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Selenium Biochemistry

Proteins containing selenium are essential components of certain bacterial and mammalian enzyme systems.

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The element selenium was discovered by Berzelius in 1817 and the first organic compound containing selenium, ethylselenol, was prepared in 1847 (*1*). However, for many years few organic selenium compounds were known and, for the most part, these were considered mere chemical curiosities. Eventually, in the 1930's, selenium was identified as a potent toxic substance for cattle and other livestock, and this focused attention on the biochemical properties of selenium compounds. In 1957 selenium was identified as the essential component of a dietary factor that protected rats from severe necrotic degeneration of the liver (*2*). This finding soon led to the recognition by animal nutritionists that several important livestock diseases are actually selenium deficiency syndromes (*3, 4*). Nevertheless, even today the toxicity of selenium and selenium-containing compounds is more generally appreciated than is the essential nature of this micronutrient. It is becoming increasingly evident that animals, bacteria, and possibly higher plants all require trace amounts of selenium, and that when available in the proper amounts the selenium is incorporated in a highly specific fashion into certain functional proteins of the cell. When organisms receive more than micromolar concentrations of selenium, those enzyme systems that cannot distinguish it from its close chemical relative, sulfur (*5*), begin to substitute the selenium indiscriminately for sulfur in many cellular constituents. Because of the greater reactivity and lower stability of selenium compounds compared to the corresponding sulfur compounds, the cell may encounter metabolic problems which eventually can lead to death of the organism. In the present article an attempt is made (i) to discuss briefly those enzymic processes which do not distinguish selenium from sulfur and therefore may be important in selenium toxicity, and (ii) to summarize the current information concerning specific enzymic reactions in which selenium participates as an essential enzyme component.

Although selenium is present in detectable amounts in all soils, it is not usually present in toxic amounts except in semiarid regions in soils derived from cretaceous shales (*6, 7*). In humid climates, or under conditions of irrigation, most of the selenium is leached from soils of this type.

There is a group of plants known as selenium indicator plants that grow in semiarid regions in soils containing large amounts of selenium (*7*). A few of these plants are normally limited in distribution to such areas and, when cultured in the laboratory in solution or moist sand, they exhibit markedly improved growth in response to the addition of selenium (*8*). Selenium indicator plants that have been studied in some detail (*7*) are *Astragalus pectinatus* (narrow-leaved vetch), *A. bisulcatus* (two-grooved poison vetch), and *Stanleya pinnata* (prince's plume). Taxonomic descriptions of these and related species (*9*) usually mention the unpleasant odor of the plants. The bad odor is due to the presence in their tissues of large amounts of various selenium-containing organic compounds which are far more malodorous than their sulfur-containing analogs. In fact the amounts of organoselenium compounds in these plants are often sufficient to cause acute selenium poisoning of grazing animals. Death of the animal may occur within a few hours after ingestion of the toxic plants.

Types of Organoselenium Compounds in Green Plants

Accumulation of the selenium analogs of methionine, *S*-methylcysteine, gamma-glutamylcysteine, and cystathionine appears to account, at least partially, for the high selenium content of some species of selenium indicator plants of the genus *Astragalus*. From specimens of *A. pectinatus* which contained 1500 to 2000 parts per million (ppm) of selenium (1.5 to 2 grams per kilogram of tissue, dry weight) Horn and Jones in 1941 (*10*) isolated a crystalline material that, on the basis of

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its elemental composition, was deduced to be a mixture of one part of cystathionine and two parts of its selenium analog. About 80 percent of the total selenium of these plants was in compounds that were readily extracted with hot water. It is noteworthy that the authors reported considerable losses of the seleno compounds when these extracts were clarified by treatment with charcoal. This high affinity for charcoal suggests that much of the selenium may have been present in organic compounds containing an aromatic system or a heterocyclic ring with aromatic character, but no such selenium-containing compounds have yet been positively identified from natural products.

In 1960 Trelease *et al.* (*11*) reported the isolation of *S*-methylcysteine and its selenium analog from water extracts of *A. bisulcatus*. In their experiments the neutral amino acid fraction from which *Se*-methylselenocysteine was isolated contained about 80 percent of the water-extractable selenium derived from the plant specimens. These plants contained 1000 to 3000 ppm of selenium (1 to 3 grams per kilogram, dry weight). *Se*-methylseleno-L-cysteine was isolated from leaves of *A. bisulcatus* by Nigam and McConnell (*12*). From extracts of the seeds of this plant they obtained a gamma-glutamyl peptide of the seleno amino acid. This dipeptide, identified as gamma-L-glutamyl-*Se*-methylseleno-L-cysteine, was the principal toxic compound found in *A. bisulcatus* seeds. The free amino acid and the peptide were almost equal in their toxicity to mice.

$CH_3 - Se - CH_2CH(NH_2)COOH$

Se-methylselenocysteine

$CH_3 - Se - CH_2CH_2CH(NH_2)COOH$

selenomethionine

Recent experiments with labeled precursors showed that *A. bisulcatus* actively catalyzes transmethylation reactions in which *S*-adenosylmethionine acts as methyl donor and selenocysteine and cysteine act as methyl acceptors (*13*). Selenocystathionine was implicated as an intermediate in the interconversion of *Se*-methylselenocysteine and selenomethionine. These transformations presumably occur by way of the known transsulfurylation pathways and thus should involve conversion of selenocysteine and homoserine to selenohomocysteine and serine. In these studies, the three-carbon chain of *Se*-methylselenocysteine was shown to be derived from labeled serine (*14*). Details of the reactions wherein hydrogen selenide, H_2Se, is generated and incorporated initially into the organic compounds in *Astragalus* have not been reported. Also unknown are the control mechanisms that these plants must have evolved to limit the extent to which selenium analogs of the sulfur amino acids are incorporated into the proteins of their cells. It is postulated that enzyme systems that convert selenocysteine to its *Se*-methyl derivative and to the dipeptide of this methylated amino acid are especially active and thus serve as detoxification mechanisms for these plants. Unfortunately, information concerning the amounts and detailed chemical structures of organoselenium compounds in the macromolecular components of these and other living organisms is fragmentary and probably incorrect for the most part. Especially suspect are claims that selenocysteine (or selenocystine) was identified in acid hydrolyzates of proteins of plant and animal origin, because about 50 percent of this amino acid is lost when it is heated for only 2 hours at 100°C in 6*N* HCl; and after 6 hours the destruction is almost quantitative (*15*). Selenocysteic acid also is unstable to acid hydrolysis (*15*).

Although assays performed on a number of species of the genus *Astragalus* indicate that these plants accumulate selenium mostly in the form of water-soluble organic compounds of low molecular weight, selenium indicator plants of some other genera have been reported to accumulate large amounts of selenium in the form of inorganic selenate (*7*).

In contrast to the selenium indicator plants, food crop plants incorporate selenium principally in their proteins in a bound form when grown in soils rich in selenium. Those that normally contain high levels of sulfur (for example, cabbage) accumulate more selenium than do those that are normally low in sulfur (*16*). This suggests that marked substitution of selenium for sulfur occurs in the normal cell constituents of nonspecialized plants and this may account for their lack of tolerance to large amounts of selenium in the soil.

Mechanism of Selenate Transport and Metabolism

Information on the mechanism of entry of selenate into living cells and its activation and reduction to the level of SeH^- is fragmentary, but the few data that are available suggest that these processes are accomplished by the same enzymes that metabolize sulfate. In a few instances, the activities of specific enzymes on corresponding sulfur and selenium substrates have been compared directly, but in other cases conclusions have been based on indirect evidence derived from studies of competition between sulfur and selenium compounds. When investigating the activation of selenate in yeast Bandurski and co-workers (*17–19*), showed that adenosine triphosphate-sulfurylase, which catalyzes the formation of 5′-adenosine phosphosulfate (APS) from adenosine triphosphate (ATP) and sulfate (Eq. 1), exhibits equal or greater activity with selenate as substrate.

$$\text{Sulfate} + \text{ATP} \rightleftharpoons \text{APS} + \text{pyrophosphate} \quad (1)$$

When the purified sulfurylase was incubated with ^{75}Se-labeled selenate, ATP, and pyrophosphatase, small amounts of a radioactive nucleotide accumulated; this nucleotide could be adsorbed on charcoal and it migrated electrophoretically in a manner similar to APS. Although this selenium-containing nucleotide, presumably 5′-adenosine phosphoselenate (APSe), was not as stable as APS under the experimental conditions employed, the amounts detected in the various experiments were much less than would be predicted on the basis of its chemical instability alone. Thus it was suggested that APSe is less stable than APS in the presence of the sulfurylase. This might be due to the tendency of enzyme-bound APSe to transfer its selenate moiety to a group on the enzyme, or to water, more rapidly than it could be liberated as APSe. When phosphate liberation from ATP in the presence of substrate and added inorganic pyrophosphatase was used to measure sulfurylase activity, the extent of reaction with selenate (10 m*M*) was greater than that with the same concentration of sulfate. An exchange assay, in which ^{32}P-labeled pyrophosphate incorporation into ATP was measured, showed that ^{32}P-labeled ATP was formed in the presence of either sulfate or selenate. This provided further evidence of the enzymic formation of an adenylic acid–selenate anhydride.

There is a specific reductase termed APS reductase which reduces the sulfate moiety of APS to sulfite with the concomitant liberation of adenosine monophosphate (AMP) (*20*). This

enzyme occurs in organisms that oxidize sulfur compounds (for example, members of the genus *Thiobacillus*) and in the sulfate-reducing bacteria (genus *Desulfovibrio*) which utilize sulfate as a terminal electron acceptor for reactions intimately connected with their energy metabolism. The overall process has been termed "dissimilatory sulfate reduction" to distinguish it from the more widespread but quantitatively less significant "assimilatory sulfate reduction" process which is utilized for sulfate reduction by most of the nonspecialized microorganisms (*20*). In the latter process it has been established that an additional activation step (Eq. 2) wherein APS reacts with another mole of ATP to form 3′-phosphoadenosine-5′-phosphosulfate (PAPS) is required to generate sulfite from sulfate (*21*). The enzyme termed adenosine phosphosulfate kinase (APS kinase) which catalyzes this reaction has been purified from yeast and characterized (*17, 18, 22, 23*)

$$\text{APS} + \text{ATP} \rightleftharpoons \text{PAPS} + \text{ADP} \qquad (2)$$

A dithiol-linked reductase of yeast which reduces the sulfate moiety of PAPS to sulfite has been described (*24*); it has also been reported to occur in *Escherichia coli* and many other bacteria (*20*). Thus it seems that the microorganisms that reduce only small amounts of sulfate to sulfite for synthesis of sulfur-containing cell constituents must synthesize first APS and then PAPS as a substrate for the reaction, whereas the specialized bacteria that form large amounts of hydrogen sulfide as the product of their terminal electron acceptor reaction utilize a reductase specific for APS. By analogy one might expect, therefore, that in most organisms the small amounts of selenium needed for specific biosynthetic reactions would follow the same pathway and require the formation of 3′-phosphoadenosine-5′-phosphoselenate (PAPSe) as an intermediate and this would be the precursor of selenite. However, there is only indirect evidence that the yeast APS kinase can react with APSe to form PAPSe (*18*), and in the original studies no accumulation of the diphosphate derivative could be detected.

Another important role of PAPS in sulfur metabolism in various animal species is to serve as a sulfate donor (*23*) for sulfurylation of phenols and other compounds that are detoxified and excreted as sulfate esters and for synthesis of sulfate esters of steroids and numerous polymeric sulfate esters such as heparin, chondroitin sulfate, and mucopolysaccharides. It has not been determined whether any of these biological systems have the ability to form PAPSe and utilize its selenate moiety in comparable reactions to produce, in a high selenium environment, the corresponding selenate esters.

In the assimilatory sulfate reduction pathway of bacteria and fungi, the further reduction of sulfite (S^{4+}) to hydrogen sulfide (S^{2-}) prior to its utilization in the synthesis of sulfur amino acids and various organic mercaptans is accomplished by sulfite reductase (*25, 26*). The precise details of this six-electron-reduction process of sulfur are not understood and no free intermediates have been detected. The enzyme has been highly purified from yeast (*26, 27*), higher plants (*28*), and *Aspergillus nidulans* (*29*), and in all cases it is a protein of high molecular weight containing a variety of bound cofactors. Among these are flavins, nonheme iron, sulfhydryl groups, and hemoprotein moieties, which suggest that sulfite reductase is in reality a multienzyme complex. Some preparations utilize either reduced viologen dyes or reduced triphosphopyridine nucleotide (TPNH) as electron donor whereas others fail to react with TPNH. Since sulfite reductase preparations are relatively nonspecific as regards the electron acceptor and reduce hydroxylamine and often nitrite, they may also have the capacity to reduce selenite but, unfortunately, none of the investigators report testing their highly purified enzyme preparations on selenite.

Although some studies show that crude bacterial extracts can reduce selenite to selenium and also to selenide (*30*), they do not establish that enzymes catalyze each individual step in the transfer of reducing equivalents from electron donor to the acceptor selenium compound. For example, the maintenance of a constant supply of a reduced thiol in the system might be sufficient to account for the observed results in view of the reported ease of reduction of selenite by thiols (*31, 32*). Critical studies with numerous nonenzymic controls will be necessary to clarify the nature of selenite reduction in the various biological systems.

In the sulfur pathway, many types of living organisms can utilize the element once it is in the form of hydrogen sulfide. A widely distributed enzyme, cysteine synthase (*20, 33*), catalyzes the formation of cysteine from serine and hydrogen sulfide. This is a two-step process in which serine is first acetylated by acetyl coenzyme A and the *O*-acetylserine is the actual substrate that is sulfurylated. This may be the major reaction for the introduction of inorganic sulfur into the pathways leading to synthesis of a great many organic compounds containing reduced forms of sulfur. If cysteine synthase can utilize hydrogen selenide in place of hydrogen sulfide, the reaction could serve also as a means of entry of inorganic selenium in the form of selenocysteine.

Metabolism of Methionine and Selenomethionine

Several enzymes that react with methionine can also efficiently metabolize its selenium analog, selenomethionine, and for at least one enzyme the selenium-containing amino acid is an even better substrate than the sulfur amino acid. Activation of methionine (*34*) by reaction with ATP to form *S*-adenosylmethionine (Eq. 3) converts the sulfur amino acid to a compound that serves as an efficient methyl donor for a wide variety of methylation processes.

$$\text{Methionine} + \text{ATP} \rightarrow \text{S-adenosylmethionine} + \text{tripolyphosphate} \qquad (3)$$

The adenosylating enzyme from yeast which catalyzes the reaction in Eq. 3 was shown by Mudd and Cantoni (*35*) to exhibit even better activity with selenomethionine as substrate. The resulting activated seleno amino acid, *Se*-adenosylselenomethionine, served as methyl donor for methylation of guanidinoacetic acid to creatine by hog liver guanidinoacetate methyltransferase (*35*). Studies of the relative activities of *S*-adenosylmethionine and *Se*-adenosylselenomethionine as precursors of polyamines, for example, whether the seleno compound can be decarboxylated by *S*-adenosylmethionine decarboxylase, seem not to have been reported.

Earlier experiments (*36*) showing that selenomethionine could substitute for methionine in reactions involved in protein synthesis have been substantiated and extended by studies on the individual enzyme steps (*37*). Thus both in *Escherichia coli* and in the rat, the methionine-activating enzyme reacts with ATP and either methionine

or selenomethionine to form methionyl-AMP or selenomethionyl-AMP, respectively. The activated amino acids are then transferred to methionyl-tRNA (transfer ribonucleic acid) indicating that neither the transferase nor the nucleic acid acceptor molecule distinguishes between the sulfur and the selenium compounds. Finally, the seienomethionyl-tRNA is utilized for polypeptide synthesis with the result that the seleno amino acid analog replaces methionine in the completed protein molecule.

Biochemistry of Other Organoselenium Compounds

Selenium-containing purine and pyrimidine bases. Both the thio and the seleno analogs of uracil, thymine, hypoxanthine, and guanine have been prepared by Mautner and co-workers and chemically characterized in some detail (*38*). Initially, interest in these thio and seleno bases was chiefly due to their inhibitory effects on growth and cell division, and their potential usefulness as antitumor agents was investigated extensively. More recently, because of recognition of the widespread occurrence of thio bases as minor constituents of tRNA, particularly in *E. coli* (*39*), experiments were conducted to see if selenium also is incorporated into nucleic acids (*40*). Growth of *E. coli* in a medium containing ^{75}Se-labeled selenite resulted in the incorporation of radioactive selenium into tRNA. After digestion of the labeled tRNA with ribonuclease the selenium was shown to be in the oligonucleotide fraction and thus presumably was present as a seleno base rather than being merely carried as a seleno aminoacyl derivative of a tRNA (for example, ^{75}Se-labeled selenomethionyl-tRNA). Although the radioactive seleno base or bases were not identified, it is possible that the selenium was incorporated into the 4-position of a uracil residue of the polymer. It is known that 4-thiouracil is a normal constituent of *E. coli* tRNA's and that it specifically occurs as the eighth base from the 5′ end of the tRNA molecules (*41, 42*). Moreover, it has been shown that the sulfur atom is introduced, after the polymer is formed, in a two-step process in which cysteine is the sulfur donor and ATP is required (*42*). The same purified enzyme system also catalyzes in vitro the transfer of selenium from selenocysteine into the *E. coli* tRNA molecule (*43*). The reaction product formed with this substrate contains 4-selenouracil as the eighth base from the 5′ end of the nucleic acid molecule. Whether the potential of this enzyme system to form the selenium analog of uracil in tRNA is realized in nature is not known. Presumably, this substitution could occur when selenocysteine or one of its precursors is present in concentrations high enough to be competitive with the corresponding sulfur compounds. A derivative of 2-thiouracil also has been found in *E. coli* transfer nucleic acids (*44*) and 5-methyl-2-thiouracil (2-thiothymine) was identified as a minor constituent of rat liver glutamic acid and lysine tRNA's (*45*). Accordingly, if the enzymes that form these modified bases also fail to distinguish between sulfur and selenium, then the corresponding seleno bases should eventually be found in the naturally occurring polymers. The biological effects of these subtle modifications in structure of these important biopolymers are not known in detail but they are probably of considerable importance.

Occurrence and metabolism of selenium analogs of sulfur-containing vitamins and coenzymes. The selenium analogs of a few of the sulfur-containing vitamins and growth factors have been synthesized and tested for biological activity, but information concerning their natural occurrence is virtually nonexistent. At best, only exceedingly small amounts of such organoselenium compounds can be expected to occur and, unless they can be marked with a radioactive isotope (*46*) or unless a specific and sensitive assay becomes available, they will be very difficult to detect.

Selenium has been substituted for sulfur in the cysteamine moieties of pantethine (*47*) and coenzyme A (*48*) and preparations of 4′-phosphoselenopantethine, *Se*-acetyl-4′-phosphoselenopantetheine, and *Se*-malonyl-4′-phosphoselenopantetheine are described (*49*). The thiol ester, *S*-malonyl-*N*-caproylcysteamine (*50*), was reacted with the selenol to form the selenol ester, *Se*-malonyl-4′-phosphopantetheine. Selenopantethine was found to be just as effective a growth factor as pantethine for *Lactobacillus helveticus* (*51*) and, presumably, utilization of the selenium analog should lead to the formation of seleno-coenzyme A. The selenium analog of coenzyme A was reported to be neither a cofactor nor an inhibitor of phosphotransacetylase of *Clostridium kluyveri* (*48*), but difficulties in maintaining seleno-coenzyme A in the reduced (—SeH) form may have been responsible for the observed inactivity. Apparently, other purified enzymes dependent on coenzyme A have not been tested for their ability to use the selenium analog. Although the biosynthesis of seleno-coenzyme A in the normal rat has been reported (*52*), this conclusion was based only on the observation that, after injection of ^{75}Se-labeled selenite, radioactivity was found associated with coenzyme A that was isolated chromatographically. Neither the purity nor the identity of the ^{75}Se-containing material was established.

Whether the selenium analogs of thiamine, biotin, and lipoic acid occur in nature seems not to be known. The exact mechanism of biosynthesis of thiamine has not been elucidated, but it is known that, in yeast, methionine provides an intact *S*-methyl group for biosynthesis of the thiazole portion of the molecule (*53*). Thus, in those organisms capable of synthesizing thiamine, it is possible that, when selenomethionine is available the seleno analog of thiazole might be formed and incorporated into the complete vitamin. In attempts to demonstrate synthesis of ^{75}Se-labeled selenothiamine in *E. coli*, Shrift (*54*) fractionated extracts of cells that had been grown in the presence of ^{75}Se-labeled selenite. Radioactive spots on chromatograms coincident with thiamine were found but the selenium-containing compound was not identified.

In view of the apparent ease of conversion of desthiobiotin to biotin by a number of microorganisms (*55*), it is not unreasonable to expect that they might insert selenium instead of sulfur in the presence of high concentrations of selenium. Organisms that synthesize relatively large amounts of biotin should be the biological material of choice in which to test this hypothesis.

Analogs of still another sulfur-containing coenzyme, lipoic acid or 6,8-dithiooctanoic acid, in which one or both of the sulfur atoms are replaced with selenium, also appear to be unknown.

The sulfur and selenium analogs of acetylcholine were synthesized and characterized by Mautner (*56*) and the effects of substitution of sulfur and selenium for oxygen on rates of hydrolysis of these esters by acetylcholine esterase were determined (*57*). Both the *S*-acetyl and the *Se*-acetyl esters were

hydrolyzed. The apparent value of the Michaelis constant (K_m) of the selenium ester was about $0.1 \times 10^{-4} M$ compared to about $1 \times 10^{-4} M$ for the oxygen ester, whereas the V_{max} (maximum velocity) value of the selenium ester was only one-half that of the normal substrate (*56*).

Effects of replacement of volatile sulfur atoms with selenium on properties of nonheme iron proteins. Several members of a family of nonheme iron electron transport proteins contain iron and sulfide bound to cysteine sulfhydryl groups. Upon acidification the bound sulfur is lost as hydrogen sulfide and hence is termed "volatile sulfur." Part or all of this volatile sulfur can be replaced with selenium without loss of biological activity in putidaredoxin (*58*), parsley ferredoxin (*32*), and adrenodoxin (*58*, *59*). Reconstitution of the apoproteins in the presence of ^{77}Se- or ^{80}Se-labeled selenite and a reducing agent (dithionite) yielded the corresponding iron-containing proteins in which sulfur was replaced by selenium; those proteins were characterized by electron paramagnetic resonance (EPR) spectroscopy. Adrenodoxins containing either one atom of ^{77}Se and one atom of ^{32}S, or one atom of ^{80}Se and one atom of ^{32}S, also were prepared and studied (*59*). By comparing the EPR signals from these reconstituted proteins with those from the native forms it was concluded that the spectral shifts detected were due to differences in the positioning of sulfur and selenium atoms in the iron centers of these proteins.

Proteins and enzyme systems in which selenium is an essential element. Selenium was recognized as an essential nutrient for the prevention of several deficiency disease syndromes, such as white muscle disease (*60*) of cattle, sheep, hogs, and poultry (*4*), exudative diathesis of chicks (*3*), liver necrosis of rats (*61*), and certain types of muscular dystrophy in a variety of animals (*62*), several years before anything was known about the nature of the biochemical reactions that might be involved.

Recently, a selenoprotein of low molecular weight was isolated from the heart and semitendinosus muscles of lambs fed a diet supplemented with selenium compounds (*63*). Little or none of this protein is found in the muscles of animals fed a selenium-deficient diet or in animals suffering from white muscle disease. To isolate it, normal animals are injected with ^{75}Se-labeled selenite and 16 hours later the heart and semitendinosus muscles are removed and homogenized. By standard protein fractionation methods, a ^{75}Se-labeled protein with a molecular weight of about 10,000 is obtained in highly purified form from the animals fed the selenium-supplemented diet and from normal animals. This ^{75}Se-labeled protein is not detected in muscle preparations from selenium-deficient animals. Although this muscle protein tentatively is termed a "selenium-binding protein" (*63*) it is also possible that the selenium is actually in an organoselenium compound covalently attached to the protein. Analyses performed on acid hydrolyzates of the purified protein show that aspartate, glutamate, lysine, glycine, and leucine are the predominant amino acids present. A small amount of methionine and traces of cysteine or cystine were found. A seleno amino acid, even if present in the protein, probably would not have been detected. The spectral properties of this interesting protein indicate that it contains a heme group similar to the chromophore of cytochrome c. Although at present the biological function of the muscle selenoprotein is unknown and there is no specific enzymic assay for its detection, its properties suggest that it may participate in an oxidation-reduction process.

A second protein of mammalian origin that appears to require selenium for its production is the glutathione peroxidase of erythrocytes (*64*). Earlier observations that oxidative damage and hemolysis of red blood cells caused by peroxide or ascorbic acid could be prevented by glucose addition in vitro (*65*) led to the finding that protection by glucose was poor if the cells were derived from selenium-deficient animals (*66*). The reaction steps that enable glucose to prevent peroxide-induced oxidative damage to erythrocytes are: (i) phosphorylation of glucose, yielding glucose-6-phosphate; (ii) oxidation of glucose-6-phosphate by TPN and glucose-6-phosphate dehydrogenase with generation of TPNH; (iii) reduction of oxidized glutathione (GSSG) to two molecules of GSH by TPNH and glutathione reductase; and (iv) utilization of the GSH by glutathione peroxidase (*67*) to reduce the hydrogen peroxide and convert it to two molecules of water (Eq. 4). The cyclic operation of this series of

$$2\,GSH + H_2O_2 \rightarrow GSSG + 2\,H_2O \quad (4)$$

reactions maintains the necessary intermediates and ensures that peroxides do not accumulate. Enzyme preparations from erythrocytes of selenium-deficient animals were low in glutathione peroxidase activity and thus were unable to use either glucose or GSH to destroy added hydrogen peroxide (*64*). The investigators at the University of Wisconsin further showed that preparations from red cells of normal animals that had been injected with ^{75}Se-labeled selenite exhibited normal levels of glutathione peroxidase activity and were radioactive. Approximately 60 percent of the ^{75}Se in these hemolyzates occurred in the glutathione peroxidase fractions isolated by a procedure that yielded highly enriched preparations of the enzyme (*64*). Meanwhile, Flohé and co-workers at Tübingen, who had studied in detail the mechanism and kinetic behavior of bovine glutathione peroxidase, reported that their crystalline enzyme, when subjected to neutron activation analysis, contained about 4 gram atoms of radioactive selenium per mole (*68*). From this they concluded that the native enzyme (molecular weight 84,000) that consists of four subunits of 21,000 probably contains 1 gram atom of selenium in each of its subunits. The amount of glutathione peroxidase in erythrocytes is very small, and the limited quantities of pure material available have not been sufficient to allow identification of the selenium-containing moiety. However, these studies (*64*, *68*) confirm that mammalian glutathione peroxidase is a selenoprotein and furnish an example of a specific oxidation-reduction reaction in which selenium plays a role.

In bacteria, as in animals, it was established as early as 1954 that selenium is a micronutrient essential to their growth (*69*). In this instance it was found that a particular metabolic activity, namely the ability to oxidize formate, depended on the presence of selenium, and also molybdenum, in the growth medium (*69*, *70*). Subsequently it was found that *E. coli* grown in the presence of ^{75}Se-labeled selenite selectively incorporated radioactive selenium into a protein fraction that exhibited formate dehydrogenase activity (*71*) but, because of the instability of the enzyme, it was not obtained in highly purified form. The amount of formate dehydrogenase in cells of *Clostridium thermoaceticum* also depends on the availability of selenium and molybdenum in the growth

Table 1. Effects of salt monovalent cations (50 m*M* solutions) on glycine reduction and ATP formation by a dialyzed glycine reductase preparation. The 0.5-ml reaction mixtures contained either 20 m*M* Na_2HAsO_4 (arsenate system) or 6 m*M* K_2HPO_4, 10 m*M* AMP, and 0.2 m*M* ADP (phosphate-adenylate system). Other reactants and conditions are described in (*77*).

Salt added	Arsenate system	Phosphate-adenylate system	
	Glycine reduced to acetate (μmole)	Glycine reduced to acetate (μmole)	ΔP_{10}* (μmole)
None	0.83	0.76	+ 0.95
Li_2SO_4	2.05	2.14	+ 2.50
$(NH_4)_2SO_4$	3.05	2.89	+ 2.30

* Amount of glycine-dependent ATP formed, measured as the amount of acid-labile phosphate cleaved in 10 minutes at 100°C.

medium (*72*). Homogenous preparations of ^{75}Se-labeled enzyme have been isolated from this organism, but the number of selenium atoms per mole of protein and the chemical form of the incorporated selenium are unknown. Preliminary studies on the formate dehydrogenases of *C. sticklandii* (*73*) and *Methanococcus vannielii* (*74*) indicate that selenium is an essential component of the enzyme from these sources also. It is likely that molybdenum is present in some or all of the formate dehydrogenases; moreover, nonheme iron is known to be a component of the *C. thermoaceticum* enzyme (*72*). Thus, during the catalytic reaction in which formate is oxidized to carbon dioxide, the iron, the selenium, and the molybdenum may participate in the electron transfer process.

The most recently discovered selenoprotein is the low molecular weight protein A of the clostridial glycine reductase system (*75–78*). This provides the first example of the obligatory participation of selenium in a specific electron transfer process that is coupled to the esterification of orthophosphate and synthesis of ATP (Eq. 5).

$$\begin{array}{l} CH_2COOH \\ | \\ NH_2 \end{array} + R(SH)_2 + P_i + ADP \rightarrow$$

$$CH_3COOH + NH_3 + R\begin{array}{l} S \\ | \\ S \end{array} + ATP \quad (5)$$

The stoichiometry of the glycine reductase reaction indicated in Eq. 5 is supported by numerous chemical balance experiments of the types shown in Table 1 [see also (*75*)]. Thus, for each two reducing equivalents utilized in the conversion of glycine to acetate and ammonia there is generated one equivalent of ester phosphate. In the enzyme system in vitro, phosphate can be replaced by arsenate without affecting the rate of the reaction, but with arsenate there is no requirement for an adenylate acceptor (Table 1). This indicates that substitution of arsenate for phosphate results in the formation of an unstable arsenate intermediate that spontaneously hydrolyzes. The activation of glycine reductase by monovalent cations (K^+ and Rb^+ can replace NH_4^+) may be related to conformational effects required for interaction of the protein components of the system.

In the purified enzyme system in vitro, a dimercaptan such as 1,4-dithiothreitol or 1,3-dimercaptopropanol serves as electron donor and bypasses a more complex series of electron carriers that normally transfer reducing equivalents from reduced pyridine nucleotides (reduced diphosphopyridine nucleotide, DPNH and TPNH). In both cases glycine is the terminal electron acceptor and is reduced to acetate and ammonia. The soluble glycine reductase system that catalyzes the reaction in Eq. 5 consists of at least three, and probably four, different proteins. Two of these have been isolated in homogenous form and a third has been considerably purified (*78*). The selenoprotein (protein A) is a heat stable, acidic protein with a molecular weight of about 12,000. It is easily obtained in pure form if a reducing agent such as dithiothreitol is included in all solutions to protect the protein from oxidation and aggregation during the isolation procedure. Preliminary studies indicate that it contains 1 gram atom of selenium per mole. Growth of *C. sticklandii* in media containing ^{75}Se-labeled selenite (1 μM) results in the synthesis of highly radioactive protein A. By contrast, ferredoxin, a protein of molecular weight of 6000, which contains approximately eight cysteine and approximately eight iron sulfide residues, and is produced by this organism in large amounts becomes only slightly radioactive. This shows clearly that little substitution of selenium for sulfur occurs under the growth conditions employed and that the incorporation of selenium in protein A is a highly specific process.

Table 2. Activation of resolved glycine reductase system by the addition of ^{75}Se-labeled protein A. For details of the steps in the assay system see (*78*). No glycine is reduced unless protein A is added.

Purification (step)	Amount of protein A added (μg)	Radioactivity (count/min)	Amount of glycine reduced (μmole)
2	416	36,800	2.13
5	7.5	19,800	1.06
5	15.0	39,600	2.12

When protein A was isolated from extracts of bacteria grown on ^{75}Se-labeled selenite, the ^{75}Se content of the fractions and their activity in the glycine reductase assay were enriched in parallel (Table 2). Addition of a few micrograms of the highly purified material obtained at step 5 of the isolation procedure (Table 2) (*78*) restores full glycine reductase activity to a protein fraction containing the remaining components of the enzyme complex. This biological assay for the selenoprotein is both precise and sensitive.

When *C. sticklandii* is grown in media deficient in selenium, protein A synthesis occurs only during the early stages of growth and ceases when the selenium is exhausted. The other proteins of the glycine reductase system continue to be formed and, if extracts of the deficient cells are supplemented with protein A, full glycine reductase activity is restored. It is not known whether an inactive form of protein A is produced by the cell in the absence of selenium or if the synthesis of the entire polypeptide chain ceases. On the basis of earlier studies it was concluded that protein A is a sulfhydryl protein since, after reduction with dithiothreitol, treatment with iodoacetamide destroys its biological activity in the glycine reductase reaction. Recent evidence suggests that an SeH group rather than an SH group may be modified by the alkylating agent. Degradation of ^{75}Se-labeled protein A by chemical and enzymic procedures, following alkylation of the protein, allows the isolation of the labeled material in good yield in a fraction of molecular weight of approximately 200 to 300 (*73*). There are some indications that the selenium-containing moiety of protein A

may be an aromatic substance or a heterocyclic compound with aromatic properties. As to the precise chemical role of the selenoprotein in the glycine reductase reaction, it is likely that it has a carrier function rather than, in the strict sense, a truly catalytic function. This conclusion is based on the fact that several other heat stable proteins of low molecular weight have proved to be electron carriers, acyl group carriers, or carbon dioxide carriers, for example.

The greater reactivity and lower oxidation-reduction potential (*1*, *15*) of some organoselenium compounds compared to their sulfur counterparts makes the selenoprotein A of the glycine reductase system an especially attractive candidate as an electron carrier that may also participate in the energy conservation portion of the reduction process.

Summary

The toxicity of selenium to animals and plants has been known and extensively documented since the 1930's, but it is only during the past 15 years that selenium has also been shown to be an essential micronutrient for animals and bacteria. Very little is known about the specific role or roles of selenium and, to date, there are only three enzyme-catalyzed reactions that have been shown to require the participation of a selenium-containing protein. These are the reactions catalyzed by (i) formate dehydrogenase of bacteria, (ii) glycine reductase of clostridia, and (iii) glutathione peroxidase of erythrocytes. The common denominator of these selenium-dependent processes is that they are all oxidation-reduction reactions. A fourth selenoprotein has been isolated from skeletal muscle of sheep but its catalytic function has not been identified. The form in which selenium occurs in these selenoproteins is unknown. The selenoprotein of clostridial glycine reductase contains selenium in a covalently bound form. Studies in progress indicate that this may be an organoselenium compound not previously detected in nature. Identification of the chemical nature of selenium in proteins participating in electron transport processes should enable us to determine its specific role and to understand the basic defects in certain cardiac and skeletal muscle degenerative diseases which are selenium-deficiency syndromes. The greater availability and ease of isolation of the selenoprotein of the bacterial glycine reductase system makes this the biological material of choice for studies on the mechanism of action of selenium. An added attractive feature of this system is that it can conserve the energy made available by the reductive deamination of glycine in a biologically useful form by synthesizing ATP.

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What Happened at Hawthorne?

New evidence suggests the Hawthorne effect resulted from operant reinforcement contingencies.

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In the folklore of behavioral science, the Hawthorne effect is cited again and again to show how variables can be unwittingly confounded in an experiment because of some aspect of the experiment itself. Certain independent variables were manipulated in the Hawthorne studies, but subjects' response rates supposedly rose regardless of any particular manipulation. Although this article will question that supposition, there was indeed a Hawthorne effect. Undoubtedly something other than what the experimenters explicitly introduced made workers' productivity increase. But what was this extraneous variable?

It is time to reexamine the Hawthorne effect in light of more recent research in human behavior, rather than speculate about the effects of morale, milieu, supervision, and group influences—although these will be discussed later. This article directs attention to circumstances in the Hawthorne experiments hitherto unreported or disregarded. A variable that had remained in obscurity emerges: the consequences of responding. The variable consisted of information feedback coupled with financial reward. Operators were told what their output rates were, and the higher the rates, the more money they earned. This interpretation of the Hawthorne effect has not been previously advanced (*1*), if only because it was not realized that the workers received knowledge of results on a daily, or even more frequent, basis.

Seven studies—not the one or two frequently mentioned in secondary sources—took place between 1924 and 1932 at the location from which the research drew its name, the Chicago plant where the Western Electric Company manufactures equipment for the Bell Telephone System. All seven were concerned with workers' productivity—their response rates. One should recognize and admire the considerable scope and pioneering nature of this research, regardless of the difficulties the investigators encountered and the myths that have accumulated over the years.

One of the myths surrounds the first three studies, which tried to determine how changes in illumination would affect the production rates of girls who inspected parts, assembled relays, or wound coils. In most cases, the subjects were reported to have worked progressively faster, regardless of changes in illumination, and some authors of secondary sources have referred to these studies as the locus of the Hawthorne effect (*2*). But is such emphasis justified? These early investigations were the impetus for the later ones and for that reason were briefly described in the principal account of the Hawthorne research (*3*). But the only published source for that account was a 12-paragraph news report (*4*) and a Western Electric memorandum (now unavailable) describing a supplementary "informal" study (*5*). No report of the research that satisfied elementary requirements of scientific description—quantitative data and experimental operations—was ever issued (*6*). The myth lies in the implication that weighty conclusions rested on sufficient evidence.

Relay Assembly Test Room Experiment

The actual source of the Hawthorne effect, the Relay Assembly Test Room experiment, lasted from 25 April 1927 to 18 June 1932. It contained 24 experimental periods in which two independent variables were manipulated (*7–11*). These were rest pauses and duration of work (*12*). Five girls performed tasks that required procedural memory and visual discrimination, finger dexterity, and hand-eye and hand-hand coordination. (A layout operator kept the others supplied with parts and assigned them work.) A relay consisted of as few as 26 parts or as many as 52, but generally between 34 and 38 (half of them dissimilar). To assemble one, a girl would take parts from a basket, an armature rack, and a coil box; reject faulty parts; arrange the parts in a jig and hold them together with pins; and, finally, replace the pins with four machine screws. One analysis showed 32 motions of the right hand and 31 of the left, 21 of each set being at the same time. According to Whitehead (*10*, pp. 62–63), "each operator employed several dozen different working patterns during the test" and "each operator showed a strong tendency to assemble relays in short runs or groups, each run typically containing anything up to about 10 consecutive relays." Operators had individual styles and tempos. Adjoining runs had different average speeds.

Over the course of the experiment, each operator assembled more than 100,

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