In the course of the PRR, 5.04 ± 0.58 nmoles Ca^{++} ions was thus released from 10^8 platelets. If this is converted to the platelet concentration usually adopted in the literature, namely 10^9 cells/ml, the figure is 50.4 ± 5.8 nmoles Ca^{++}, or about 20% of the total content in human platelets.

The results for the kinetics of Ca^{++} release under the influence of thrombin thus obtained indicate that this process can be recorded by means of ion-selective electrodes and they open up definite prospects for the study of PRR and the mechanism of aggregation of platelets and their activity in various pathological states and under the influence of certain drugs.

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AGE CHANGES IN SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE ACTIVITY IN CYTOSOL AND MITOCHONDRIA OF RAT LIVER

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Processes of free-radical lipid peroxidation (LPO) may play an essential role in disturbance of the function of cell systems [1, 2]. The writers previously found a considerable increase in activity of enzymic and nonenzymic LPO liver membranes in various pathological states, including experimental malignant growth [3, 4], chemical carcinogenesis [4, 5], and experimental atherosclerosis [6, 7]. It was shown that activation of LPO systems in the liver is observed not only in pathological states, but also during normal physiological processes and, in particular in the early stage of postnatal development [7, 8]. This finding was confirmed in investigations by various workers in recent years, both *in vitro* [9-12] and *in vivo* [13]. The intensity of LPO in the subcellular organelles of the rat liver falls with age [7-10], simultaneously with an increase in NADPH and in the SH-dependent antioxidant action of the cytosol [10]. It can be postulated on the basis of results so far obtained [7, 8, 10] that LPO in the cell at various stages of development and aging does not lead to induction of pathological changes, for it is under the effective control of "antioxidant" enzymes [2, 7], namely superoxide dismutase (SOD) and glutathione peroxidase (GP). The investigation described below was devoted to an experimental test of this hypothesis.

EXPERIMENTAL METHOD

Male Wistar rats of different ages (newborn, aged 1 and 2 weeks and 1, 2, 3, 6, 12, 18, and 24 months), kept under standard conditions, were used. Embryos were obtained by autopsy of the pregnant females of the same line one week before the expected date of birth. The rats were killed by decapitation and, after perfusion with cold 0.154 M KCl, pH 7.4 (the liver of the embryos and of the newborn and week-old rats was not perfused) the liver was homogenized

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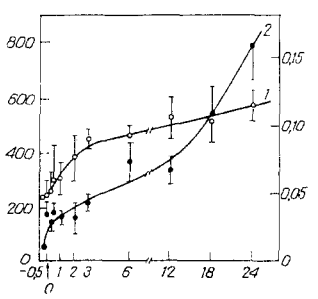

Fig. 1

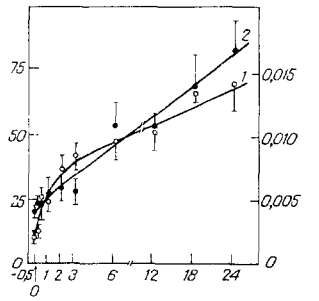

Fig. 2

Fig. 1. Age changes in SOD (1) and GP (2) activity in rat liver cytosol. Here and in Fig. 2, ordinate — age (in months), abscissa — on left, SOD activity (in units/mg protein), on right, GP activity (in units/mg protein).

Fig. 2. Age changes in SOD (1) and GP (2) activity in rat liver mitochondria.

in medium containing 250 mM sucrose, 10 mM Tris-HCl, pH 7.4, and 0.1 mM EDTA [8]. The mitochondrial (8000g × 10 min) and cytosol (105,000g × 10 min) fractions of the liver were obtained by centrifugation as described previously [8]. The residue of mitochondria was washed with isolation to remove cytosol. At each experimental point 20 embryos, 30 newborn or week-old rats (the pooled liver homogenate from ten animals), or five rats starting from the age of 2 weeks were used. Subcellular fractions of liver were frozen in polyethylene containers and kept until analysis in liquid nitrogen, and immediately before determination of activity of the enzymes they were quickly thawed at 37°C. Activity of SOD and GP (using tert-butyl hydroperoxide as substrate) was measured as described previously [14] on the Amico DW-2A (USA) spectrophotometer. The protein concentration in the preparations was determined by Lowry's method.

EXPERIMENTAL RESULTS

SOD and GP activity increased with age of the rats both in the cytosol (Fig. 1) and in the mitochondria of the liver (Fig. 2). SOD activity in the liver of the mature animals (3 months) was 1.8 times higher in the cytosol and 3.5 times higher in the mitochondria than in newborn animals. As the rats subsequently aged (3-24 months) the SOD activity in the cytosol and mitochondria of their liver increased by a smaller degree — by 1.3 and 1.7 times respectively (Figs. 1 and 2). GP activity, on the other hand, showed a smaller increase from birth to maturity, both in the cytosol and mitochondria of the rat liver, by 1.2 and 1.4 times respectively (Figs. 1 and 2). SOD activity in the liver cytosol of old rats (24 months) was 2.4 times higher than in the newborn animals, whereas GP activity was 4.6 times higher; in the mitochondria the activity of these enzymes increased during the same period by 6.1 times for SOD and by 4.2 times for GP (Figs. 1 and 2). These results confirm the conclusion that one of the main causes of the decrease in the intensity of LPO in the postnuclear fraction of the liver homogenate of rats during aging [10] is a sharp increase in GP activity in the cytosol. An increase in the steady-state concentration of lipid peroxides in the cell is most likely at puberty, for it is at this stage of development that the highest rate of enzymic generation of lipid peroxides is observed [7, 8, 12] coupled with a comparatively low rate of enzymic utilization of the glutathione peroxidase system. The physiological importance of intensification of LPO at the stage of puberty may thus be determined by the role of free-radical processes in structural and functional modifications in biological membranes.

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