# DECOMPOSITION OF $H_2O_2$ BY HUMAN LENSES AFFECTED BY CATARACT

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The hydrogen peroxide ($H_2O_2$) concentration in the human aqueous humor is 25 μM, but when cataract develops the $H_2O_2$ level in the aqueous rises significantly, to reach 660 μM in some cases [6]. Considering that the lens, which is an avascular tissue, carried on its metabolism entirely through direct contact with the aqueous in the anterior chamber, it can be postulated that cumulation of $H_2O_2$ in the aqueous constitutes a real threat to oxidative injury of the lenticular membranes and of the protein groups of the lens. However, the problem of the causes of $H_2O_2$ accumulation in the aqueous in the anterior chamber of the eye still remains unsolved. Pirie [4] suggested in 1965 that $H_2O_2$ is formed in the aqueous by oxidation of ascorbic acid, which is normally present in a high concentration in this fluid. However, $H_2O_2$ accumulation in cataract may also be the result of reduced ability of the lenses to decompose $H_2O_2$.

The aim of this investigation was to study the ability of the human lens, at different stages of cataract development, to regulate the $H_2O_2$ level in the surrounding medium.

## EXPERIMENTAL METHOD

Opaque human lenses were obtained in the course of the operation of intracapsular cryoextraction of cataract. Depending on the clinical picture of opacity of the lens the cataract was described as unripe (n = 11) and ripe (n = 7). Transparent human lenses (n = 3) were obtained from eyes donated for corneal grafting. Rabbit lenses (n = 3) were obtained from freshly enucleated eyes of these animals. In all cases the integrity of the capsule of the lens was preserved. Quantitative assessment of the degree of opacity of the lens was undertaken by determination of relative areas of zones with maximal optical density during investigation of the lens by quantitative morphometry on the Leitz (West Germany) television image analyzer. To assess the ability of the lens to decompose $H_2O_2$, it was placed in 1 ml of Hanks' medium (without bicarbonate), pH 7.0, containing 7 mM glucose and $10^{-4}$ M $H_2O_2$. Incubation was carried out at room temperature. After fixed time intervals samples of incubation medium (50 μl) were taken for determination of the $H_2O_2$ concentration, using a highly sensitive chemiluminescence method in a system of luminol–horseradish peroxidase (HRP) [5]. The light sum of chemiluminescence (CLS) was recorded in samples containing 50 μl of the test sample for analysis, 930 μl of luminol (60 μM), and 20 μl of HRP with 1750 activity units/ml. The graph of CLS as a function of $H_2O_2$ concentration was plotted for standard $H_2O_2$ solutions

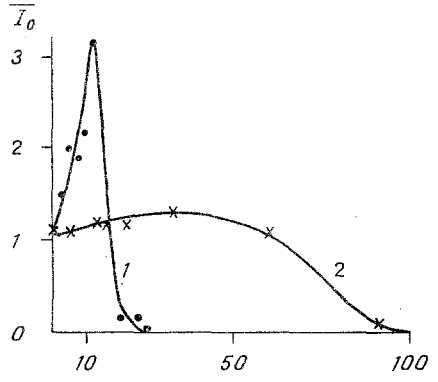


Fig. 1. Typical curves showing changes in CLS of samples of medium surrounding the lens, taken for analysis. Abscissa, incubation time (in min); $I_0$, I) light sums at initial ($10^{-4}$ M $H_2O_2$) and subsequent times, respectively. 1) Initial cataract; 2) ripe cataract.

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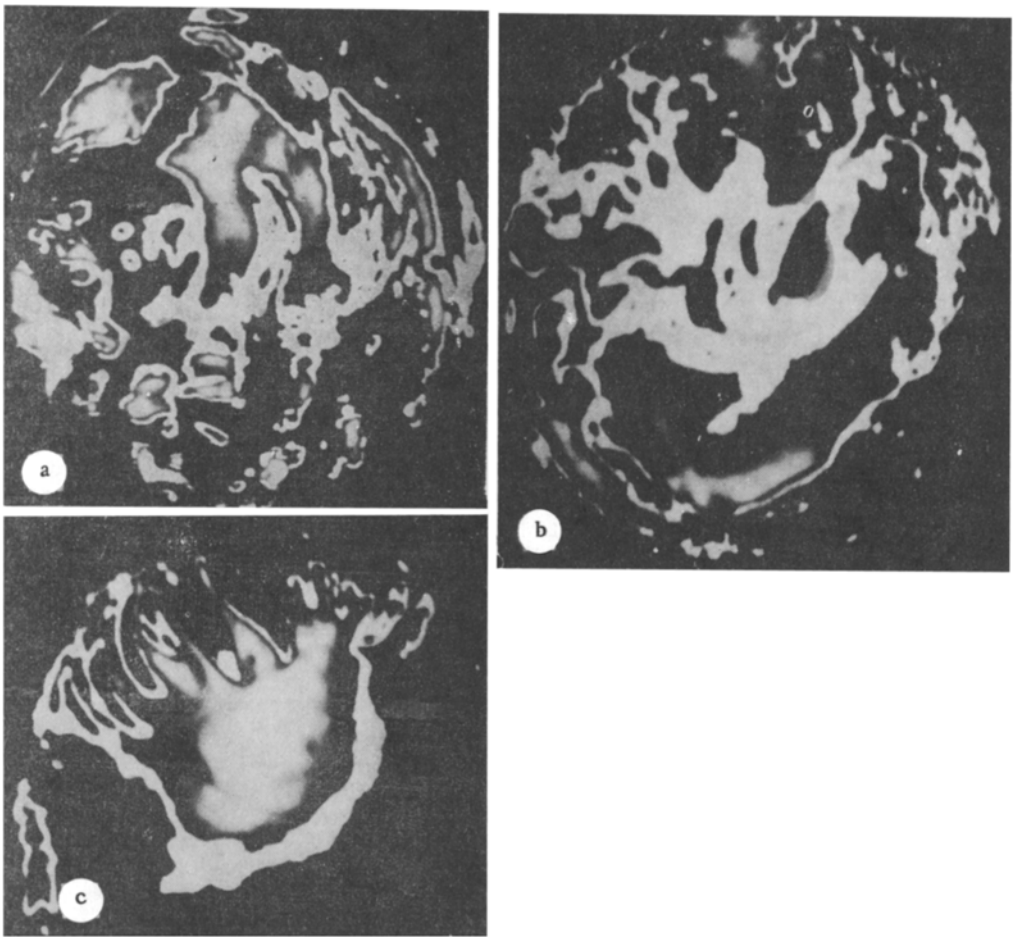


Fig. 2. Estimation of opacity of the lens by quantitative morphometric analysis. a) Unripe cataract, image of lens with binary isolation of zone with 2nd degree of optical density; b) unripe cataract; c) almost ripe cataract. Optical densities (OD) of different zones of the lens subdivided in diminishing order into 10 degrees corresponding to different degrees of opacity of the lens [scale of optical densities omitted in Russian original]. Numbers [also omitted] on photographs express absolute area of zones with assigned value of OD. Dark areas correspond to higher value of OD.

(1 μM to 0.7 mM). Chemiluminescence was recorded on a "Chemiluminometer 1250" instrument (from LKB, Sweden). Reduced glutathione (GSH) used in the work was obtained from Calbiochem-Behring Corp. (USA) and the 3-amino-1N-1,2,4-triazole was obtained from Serva (West Germany).

## EXPERIMENTAL RESULTS

Since the $H_2O_2$ level in the aqueous of the anterior chamber in cataract averages 82 μm [6], the $H_2O_2$ concentration in the incubation medium of the lens was chosen to be 100 μM. It was found that the $H_2O_2$ concentration in the control solution (without lens) remains virtually constant for 100 min. Meanwhile a considerable fall of the $H_2O_2$ level was observed with the course of time after addition of the lens to the medium. A similar approach was used previously to examine the protective systems of normal lenses [3]. In the present case the ability of opaque human lenses to catalyze the decomposition of exogenous $10^{-4}$ M $H_2O_2$ was estimated on the basis of the time taken to reduce CLS of $H_2O_2$. The kinetics of the change in CLS of the samples of medium surrounding the lens taken for analysis can be judged from data given in Fig. 1. Decomposition of $H_2O_2$ was preceded by an increase in CLS. Although the cause of this increase was not specially investigated, it can be tentatively suggested that it was due to the outflow of thiol compounds from the lenticular cells due to a disturbance of membrane permeability in the presence of $H_2O_2$. This hypothesis is supported by the following facts. Addition of exogenous glutathione to the incubation medium of the lens led to a sharp increase in CLS which, however, was followed by rapid decomposition of $H_2O_2$ by the

TABLE 1. Decomposition of $H_2O_2$ ($10^{-4}$ M) by Human Lenses with Different Degrees of Opacity at Room Temperature (M ± m)

| Experimental conditions | Rate of decomposition of $H_2O_2$, nmoles/h | Relative area of zone of opacity of lens, relative units |
|---|---|---|
| Transparent lens (3) | 270±60 | 0,0—0,1 |
| Unripe cataract (11) | 245±40 | 0,1—0,7 |
| Ripe cataract (7) | 71±7* | 0,8—1,0 |
| Ripe cataract + 10 μM GSH (3) | 806±300** | — |
| Unripe cataract + 10 mM 3-amino-1N-1,2,4-triazole (3) | 220±20 | — |
| Unripe cataract + 2nd addition of $H_2O_2$, ($10^{-4}$ M) (3) | 190±20 | — |
| Rabbit lens | 400±60 | — |

Legend. Number of lenses given in parentheses. *P < 0.01 compared with transparent lens; **P < 0.01 compared with ripe cataract.

lens. The increase in CLS was more marked for transparent lenses and lenses in the stage of initial cataract compared with this parameter for ripe cataracts. These stages of cataract, as we know, differ significantly in the GSH level in the lenses [1]. Repeated incubation of the lenses with $H_2O_2$ appreciably weakened the effect of the increase in CLS, and this may be explained by utilization of glutathione during the first incubation.

The rate of decomposition of $H_2O_2$ by the lenses was estimated by the time during which CLS fell by 90% of its initial value. With an increase in the relative area of opacity of the lens from 0.1 to 0.8-1 relative unit the rate of $H_2O_2$ decomposition fell from 200-300 to 65-80 nmoles/h (Table 1, Fig. 2).

We know that the principal enzymes of antioxidant protection of tissues, utilizing $H_2O_2$ as their substrate, are catalase and gluthathione peroxidase. Addition of 3-amino-1N-1,2,4-triazole, a specific catalase inhibitor, to the incubation medium of the lens in a concentration of 10 mM did not lead to any significant decrease in the rate of $H_2O_2$ decomposition. This suggests that the main cause of the rapid destruction of $H_2O_2$ by normal lenses was not catalase, but rather the glutathione peroxidase + GSH system, and that, consequently, activity of this system actually falls during cataract development. As will be clear from the results, addition of exogenous glutathione to the incubation medium of the lens caused a marked increase in the rate of $H_2O_2$ decomposition (Table 1). Meanwhile, after repeated addition of the same quantity of $H_2O_2$ ($10^{-4}$ M) to the lens the rate of its decomposition (190 ± 20 nmoles/h) was rather lower than the rate of decomposition of the first portion of $H_2O_2$ (245 ± 40 nmoles/h). This evidently can be explained by utilization of some of the glutathione in the course of $H_2O_2$ reduction, catalyzed by glutathione peroxidase.

The results as a whole are evidence that the rate of $H_2O_2$ decomposition by human lenses with different degrees of opacity is primarily dependent on the GSH pool in the lens, whose level, as was shown previously [1, 2], falls during cataract development. Activity of the protective antiperoxide systems of the lens is thus determined by the possibility of maintaining high concentrations of reduced glutathione in the cells of the lens. This feature must be taken into account during implantation of an intraocular lens after cataract extraction. In this case, one cause of loss of the endothelial cells of the cornea (the most frequent complication of this operation) may be indirect injury to the endothelium by hydrogen perioxide, associated with absence of the system for protection against $H_2O_2$ from the intraocular lens.

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# *IN VITRO* MODULATION OF STIMULUS-INDUCED CYCLIC AMP FORMATION BY A SYNTHETIC ANTIOXIDANT

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There is evidence in the literature of the important role of the composition and physicochemical properties of cell membrane lipids in regulation of the interaction between receptor and adenylate cyclase (AC) [12, 13]. Data of this kind have been obtained for AC in the membranes of peripheral organs [4, 7] and brain of mammals [3]. In particular, activation of AC of synaptic membranes of the rat brain has been found when lipid peroxidation (LPO) is intensified [2, 8].

In the investigation described below changes in basal and stimulus-induced AC activity were studied in membranes of various internal organs of rats when exposed *in vitro* to a synthetic water-soluble antioxidant.

## EXPERIMENTAL METHOD

By the use of techniques described previously fractions of membrane preparations of rat internal organs were obtained: plasma membranes of hepatocytes [5], plasma membranes of enterocytes from the small intestine [10], and also brain synaptosomes [9]. The preparations used to stimulate cyclic AMP (cAMP) formation included glucagon (10 μM), isoproterenol (100 μM), and the opioid receptor agonist dalargin (D-Ala$^2$,Leu$^5$,Arg$^6$-enkephalin; 10 μM), obtained in the Laboratory of Peptide Synthesis (Head, M. I. Titov), All-Union Cardiologic Science Center, Academy of Medical Sciences of the USSR.

To determine AC activity the incubation medium for plasma membranes of the liver contained 50 mM Tris-HCl (pH 7.5), 2.5 mM cAMP, 3 mM $MgCl_2$, 1 mg/ml creatine phosphokinase, 50 mM creatine phosphate, 0.3 mM ATP, 0.25 mM GTP, 3 mM dithiothreitol, 0.5 mM theophylline, and [$^{32}$P]-ATP with activity of $0.5 \times 10^6$ cpm. In experiments with enterocyte membranes and brain synaptosomes, the incubation medium was of the same composition, but concentrations of some compounds were different: 0.3 mM dithiothreitol, 10 mM theophylline, and 1 mM EGTA. The final protein concentration of the membrane preparations was 0.05 mg/ml; protein was determined by the method in [11].

Membranes were incubated in the reaction medium for 15 min at 37°C. The reaction was stopped by addition of 0.5 N HCl. The samples were subjected to hydrolysis at 100°C for 7 min and then neutralized with 1.5 M imidazole. The labeled cAMP thus formed was removed by filtration on columns with neutral aluminum oxide [15]. A water-soluble antioxidant belonging to a class of screened phenols (phenozan-1K), synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR, was used. Interaction with the antioxidant took place during preincubation of the membrane preparation with phenozan-1K for 30 min at 25°C, after which the membranes were incubated for 15 min in the reaction mixture at 37°C.

## EXPERIMENTAL RESULTS

The action of phenozan-1K *in vitro* increased both the basal and glucagon-stimulated AC activity of the hepatocyte plasma membranes, with a maximum when the phenozan-1K concentration was $10^{-5}$ M (Fig. 1, I).

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