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RESPONSE OF HUMAN BLOOD PLATELET MEMBRANE TO SODIUM SELENITE

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It was demonstrated that incubation of blood platelets with sodium selenite (1—100 μM) resulted in a dose- and time-dependent loss of platelet thiols (both glutathione and protein −SH groups). The effects of sodium selenite on platelet membrane lipid fluidity by the EPR spin-labelling method was also investigated. We showed there were no alterations in membrane fluidity at the deeper regions (12-DOXYL-Ste) in lipid bilayer, a slight increase (approx. 7%, $p < 0.03$) of h_{+1}/h_0 for spin probe 5-DOXYL-Ste was monitored. The amount of Triton-insoluble protein fraction isolated from platelets after incubation (60 min) with selenite was significantly elevated ($p < 0.006$). It has been suggested that limited increase in lipid fluidity at the surface regions in the lipid bilayer of the platelet membrane in selenite-treated platelets may be the result of alteration in lipid-protein interactions caused by protein conformational changes.

Key words: *blood platelets, membrane fluidity, thiols, lipid peroxidation, sodium selenite*

INTRODUCTION

Selenium (Se) is recognized as an essential dietary nutrient for all mammalian species. On the other hand Se compounds are highly toxic (1, 2). Several pieces of evidence indicate that selenium deficiency may be related to cardiovascular diseases (3) and may be a risk factor for cancer in humans (4). The protective effects of selenium during myocardial ischemia as well as in the prevention of acute myocardial infarction has been documented (5, 6). However, the mechanism of this beneficial action of Se has not been defined. The biological functions of Se are mediated via selenoproteins that contain Se as a selenocysteine (7). The function of Se in human body, with the exception of Se-dependent enzymes — glutathione peroxidases and iodothyronine deiodinases — is largely unknown.

Blood platelets are among other blood cells the first target of Se action. Recently we have shown that sodium selenite exerted an inhibitory effects on blood platelet activation (8). In particular, sodium selenite in toxic doses

inhibited thrombin- and ADP-induced platelet aggregation. Preincubation of platelets with sodium selenite (even with sub-toxic concentrations) resulted in the decrease of adenine nucleotide secretion as well as the reduction of maloyldialdehyde/thromboxane A_2 (MDA/TXA_2) production in thrombin-stimulated platelets (8).

Platelet aggregation, secretion of its granule content and TXA_2 generation are mediated by signal transduction mechanisms and many specific, membrane receptors are involved in agonist-induced platelet activation (9, 10). In this study we monitored the fluidity of blood platelet membrane after pre-treatment of cells with sodium selenite using the EPR spin-labelling technique. Sodium selenite is thought to react with protein $-SH$ groups (1). The skeletal and the membrane proteins are probably the main target of the action of selenium. Therefore, the effects of selenite on the level of Triton-insoluble fraction of platelet protein as well as on the amount of platelet thiols was also determined.

MATERIALS AND METHODS

Sodium selenite, Hepes, bovine serum albumine (BSA), 5-doxylstearic acid (5-DOXYL-Ste) and methyl-12-doxylstearate (12-DOXYL-Ste) were purchased from Sigma Chemical Co (USA). All other chemicals were of A.R. grade from POCh (Gliwice, Poland).

Preparation of platelet suspensions

Blood was obtained from healthy human volunteers under the guidelines of the Helsinki Declaration for human research and the studies were approved by the committee on the Ethics of Research in Human Experimentation at Medical University of Łódź Platelets wwre preparated by the routine method of differential centrifugation (11). Acid-citratedextrose (ACD) anticoagulated blood was centrifuged for 20 min at $200 \times g$ and collected platelet-rich plasma (PRP) was then centrifuged for 15 min at $100 \times g$ to sediment platelets. The resulting pellet was resuspended in the modified Tyride's buffer (140 mM NaCl, 15 mM Hepes, 10 mM KCl, 10 mM glucose, 0.35% BSA, 0.1 g/l apyrase, pH 7.4) and platelets were washed three times with the buffer.

EPR measurements

Washed platelets (9×10^8 cells/ml) were incubated for 30 min at room temperature with 5-DOXYL-Ste or 12-DOXYL-Ste added to a final concentration of 50 μM. Sodium swlenite (at the final concentration 1, 10 or 100 μM) was added to spin-labelled platelets and samples were incubated at 37°C for indicated time. EPR measurements were performed at 25°C in a Bruker ESP 300 E spectrometer.

Estimation of platelet acid-soluble and acid-insoluble thiols

Pig platelets were separated by differential centrifugation according to Wachowicz and Krajewski (12). Briefly, blood collected from pig into ACD solution (5 : 1, v/v) was centrigugated for 20 min at $750 \times g$ (PRP) and then platelets were sedimented (15 min at $1000 \times g$). The platelet pellet was gently suspended and washed twice in modified (Ca^{2+} free) Tyrode's buffer (0.14 M NaCl, 0.014 M Tris/HCl, 5 mM glucose, pH 7.4). The platelets in suspension (10 mg of platelet

protein/ml) were preincubated without (control) or with sodium selenite at final concentration 0.1 or 100 μM (5—30 min, 37°C), and then centrifuged for 15 min at 1.200 × g. To frozen platelet pellets 1 ml of precipitating solution (30% NaCl, 0.85% H_3PO_4, 0.2% EDTA) were added, suspension were frozen (−20°C) and thawed twice and then centrifuged (20 min, 3.800 × g). Thiols in acid-soluble (supernatant) and acid-insoluble (precipitate) fractions were estimated by the method of Ando and Steiner (13) as previously described (14).

Determination of platelet protein

The platelets in suspension (10 mg of platelet protein/ml) after an hour incubation with sodium selenite (final concentrations: 0.1, 1, 10 or 100 μM) at 37°C were centrifuged for 2 min in an Eppendorf microcentrifuge. Platelet pellets were disolved in 10 mM phosphate buffer (pH 7.0) containing 0.25 M sucrose and 0.25% Triton X-100. Platelet protein concentration was determinated by a modified Lowry method (15).

Statistical analysis was performed by Student's t-test paired data. P values < 0.05 were considered to be statistically significant.

RESULTS

It was demonstrated that incubation of pig blood platelets with sodium selenite resulted in a decreased concentration of thiols both in acid soluble (*Fig. 1*) and acid insoluble (*Fig. 2*). fraction. The loss of thiols was dose- and time-dependent, although within first ten minutes there was a slight rise in the level of thiols in acid-soluble fraction (*Fig. 1*).

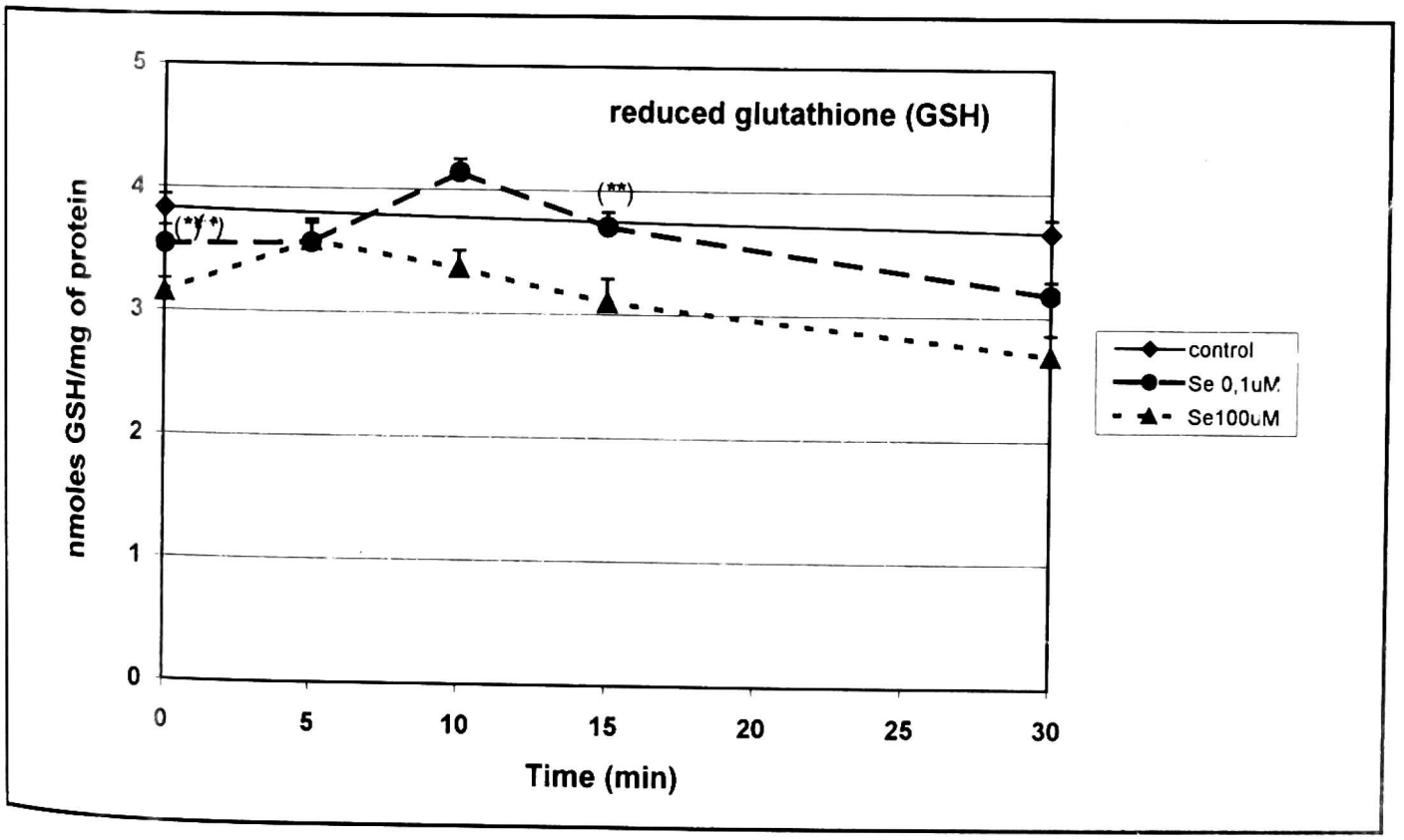

Fig. 1. Time dependency of the effects of sodium selenite on the level of reduced glutathione in washed pig blood platelets. The results are presented as mean values ± SEM of n = 9 experiments. *** $p < 0.02$; ** $p < 0.05$ (two-tailed paired t-test), for all other values the significance of the differences was < 0.01.

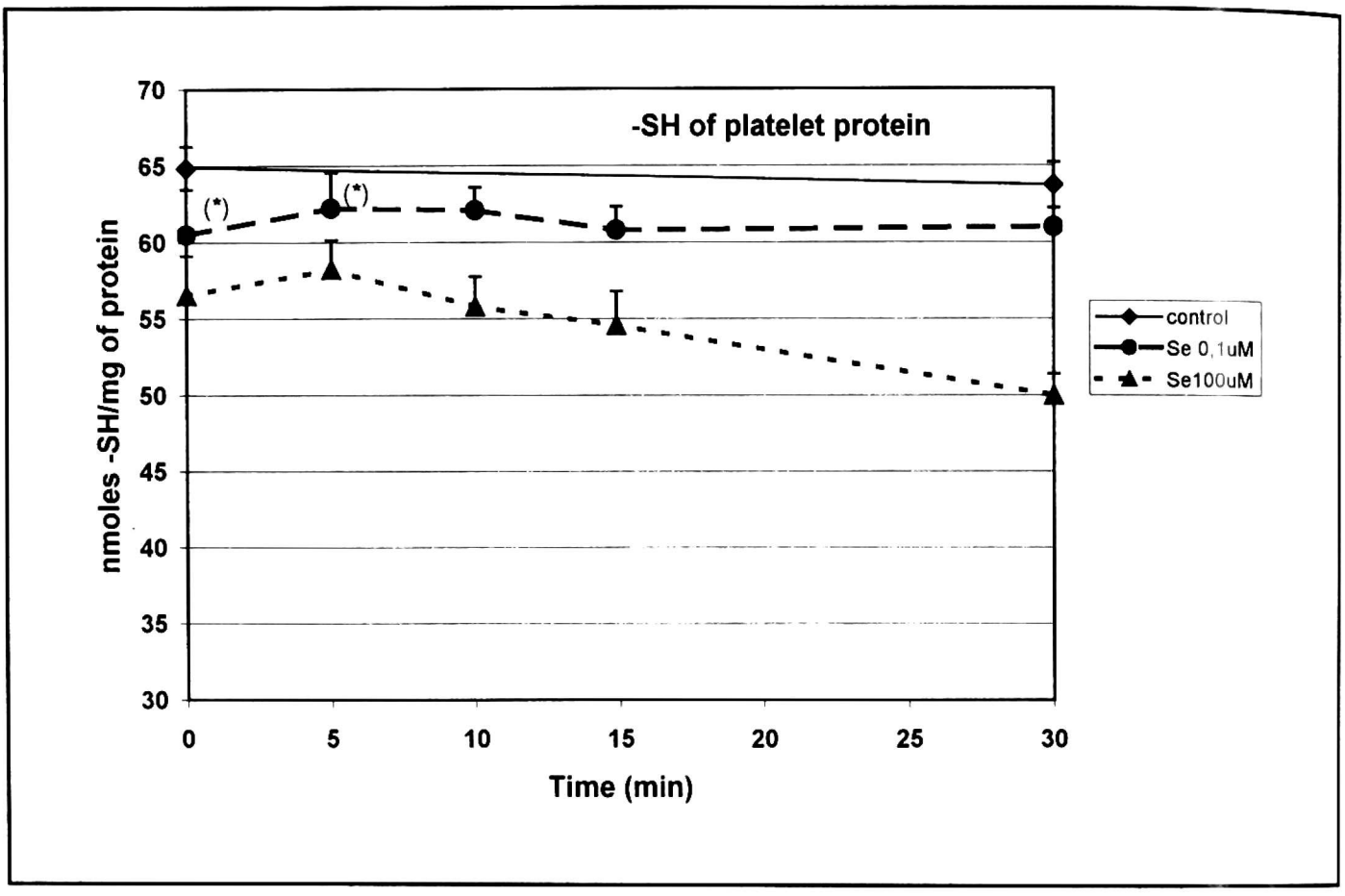

Fig. 2. Time dependency of the effects of sodium selenite on the amount of protein thiols in washed pig blood platelets. The results are presented as mean valueas ± SEM of n = 8 experiments. *not significant (two-tailed paired t-test), for all other values the significance of the differences was < 0.01.

For the EPR study we used two spin-laćel probes to monitor lipid fluidity at the surface (5-DOXYL-Ste) and deeper (12-DOXYL-Ste) regions in the lipid bilayer of the membrane. The mobility of the spin-label probe was measured as k_{+1}/h_0, where h_{+1} represents the height of the low- and h_0 the height of the middle-field lines spectra, respectively (16, 17). *Table 1* presents the mobility of 5-DOXYL-Ste probe in platelets preincubated with sodium selenite. It appeared that Se caused a slight increase of h_{+1}/h_0 (indicating a slight increased lipid fluidity of platelet membrane), although the increase was neither dose- nor time-dependent and it was statistically significant ($p < 0.03$) only after 30 min incubation with a high dose of the tested compound (100 μM). The order parameter (S) calculated from the spectra of 5-DOXYL-Ste remained unchanged after pretreatment of platelets with sodium selenite (*Table 1*). In contrast to the small fluctuations in g_{+1}/h_0 monitored for 5-DOXYL-Ste there were no significant differences in the mobility of 12-DOXYL-Ste probe between control and selenite-treated platelets (*Table 2*).

Table 1. The mobilities (h_{+1}/h_0) of 5-DOXYL-Ste and the order parameter (S) alculated for membranes of human blood platelets. The data were expressed as % of control (values obtained for selenite-untreated platelets)

Sodium selenite	Spin-probe mobility (h_{+1}/h_0) for 5-DOXYL-Ste[a]			Order parameter (S) (% control)[a]	
	Incubation time			Incubatiom time	
	5 min	30 min	60 min	5 min	30 min
1 μM	101.73 ± 1.42 (7) *	105.10 ± 3.51 (7) *	nd [b]	98.68 ± 0.39	100.58 ± 1.82
10 μM	102.00 ± 1.68 (3) *	101.97 ± 2.13 (3) *	nd [b]	98.71 ± 0.58	100.21 ± 1.81
100 μM	100.92 ± 4.54 (8) *	107.15 ± 2.31 (6) **	106.57 ± 2.30 (3) *	98.87 ± 0.36	100.00 ± 0.61

[a] Values are means ± SEM for number of experiments indicated in brackets.
[b] nd, not done
* not significant
** significant ($p < 0.03$)

Table 2. The mobilities (h_{+1}/h_0) of 12-DOXYL-Ste calculated for membranes of human blood platelets. The data were expressed as % of control

Sodium selenite	Spin-probe mobility (h_{+1}/h_0) for 12-DOXYL-Ste[a]	
	Incubation time	
	5 min	30 min
1 μM	102.35 ± 2.70 (6) *	95.97 ± 3.63 (6) *
100 μM	99.42 ± 2.46 (6) *	99.51 ± 3.11 (6) *

[a] Values are means ± SEM for number of experiments indicated in brackets.
* not significant

As shown in *Fig. 3*, after incubation (1 hour) of blood platelets with sodium selenite up to a concentration of 1 μM, the amount of Triton-insolute proteins (measured as % of total) was significantly elevated. For example, the highest concentration of selenite (100 μM) caused an increase of Triton-insoluble fraction from 37.25 ± 1.99% of total obtained for Se-untreated platalets to 51.12 ± 3.66% ($p < 0.006$).

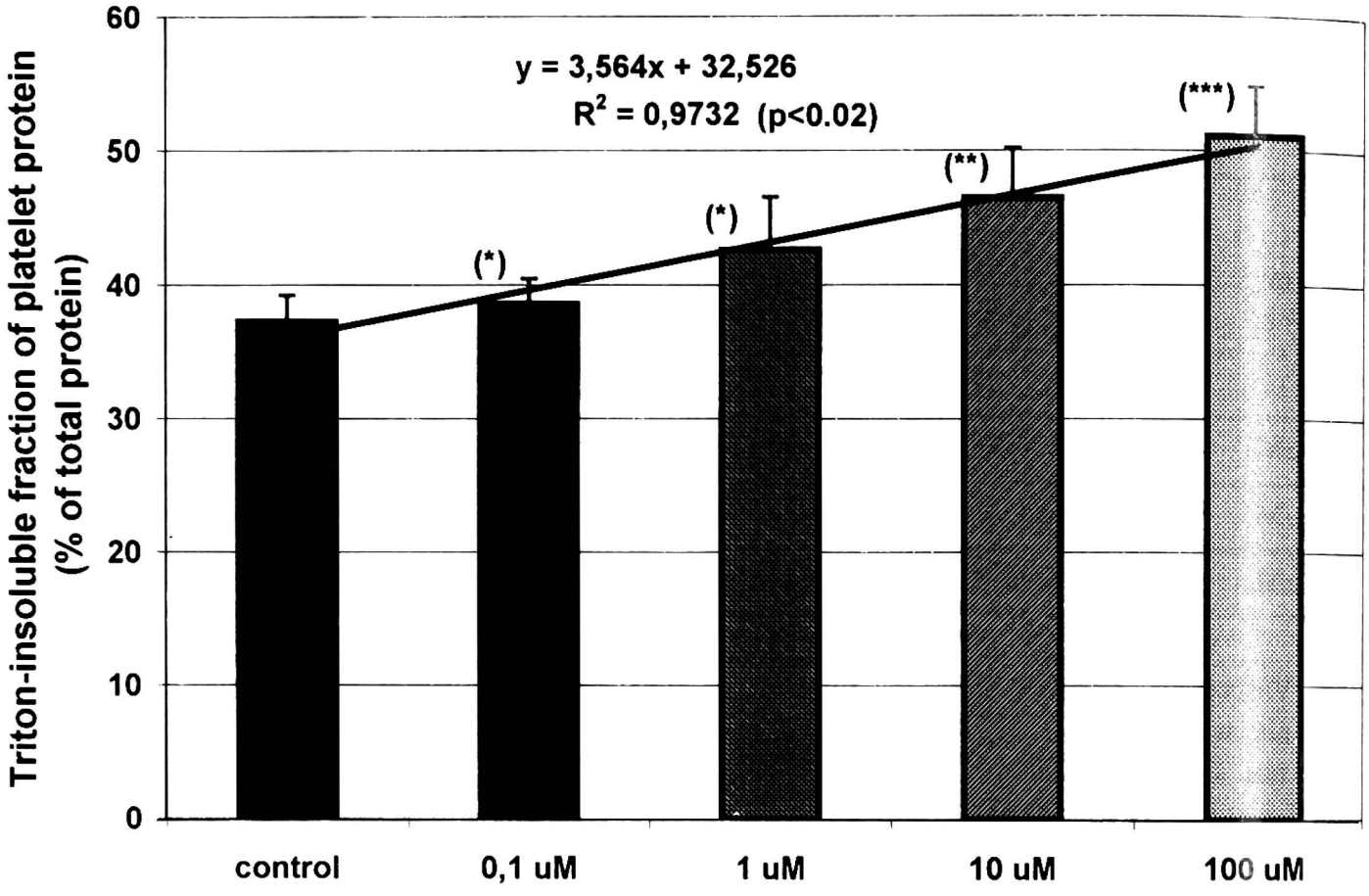

Fig. 3. The amount of Triton-insoluble fraction of platelet protein after an hour preincubation of pig blood platelets with different concentrations of sodium selenite. All data shown are mean ± SEM of n = 4 experiments (carried out in triplicate). Data were calculated as % of total platelet protein. * not significant; ** $p < 0.02$; *** $p < 0.006$. Regression line was calculated by means of the least squares method.

DISCUSSION

The role of selenium in human body is largely unknown. Nevertheless, moderate Se supplementation (50—100 μg/day) has been recommended to populations exposed to marginal selecium deficiency (18, 19). Selenium deficiency increases lipid peroxidation by reducing the activity of glutathione peroxidase which promote oxidative damage in cell membranes. On the other hand elevated level of Se (10—100-fold) is highly toxic. It is believed that sulphydryl groups important to oxidative processes and reactive intermediates are possibly involved in Se toxicity (1). It has been well established that thiols are primary tarhet for selenite in cells (platelet, erythrocyte) 14, 20). Selenite causes a concentration-dependent oxidation of glutathione (GSH) as well as oxidation of certain protein thiols. A consequence of the reaction of selenite with GSH is the production of H_2O_2 and superoxide anion (O_2^-) involved in the toxicity of selenite (1). In the present study we demonstrated within first minutes there was a slight rise in the level of thiols in acid-fraction (*Fig. 1*). This phenomen may confirm that in platelets selenite reacts with thiols forming

highly reactive intermediates e.g. glutathioselenol (GSSeH) that then undergo rapid reduction to selenide (Se^{-2}). It was documented by Zhu et all (21) that DTNB (5,5'-dithio-bis-2-nitrobenzoic acid) used for the estimation of platelet thiols can react with $-SeH$ groups of proteins.

Since the cell response to selenite alone and its metabolites (GSSeH, Se^{-2}) may be different we examined the lipid fluidity in platelet membrane after a short (5 min) and a long (30 or 60 min) exposure of cells to sodium selenite. It was shown by other authors that sodium selenite is rapidly taken up by red cells (20). The uptake of radiolabelled selenite into murine melanoma cells was maximal in the first hour of incubation (22). It seems that blood platelets also can accumulate Se since selenite induces a fast consumption of GSH. Nevertheless, we demonstrated that selenite does not significantly influence platelet membrane fluidity since neither values of h_{+1}/h_0 monitored for spin probes 12-DOXYL-Ste nor order parameter (S) calculated for 5-DOXYL-Ste were changed. Microviscosity of platelet membrane may be affected by alterations in lipid components of platelet membrane (i.e. when lipid peroxidation occurs) or by changes in lipid-protein interations. Our earlier results indicated that incubation of washed pig blood platelets with 1 or 10 μM sodium selenite reduced the platelet lipid autoperoxidation measured by the thiobarbituric acid method (23).

On the other hand selenite treated platelets contained elevated level of Triton-insoluble fraction of platelet protein (*Fig. 3*). In addition, we have shown elsewhere (14) by SDS PAGE the presence of high molecular weight aggregates in the cytoskeleton of platelets incubated with toxic dose of sodium selenite. Taking previous and presented here results into consideration we suggest that the limited increase in lipid fluidity at the surface regions in the lipid bilayer of the membrane in selenite-treated platelets may be partly the result of protein conformation changes. Due to the reactivity of selenite with thiols, the reaction of selenite with cysteine and pairs within membrane and/or skeletal proteins may occur before selenite enters the cell and reacts with GSH. Reaction of selenite with proteins containing pairs of cysteine leads to production of high molecular weight protein-S-Se-S-protein stable adducts.

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