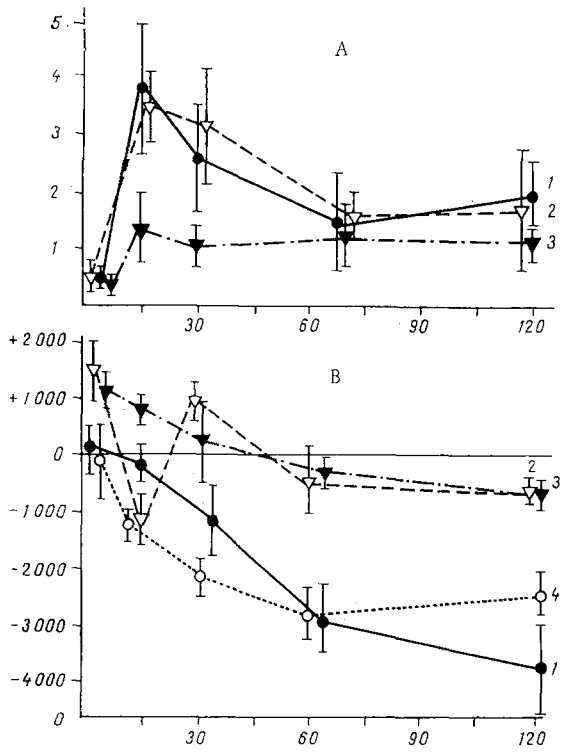


Fig. 1

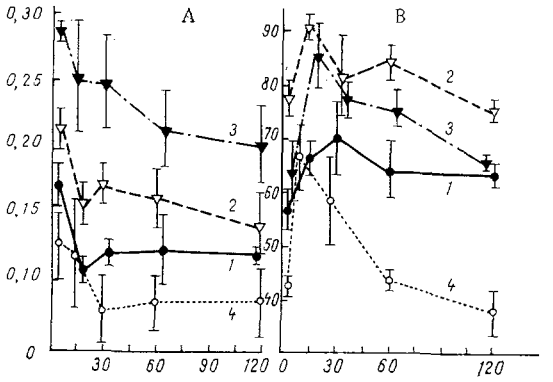


Fig. 2

Fig. 1. Peroxidation of lipids in homogenate and in subcellular fractions of ischemized liver. Abscissa, duration of ischemia (in min). A) Changes in lipid peroxide concentration. Ordinate, concentration of lipid peroxides (in neq/mg). 1) Homogenate, 2) mitochondrial fraction, 3) microsomal fraction. B) Changes in antioxidative activity of lipids. Ordinate, AOA (in ml·h/g). 1-3) As in A. 4) Nuclear fraction.

Fig. 2. Changes in content of lipids and phospholipids in homogenate and subcellular fractions of ischemized liver. A: ordinate, content of total lipids (in g/g protein); ordinate, content of total phospholipids (in % of total lipids). Remainder of legend as in Fig. 1.

for 15 min there was a sharp fall in AOA of lipids, and after 30 min a tendency was observed for a return to the original level (Fig. 1B). This tendency could evidently be linked with redistribution of antioxidants between subcellular particles as the result of the adaptive reaction of the cells. However, in later stages of ischemia, as a result of exhaustion of the intracellular reserves of antioxidants, AOA of the mitochondrial lipids fell to negative values. These negative values of AOA indicate that the concentration of products stimulating LP processes in the lipids was higher than the level of natural antioxidants.

The concentration of total lipids in the homogenate, nuclei, mitochondria, and microsomes after ischemia for 2 h was reduced by one-third (Fig. 2). The total phospholipid content relative to protein also fell gradually during ischemia, but relative to total lipids it increased in the early periods of ischemia (15-30 min), evidently because of the faster oxidative destruction of neutral lipids compared with phospholipids at these periods of ischemia. After 1-2 h the ratio between the content of phospholipids and that of total lipids returned to its original level. At these stages of ischemia the rate of oxidation of phospholipids was probably equal to the rate of oxidation of neutral lipids.

The dynamics of the concentration of individual phospholipids in the homogenate and subcellular fractions during ischemia depended on the degree of their unsaturation and on their role in LP reactions. The concentration of cardiolipin, composed to the extent of about 90% of UFA [12], fell after ischemia for 1 h in the mitochondria by 3.5 times and in the nuclei and homogenate by 2-3 times (Fig. 3). Conversely, the concentration of sphingomyelin, containing only saturated fatty acids, showed no significant change and in the late stages of ischemia had a tendency to rise relative to other phospholipids. The concentrations of phos-

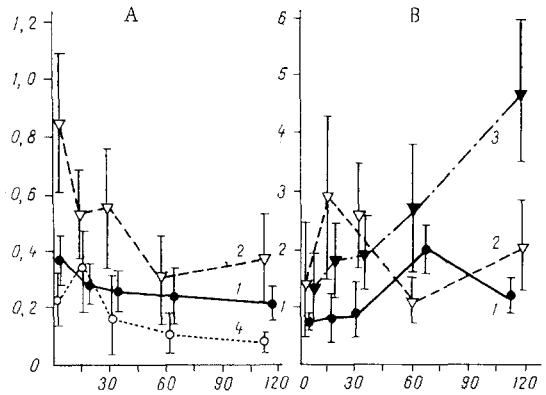


Fig. 3. Changes in composition of phospholipids in homogenate and subcellular particles of ischemized liver. A: ordinate, content of cardiolipin (in mg/g protein); ordinate, content of lysophosphatides (in % of lipid phosphorus). Remainder of legend as in Fig. 1.

phatidylcholine and phosphatidylethanolamine fell gradually in all subcellular fractions studied throughout the period of ischemia. Lysophosphatides accumulated relative to the content of total phospholipids in lipids of the homogenate and mitochondria (Fig. 3). In the microsomes, their concentration after ischemia for 2 h was increased by 3.6 times, which could lead to an increase in membrane permeability.

Marked activation of LP, correlating with changes in the composition of lipids and phospholipids in the liver cells and, in particular, in the mitochondrial fraction of the liver, is in harmony with the low resistance of the liver as a whole, and in particular, of the hepatic mitochondria to ischemia.

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