cyclase. An immunohistochemical survey of the entire brain will be required to determine if other central adrenergic responses are also accompanied by elevations in neuronal cyclic AMP.

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7. In rats, the nucleus locus coeruleus is composed almost entirely of catecholamine-containing neurons (*6*), and is situated bilaterally just below the 4th ventricle in the anterior pons, immediately medial to the mesencephalic root of 5th cranial nerve, and just dorsal to the brachium conjunctivum.
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13. Additional controls for stimulation experiments consisted of 6-hydroxydopamine–treated rats, in which the stimulating electrode was inserted into the region of the locus coeruleus and currents applied which would have been supramaximum in normal rats, or normal rats in which stimulating electrodes were inserted into the locus coeruleus but no stimulating currents were applied (sham-stimulation).
14. As the immunocytochemical staining method is now applied, we could not objectively evaluate gradations of staining intensity when comparing different experimental specimens. Therefore, all unequivocally reactive neurons were grouped together and the results were expressed as a percentage of the total number of Purkinje neurons visualized within the section. In the results of Fig. 2, since the response is evaluated only in terms of a single all-or-none detection threshold, rather than by graded measurement, it is not surprising that $10^{-6}M$ norepinephrine and control values are equivalent, and that at 10^{-5}, 10^{-4}, and $10^{-3}M$ norepinephrine, cell counts are similar. A dose-dependent rise would be unlikely if a homogeneous population of Purkinje cell receptor content (*8*) and distribution exists from cell to cell. Granule cells appear to be maximally reactive even in the control conditions and it is difficult to determine whether the reactive granule cells are qualitatively changed by any of the experimental conditions studied.
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Selenium: Biochemical Role as a Component of Glutathione Peroxidase

Abstract. *When hemolyzates from erythrocytes of selenium-deficient rats were incubated in vitro in the presence of ascorbate or H_2O_2, added glutathione failed to protect the hemoglobin from oxidative damage. This occurred because the erythrocytes were practically devoid of glutathione-peroxidase activity. Extensively purified preparations of glutathione peroxidase contained a large part of the ^{75}Se of erythrocytes labeled in vivo. Many of the nutritional effects of selenium can be explained by its role in glutathione peroxidase.*

Although the nutritional importance of selenium and its relation to vitamin E are well known (*1*), definition of a specific biochemical role for selenium has so far proved elusive. We present evidence here for a role of Se in glutathione (GSH) peroxidase (glutathione: H_2O_2 oxidoreductase, E.C. 1.11.1.9; $2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$) which provides further evidence for the essentiality of Se for animals and for one of its biochemical functions, and provides a plausible explanation for its relation to vitamin E.

Earlier, we found that dietary Se helped prevent oxidative damage to rat erythrocytes incubated in vitro, as evidenced by decreased hemolysis and decreased hemoglobin oxidation (*2*). These effects of dietary Se were dependent on the addition of glucose in vitro, and the well-known protection against hemolysis and hemoglobin oxidation afforded by glucose (*3*) was virtually absent in erythrocytes from rats deficient in Se. A related dietary inhibitor of oxidative damage, α-tocopherol (vitamin E), protected against hemolysis whether or not glucose was present, but did not protect against hemoglobin oxidation. This result demonstrated that the effect of Se was specific and distinct from that of vitamin E (*2*).

Protection by glucose against oxidative damage to red blood cells has been attributed to the maintenance of the intracellular concentration of reduced glutathione (GSH) through the combined actions of the enzymes, glucose-6-phosphate dehydrogenase [which generates reduced nicotinamide adenine dinucleotide phosphate (NADPH)] and glutathione reductase [which generates GSH from NADPH and oxidized glutathione (GSSG)] (*3*). At least in part, GSH acts by destroying hydrogen peroxide and fatty acid hydroperoxides through reactions catalyzed by GSH peroxidase (*3, 4*). In our earlier work (*2*) we found that the GSH concentration was higher in Se-deficient than in Se-adequate erythrocytes and that it was as effectively maintained during incubation in vitro. This suggested that the defect in Se deficiency was not in the maintenance of GSH but rather in the utilization of GSH in protecting the cell (*2*). We therefore focused on a possible role for Se in the GSH protection against hemoglobin oxidation and ultimately on the enzyme (GSH peroxidase).

All experiments were performed with

erythrocytes from rats fed either a Se-deficient (Se content, < 0.01 ppm) or Se-supplemented (Se content, 0.5 ppm; supplied as sodium selenite) diet (*2*). Erythrocyte hemolyzates prepared from rats receiving the respective diets are called (for convenience) Se-deficient or Se-sufficient hemolyzates. Because previous experiments showed that the effects of Se in preventing oxidative damage were independent of vitamin E (*2*), all diets in our studies contained 50 mg of *dl*-α-tocopherol per kilogram of diet. The rats received the respective diets for at least 6 weeks before blood samples were taken. Erythrocytes were centrifuged from heparinized blood taken by heart puncture, the buffy coat was removed by aspiration, and the erythrocytes were washed three times with isotonic saline-phosphate buffer at *p*H 7.4 (*2*). The cells were then hemolyzed with an equal volume of water; hemoglobin content was measured by the cyanomethemoglobin method (*5*), and hemoglobin oxidation was followed by the methods described by Mills (*6*); GSH was measured as described previously (*2*).

Ascorbate- and H_2O_2-induced hemoglobin oxidations were quantitated in hemolyzates incubated in vitro by measuring, respectively, choleglobin formation and increase in absorbance at 627 nm by slight modifications of methods described by Mills (*6*). As shown in Table 1, GSH decreased both ascorbate- and H_2O_2-induced oxidations of hemoglobin in Se-sufficient hemolyzates, but GSH did not decrease hemoglobin oxidation in Se-deficient hemolyzates. Without added GSH, hemoglobin oxidations were similar in Se-sufficient and Se-deficient hemolyzates. The oxidation of hemoglobin by H_2O_2 was easily visible as a brownish-green discoloration within 2 minutes after H_2O_2 was added. These studies suggested that the activity of the GSH peroxidase may be much lower in Se-deficient hemolyzates.

Table 1. Effect of glutathione (GSH) on the oxidation of hemoglobin (*13*) induced by ascorbate and by H_2O_2 in Se-sufficient (Se+) and Se-deficient (Se−) erythrocyte hemolyzates. Ascorbate-induced oxidation is expressed as the percentage of choleglobin formed; H_2O_2-induced oxidation is expressed as the increase in optical density at 627 nm.

Source of hemolyzate	GSH	Hemoglobin oxidation	
		Ascorbate-induced (%)	H_2O_2-induced
Se−	−	19.4	0.107
Se−	+	27.7	0.114
Se+	−	20.4	0.100
Se+	+	6.5	0.036

It has been shown that Se-deficient hemolyzates have much lower GSH peroxidase (method in legend of Fig. 1) as compared to Se-sufficient hemolyzates [for example, at 66 days on experiment the value was 10.0 ± 0.5 (S.E.) compared to 55.5 ± 2.1 enzyme units per milligram of hemoglobin] (*7*).

The Se-dependent GSH peroxidase activity could not be restored to the Se-deficient hemolyzates by incubating for 30 minutes with either 6.3 μM sodium selenite alone, or combined with 25 mM GSH or 25 mM dithiothreitol, suggesting that the enzyme had not simply been oxidatively deactivated in Se-deficient hemolyzates. Enzyme activity did not disappear upon dialysis against any of the following: saline-phosphate buffer (*2*), 5 mM GSH in buffer, or 5 mM GSH plus 1 mM EDTA in buffer. Also the enzyme activity was not retarded on G-25 Sephadex. These observations suggested that Se may be an integral part of GSH peroxidase rather than a loosely bound cofactor of low molecular weight.

To test the hypothesis that Se is a component of GSH peroxidase, we partially purified GSH peroxidase from erythrocytes of Se-adequate rats which 2 or 4 weeks earlier had been injected with ^{75}Se as sodium selenite. By DEAE-Sephadex chromatography (Fig. 1, top), GSH peroxidase was separated from the large hemoglobin peak and some minor erythrocyte proteins. Approximately 60 percent of the ^{75}Se in the initial hemolyzate cochromatographed with the GSH peroxidase activity. There were no other substantial ^{75}Se-containing peaks. Fractions containing GSH peroxidase were concentrated and rechromatographed on Sephadex G-150 (Fig. 1, bottom). Two ^{75}Se-containing peaks were obtained corresponding to approximate molecular weights of 90,000 and 25,000. The lower-molecular-weight peak, which has not been identified, contained insignificant GSH peroxidase activity. The peak of 90,000 molecular weight corresponded to the GSH peroxidase activity and contained about 70 percent of the ^{75}Se applied to

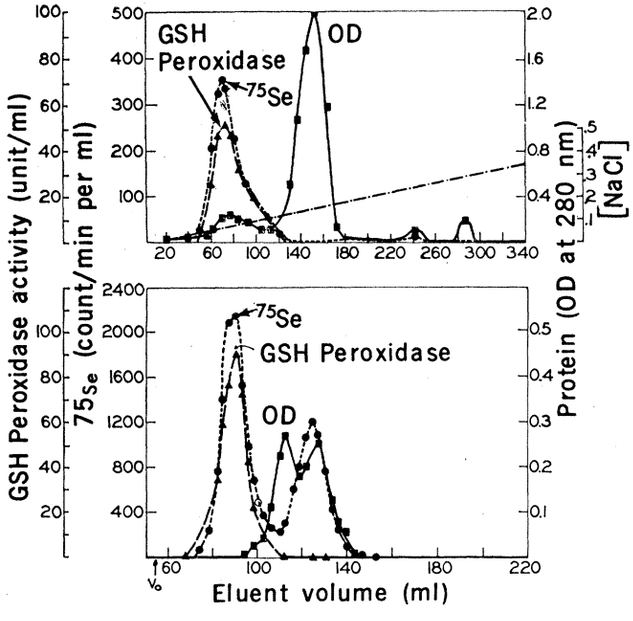

Fig. 1. Purification of GSH peroxidase. (Top) Erythrocytes separated from 3 ml of heparinized blood from a rat injected 2 weeks earlier with 10 μc of sodium [^{75}Se]selenite were hemolyzed with water. Approximately 90 percent of the hemoglobin precipitated on overnight storage at 4°C, with minimal loss of GSH-peroxidase activity. The supernatant containing 75 percent of the ^{75}Se was chromatographed on DEAE-Sephadex A-50, eluting with 0.05M tris buffer, *p*H 8.0, and a linear gradient of 0.0 to 0.5M NaCl (dashed line). The GSH peroxidase was assayed by a modification of Mills' procedure 2 (*14*). The incubation mixture at 37°C contained 0.08M sodium phosphate (*p*H 7.0), 0.08 mM EDTA, 1.0 mM sodium azide, 0.40 mM GSH and 0.25 mM H_2O_2. GSH was determined at 3-minute intervals (*15*). An enzyme unit represents a decrease in GSH concentration of 0.001 log unit per minute, after subtraction of the nonenzymic rate. The ^{75}Se was measured with a crystal scintillation spectrometer. (Bottom) The DEAE-Sephadex A-50 column fractions containing GSH peroxidase activity from 3 ml of blood from a rat injected 4 weeks earlier with 100 μc of ^{75}Se were pooled, concentrated by ultrafiltration, and rechromatographed on Sephadex G-150, eluting with 0.05M tris buffer, *p*H 8.0. OD, optical density; V_o, void volume.

the column. At the concentration present in the eluate, no protein could be detected in the active peak by absorbance at 280 nm, suggesting that extensive purification of the enzyme had been achieved. Although we have not yet completely purified the rat enzyme, cochromatography of ^{75}Se with GSH peroxidase activity through two highly effective purification steps suggests that Se is an integral and necessary part of the enzyme. In our laboratory the enzyme from ovine blood has been purified, yielding a preparation containing at least 2 gram-atom of Se per mole of enzyme (*8*).

Flohé (*9*) reported that bovine erythrocyte GSH peroxidase contains no nonprotein prosthetic group; however, a Se moiety would probably not be detected by the spectrophotometric methods used by Flohé. Selenite (*10*) or Se–amino acids (*11*) enhance the reducing ability of GSH in model systems, and SH groups in GSH peroxidase appear to change their redox state during the catalytic process (*9*). Whether Se in the enzyme participates in these redox reactions or has some other function is not known.

Several groups of investigators (*3*) have emphasized the role of GSH peroxidase as the primary mechanism for degrading low levels of H_2O_2 in cells. Since GSH peroxidase also acts on hydroperoxides of unsaturated fatty acids (*4*), the enzyme plays an important role in protecting membrane lipids, and thus the cell membranes, from oxidative disintegration. Failure of peroxide destruction can explain the hemolysis in vitro and oxidative damage to hemoglobin and possibly the wide variety of degenerative conditions that occur in Se deficiency. A role for Se in GSH peroxidase may also account for the apparent "antioxidant" effects of dietary Se observed by previous workers (*12*).

Our work offers new insight into the interactions of Se, vitamin E, and the sulfur-containing amino acids in preventing some of the same nutritional diseases. If vitamin E prevents fatty acid hydroperoxide formation, and the sulfur amino acids (as precursors of GSH) and Se are involved in peroxide destruction, these nutrients would produce a similar biochemical result—that is, lowering of the concentration of peroxides or peroxide-induced products in the tissues. Protection against oxidative damage to susceptible nonmembrane proteins by dietary Se but not by vitamin E (*2*) might explain why some nutritional diseases respond to Se but not to vitamin E (*1*). On the other hand, certain tissues or subcellular components may not be adequately protected from oxidant damage because they are inherently low in GSH peroxidase even with adequate dietary Se. Damage to such tissues would be expected to be aggravated by diets high in unsaturated fatty acids and to respond adequately to vitamin E but not to Se.

Measurement of GSH peroxidase may provide a useful means for defining Se requirements and for identifying Se deficiency in animals and humans. With purified GSH peroxidase it should be possible to identify the active form of Se and further clarify its role.

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Haptic Illusion: Apparent Elongation of a Disk Rotated between the Fingers

Abstract. *A disk (coin) turned end over end between thumb and forefinger feels longer to the turning hand. The illusion grows rapidly for 30 seconds but does not become asymptotic within 60 seconds. The illusion increases with coin size and turning rate, and is independent of holding pressure. It appears to involve illusory mechanisms in both hands.*

This report describes an illusion which I first observed while idly turning a penny end over end between my thumbs and forefingers, using two hands. The coin was held on edge so that the balls of the thumb and forefinger of the holding hand were separated by its diameter. This diameter served as the axis about which the same digits of the other hand turned the coin. The thumb of the turning hand pushed on one rim and the forefinger pulled the opposite rim around. As the fingers came together, the thumb slid back so that it could engage the other rim while the index finger moved forward to catch the edge released by the thumb. This operation was repeated over and over, turning the coin end over end. The coin seemed to stretch