Ann. Rev. Nutr. 1983. 3:53–70

BIOLOGICAL ACTIVITY OF SELENIUM

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CONTENTS

INTRODUCTION

Although the nutritional essentiality of selenium was recognized as early as 1957, attempts to uncover biochemical functions of the element in animals were unsuccessful until 1973. In that year selenium was shown to be a component of glutathione peroxidase (67). No additional specific biochemical functions of selenium have been discovered in animals in the intervening years.

0199–9885/83/0715–0053$02.00

However, several important metabolic effects have been described, and it seems likely that future work will identify selenoenzymes underlying them.

The purpose of this chapter is to review some of the physiological metabolic effects of selenium, and to relate them to effects of the element in intact animals. Pharmacological and toxic doses of the element are not considered here. Selenium metabolism and function in bacteria (75) and in plants (7) are also outside the scope of this review. Diplock (30) reviewed some other effects of selenium and Bopp et al reviewed selenium metabolism (5).

EFFECTS OF SELENIUM ON GLUTATHIONE METABOLISM AND GLUTATHIONE-DEPENDENT ENZYMES

It seems quite logical that the biological roles of selenium and glutathione should be intertwined. Glutathione is the most abundant sulfhydryl compound in most cells and plays a prominent redox role. Selenium is in the same column of elements in the periodic table as sulfur, and thus shares many chemical properties with it. More reactive than sulfur chemically, selenium occurs much less abundantly in nature. Thus, it is not surprising that it has a catalytic role with respect to glutathione.

Glutathione Peroxidase

Studies of selenium-dependent protection against rat red blood cell hemolysis conducted in the laboratory of W. G. Hoekstra culminated in 1973 with the discovery that rat red cell glutathione peroxidase is a selenoenzyme (67). Shortly thereafter, Flohé et al reported that bovine blood glutathione peroxidase contained one selenium atom per subunit (36). Since then, this enzyme has been purified from a number of sources, including human tissues, and shown to contain selenium in each case. It has a wide distribution in animal cells and is present in mitochondria and in the cytosol.

Nutritional deficiency of selenium results in a decline in tissue selenium-dependent glutathione peroxidase activity. Rat liver activity falls to undetectable levels after only four weeks of a selenium-deficient dietary regimen (40). Other tissues experience a more gradual decline in glutathione peroxidase activity. These observations indicate that a major biochemical effect of selenium is to maintain glutathione peroxidase activity.

The understanding of selenium-dependent glutathione peroxidase was complicated in 1976 by the discovery of a nonselenium-dependent glutathione peroxidase activity (51). The glutathione S-transferases account for non-selenium-dependent glutathione peroxidase activity (65). These enzymes are found in fewer cell types than selenium-dependent glutathione peroxidase. In rat liver, glutathione S-transferases are present in cytosol, mitochondria, and

microsomes. Their glutathione peroxidase activity in microsomes appears to be less than that in the other sites (19).

Rat liver glutathione S-transferase activity—and presumably also non-selenium-dependent glutathione peroxidase activity—rises in selenium deficiency (52). The reason for this increase is unknown, but it may occur to compensate for the loss of selenium-dependent glutathione peroxidase.

Although the selenium-dependent and nonselenium-dependent glutathione peroxidases carry out the same reaction, many of their characteristics as enzymes differ (15). Selenium-dependent glutathione peroxidase destroys both H_2O_2 and organic hydroperoxides. Nonselenium-dependent glutathione peroxidase does not metabolize H_2O_2. It also has higher apparent Kms toward organic hydroperoxide substrates than selenium-dependent glutathione peroxidase does. Table 1 summarizes some of the properties of these activities.

The major physiological function of the glutathione peroxidases is thought to be the maintenance of appropriately low levels of H_2O_2 and other hydroperoxides in the cell. Accumulation of these reactive substances could lead to impaired function or death of the cell. Experiments in which H_2O_2 or organic hydroperoxides were added to isolated cells or perfused livers showed that the glutathione peroxidases function in intact cells (31, 72). Thus, H_2O_2 concentration in the cell may be regulated by selenium-dependent glutathione peroxidase acting in concert with catalase (48).

The role of the glutathione peroxidases in the metabolism of organic hydroperoxides is not as clear. Fatty acid hydroperoxides should be the major organic hydroperoxides formed in the cell. They serve as substrates for both glutathione peroxidases when present in unesterified form (63), but may not be available to the enzymes when esterified in phospholipids (57), as they are likely to be found in the cell. As a result, it cannot be stated with certainty that the glutathione peroxidases metabolize fatty acid hydroperoxides in the cell.

Table 1 Comparison of rat liver selenium-dependent and nonselenium-dependent glutathione peroxidases

Activity	Enzymes with activity	Substrates		Effect of Selenium Deficiency
		H_2O_2	Organic hydroperoxides	
Selenium-dependent Glutathione Peroxidase	Seleno-glutathione peroxidase	+	+	↓ ↓ ↓
Nonselenium-dependent Glutathione Peroxidase	Glutathione S-transferases	–	+	↑

Recent evidence suggests that selenium-dependent glutathione peroxidase may help regulate mitochondrial substrate oxidation (73). Oxidation of pyruvate decreased when hydroperoxide was being metabolized by selenium-dependent glutathione peroxidase, but no decrease was found when selenium-deficient mitochondria were used. This indicates that the selenoenzyme may have subtler functions than the destruction of hydroperoxides.

Glutathione S-Transferase (49, 80)

Glutathione S-transferase activity is 50–100% higher in selenium-deficient male rat liver cytosol than in the control (52). Therefore, the functions of these enzymes should be increased by selenium deficiency. The glutathione S-transferases catalyze the conjugation of electrophilic compounds and metabolites with glutathione, and thereby constitute one of the major detoxification mechanisms in the liver. They can also bind nonsubstrate compounds such as bilirubin and heme and are considered to be storage proteins. Some of these enzymes have nonselenium-dependent glutathione peroxidase activity, as discussed above.

Glutathione

Until recently, researchers knew little of the effect of selenium deficiency on glutathione metabolism. An early study indicated that selenium deficiency increased rat red blood cell glutathione (66). Others found no effect of selenium deficiency on liver glutathione concentration (18).

Liver glutathione concentration is determined by the rate of synthesis of the compound in the liver and the rate of glutathione release into the bile and the blood (2). Experiments with isolated hepatocytes and perfused livers indicate that selenium deficiency markedly accelerates glutathione synthesis in the rat liver (42)—which is balanced by an increased release of glutathione into the blood. There appears to be no effect of selenium deficiency on release of glutathione into bile.

Liver cysteine concentration is lower in the selenium-deficient rat than in the control (42), presumably because cysteine is used in the synthesis of glutathione. Glutathione concentration in blood plasma is two to three times higher in selenium-deficient rats than in controls as a result of the increased release of the compound into the blood.

At present it is not known why selenium deficiency increases hepatic glutathione turnover. The increase may function to raise the plasma glutathione level in compensation for the loss of plasma glutathione peroxidase activity. Alternatively, glutathione could be lost from the liver cell as a consequence of faulty regulation of its release. Little is known about the mechanism and regulation of glutathione release into the blood.

Recapitulation

Selenium deficiency markedly affects glutathione metabolism and some glutathione-dependent enzymes. Although the study of these effects is incomplete, some indications of their physiological consequences can be given.

Selenium deficiency causes a drastic fall in selenium-dependent glutathione peroxidase activity, which is likely to result in a rise in cellular H_2O_2 concentration. Higher levels of H_2O_2 can still be disposed of through catalase (48), so a new, but higher, steady-state level of H_2O_2 probably will emerge in selenium deficiency. This has not been measured directly. It is not clear what effect loss of selenium-dependent glutathione peroxidase has on fatty acid hydroperoxides in the cell because of the uncertainty over their metabolism and the presence of nonselenium-dependent glutathione peroxidase. Selenium-dependent glutathione peroxidase may play a metabolic regulatory role as proposed by Sies & Moss (73). Thus, selenium deficiency may decrease the ability of the mitochondria to adjust to changes in substrate concentrations.

Selenium deficiency causes an increase in hepatic glutathione S-transferase activity. This should provide more binding sites to allow increased "storage" of compounds such as bilirubin, heme, and other organic anions. More importantly, it and the increased glutathione synthesis should increase the ability of the liver to detoxify substances via the glutathione conjugation pathway.

The selenium deficiency–induced increase in hepatic glutathione synthesis depletes cellular cysteine, so it may impair cellular processes such as protein synthesis that require cysteine. It increases plasma glutathione concentrations due to an increased rate of glutathione release by the liver.

EFFECT OF SELENIUM ON XENOBIOTIC METABOLISM

There has been an increasing interest in the effect of selenium on xenobiotic metabolism, stimulated by reports that selenium can influence the toxicity and the carcinogenicity of a number of compounds. They show that selenium significantly affects the metabolism of xenobiotics. However, interpretation and comparison of these reports are often difficult owing to differences in experimental design.

Species and sex-related variations in xenobiotic metabolism must be considered. In some studies, excess selenium was administered to animals that had a normal selenium status. This was not always made clear by the authors. Since this is considered to be a pharmacological use of the element, it will not be considered further in this review. Here the focus is on the effects of selenium deficiency on xenobiotic metabolism.

An assessment of the severity of the selenium deficiency produced in a study presents a major interpretive problem. Most investigators use liver selenium-

dependent glutathione peroxidase activity as an index of selenium deficiency. Complete loss of this activity occurs after weanling male rats have been fed a selenium-deficient diet for only three to four weeks (40, 81). Therefore, this measurement has limited application in studies extending beyond a month. Since no other biochemical parameters are readily available for assessment of selenium status, the length of time the animals are fed the deficient diet appears to be the best practical index available. We note that male rats show a higher selenium requirement than females (71) and that feeding rats the deficient diet from weaning most efficiently produces selenium deficiency.

Cytochrome P-450

The hepatic microsomal cytochrome P-450 system consists of NADPH-cytochrome P-450 reductase (also known as NADPH-cytochrome *c* reductase because it is usually assayed with cytochrome *c* as the substrate) and a family of hemoproteins, the cytochrome P-450s. The cytochrome P-450 confers substrate specificity. It can be induced up to threefold by certain xenobiotics such as phenobarbital and 3-methylcholanthrene. A given xenobiotic characteristically affects certain forms of cytochrome P-450.

Two groups presented evidence that induction of the cytochrome P-450 system increases the selenium requirement, implying that selenium is necessary for proper function of the system. Siami et al demonstrated that phenobarbital induction of the system in rats was accompanied by an increased requirement for selenium as determined by growth rate studies (71). Combs & Scott, using plasma glutathione peroxidase activity, showed an increased selenium requirement in chicks caused by administration of polychlorinated biphenyls (28). Neither of these groups found effects of selenium deficiency on the cytochrome P-450 system. However, they did not measure the components of the system, but relied instead on rates of drug metabolism. One disadvantage of this approach is that a drug metabolism assay may reflect the amount of one form of cytochrome P-450 present, and changes in other forms of the hemoprotein may be missed unless substrates appropriate for them are used.

In 1975, Burk & Masters reported a detailed study of the effect of selenium deficiency on the hepatic microsomal cytochrome P-450 system (17). They used male rats fed the experimental diets for at least 12 weeks from the time of weaning. Selenium deficiency impaired the induction of cytochrome P-450 by phenobarbital, but had no effect on basal levels of the hemoprotein. NADPH-cytochrome *c* reductase activity was not affected by selenium deficiency. One drug metabolism test, ethylmorphine demethylase, was affected by selenium deficiency in the same way as cytochrome P-450. Another activity, biphenyl 4-hydroxylase, was not affected by selenium deficiency. When 3-methylcholanthrene was used as the inducing agent, selenium deficiency had no effect on the system. These results suggested that the predominant effect of

selenium deficiency was on the phenobarbital-induced form of cytochrome P-450.

Shull et al (70) conducted studies in rats maintained on a selenium-deficient diet for 11 months (first generation), and on rats born to selenium-deficient mothers and then fed a selenium-deficient diet for 15 weeks (second generation). The second generation rats had signs of severe selenium deficiency, including depressed weight and alopecia. Shull and associates made no measurements of cytochrome P-450 or of NADPH-cytochrome *c* reductase, but they assayed several drug metabolism functions dependent on that system. Selenium deficiency depressed aminopyrine N-demethylation, monocrotaline metabolism, and aniline hydroxylation activities in males. No effect was found in females. Second-generation males had greater impairment of these functions than first-generation males. Limited studies after phenobarbital treatment of the rats showed that first-generation males and second-generation females had subnormal induction of monocrotaline metabolism, but normal inductions of aminopyrine N-demethylase and aniline hydroxylase. Second-generation males, the most selenium-deficient rats in the study, were not used in the phenobarbital induction experiment.

Two other groups have measured cytochrome P-450 in microsomes from selenium-deficient rats. Neither noted an effect, but one group fed the rats the experimental diet only two weeks (24). The other group started the diet when the rats weighed 200 g and did not deplete liver glutathione peroxidase (23).

These studies indicate that selenium is required for the normal function of the rat hepatic microsomal cytochrome P-450 system. Moderate selenium deficiency impairs induction of cytochrome P-450 by phenobarbital. Severe selenium deficiency, as studied by Shull et al (70), and more recently by Burk & Correia (11), causes a decrease in cytochrome P-450 and some of its associated activities in the uninduced state.

Pascoe & associates have shown that selenium in the diet is necessary for the maintenance of rat small intestinal mucosal cytochrome P-450 levels (61). Institution of a selenium–deficient diet in normal adult rats caused a 45% fall in intestinal cytochrome P-450 content within a week. Pascoe et al found even more profound decreases in aryl hydrocarbon hydroxylase and ethoxyresorufin O-deethylase activities. This suggests that the intestinal mucosa obtains its selenium from the gut lumen rather than from the blood. It also demonstrates that selenium helps maintain cytochrome P-450 in sites other than the liver.

The liver microsomal cytochrome P-450 system was studied by Pilch & Combs in selenium-deficient chicks (64). They found no effect of the deficiency on NADPH-cytochrome *c* reductase or on cytochrome P-450 in uninduced or in phenobarbital-treated chicks. Both sexes studied gave similar results. The severity of the deficiency produced is hard to estimate from the data given but

appeared to be moderate. Based on this study, chicks may need less selenium than rats to maintain cytochrome P-450 levels.

The biochemical function underlying the support of cytochrome P-450 levels by selenium has not been identified. Heme is the prosthetic group of cytochrome P-450. Correia & Burk compared hepatic heme metabolism in selenium-deficient rats and in controls (11). They showed that selenium deficiency increased hepatic microsomal heme oxygenase activity, indicating that heme catabolism was occurring. They found no defect in heme synthesis (10). After phenobarbital administration, heme synthesis and catabolism increased strikingly in selenium-deficient liver (29). In the control liver, phenobarbital administration stimulated heme synthesis, but it diminished heme catabolism. These findings were interpreted as follows: Phenobarbital induces the synthesis of heme to be used primarily in the assembly of cytochrome P-450. In control animals heme and apocytochrome P-450 are produced and assembled; little heme is catabolized. In selenium-deficient animals the heme is produced but not efficiently assembled with the apoprotein. Consequently, less cytochrome P-450 is produced, and excess heme is present in the hepatocyte. This excess heme induces the catabolic enzyme microsomal heme oxygenase, which disposes of it.

Whether the impairment of cytochrome P-450 assembly in selenium deficiency is due to defective heme metabolism or to defective apocytochrome P-450 metabolism—i.e. impaired synthesis of the apoprotein—is not clear. Correia & Burk found that selenium deficiency caused a delay in amino acid incorporation into cytochrome P-450 after phenobarbital treatment in comparison with controls (29). However, the ultimate incorporation of amino acids into cytochrome P-450 was the same in selenium-deficient and in control rats. These investigators favored the idea that a defect in heme attachment to the apoprotein existed.

Newman & Guzelian examined the effect of selenium addition to cultured hepatocytes (59). They used immunochemical assays for specific cytochrome P-450 isoenzymes, whereas Correia & Burk had separated them as a group with polyacrylamide gel electrophoresis. Their findings indicate that selenium can increase the synthesis rate of the cytochrome P-450 isoenzyme induced by phenobarbital. Thus, whether selenium is necessary for apoprotein synthesis remains open.

Selenium does not exert its effect on cytochrome P-450 through selenium-dependent glutathione peroxidase. Injection of 50 μg of selenium into a rat corrects the abnormality in heme metabolism within 12 hr (29). There is no detectable recovery of glutathione peroxidase activity in this period. Therefore, the abnormality in heme metabolism appears to be due to an undiscovered function of selenium.

Other Xenobiotic-Metabolizing Enzymes

Selenium may affect drug-metabolizing enzymes other than cytochrome P-450 and glutathione S-transferase. In two separate studies, rats fed selenium deficient diets for 4 weeks showed increased UDP glucuronyl transferase activity (4, 26). In another study, where hepatic selenium-dependent glutathione peroxidase activity only decreased to 50% of control, UDP glucuronyl transferase activity reportedly fell in selenium deficiency (23). Although it seems likely that microsomal UDP glucuronyl transferase activity is increased by selenium deficiency, additional studies are warranted. Feeding weanling rats a selenium-deficient diet for 3 to 4 weeks did not affect hepatic soluble sulfotransferase activity. Table 2 lists the known effects of selenium deficiency on xenobiotic-metabolizing enzymes in rat liver. It is evident that this element has many effects on drug metabolism that could influence toxicity or carcinogenicity of certain chemicals.

OTHER EFFECTS OF SELENIUM

Vitamin E

Clearly there exist nutritional and biochemical relationships between selenium and vitamin E. In the most straightforward relationship, both nutrients are antioxidants and are therefore related in function (44). The attractiveness of this theory has pushed other hypotheses into the background in recent years. Observations by several groups indicate that selenium deficiency affects α-tocopherol metabolism. Two groups studying vitamin E–deficient rats reported that the low plasma vitamin E level found under these conditions was increased significantly by the simultaneous induction of selenium deficiency (20, 25). These workers felt that selenium influenced the distribution of vitamin E in the body. Fisher & Whanger recently showed a greater rate of tritium excretion in the urine of selenium-deficient rats than in controls after administration of tritiated α-tocopherol(34). They concluded that vitamin E is metabolized more rapidly in selenium-deficient rats than in controls. This hypothesis is supported by the recent report that selenium deficiency in the rat depresses hepatic microsomal α-tocopherol content (43). Thus, selenium deficiency appears to

Table 2 Effects of selenium deficiency on some rat hepatic xenobiotic-metabolizing activities

Activity	Effect of selenium deficiency
Glutathione S-transferase	↑
Cytochrome P-450	↓ (some isoenzymes)
UDP glucuronyl transferase	↑
Soluble sulfotransferase	0

favor the loss of α-tocopherol from the body. The cause of this loss has not been established but most likely relates to increased α-tocopherol oxidation under conditions where selenium-dependent antioxidant mechanisms are impaired.

Mercury

Inorganic mercury and methylmercury more severely affect selenium-deficient animals than controls (37, 60). Yet, despite the hundreds of published studies on selenium interrelations with mercury, the mechanisms by which selenium ameliorates the toxic effects of this heavy metal remain uncertain.

One hypothesis for selenium protection against mercury toxicity is that selenium causes mercury to be bound in tissue in an inactive form (37, 46). The selenium status of the animal affects the metabolism and distribution of mercury (12). These alterations could be related to the changes in glutathione and cysteine metabolism discussed above (1, 42). For example, the decrease in cysteine concentration could impair metallothionein synthesis, thus reducing mercury sequestration and leading to increased toxicity.

Mercury metabolism may lead to oxidant stress, either by inactivating protective enzymes or by producing free radicals (37). This hypothesis suggests that selenium prevents oxidant injury.

Others

Others ascribe different biological activities to selenium. Fischer & Whanger described an increased rate of [^{14}C]glucose oxidation to $^{14}CO_2$ in selenium-deficient rats (33). They estimated hepatic phospholipid turnover after administration of [1-^{14}C]acetate and found it to be more rapid in selenium-deficient rats than in controls. They suggested that selenium-deficient rats may have a higher metabolic rate than controls. We examined this proposition directly by measuring basal O_2 consumption in male rats. We fasted the rats overnight and put them into chambers and measured O_2 consumption hourly for 6 hr (13). Selenium-deficient rats consumed 1.22 ± 0.07 liters of 0_2/hr/kg body weight; controls consumed 1.23 ± 0.07. Thus, it appears that selenium deficiency does not increase the basal metabolic rate. This leaves unexplained the increased glucose oxidation and phospholipid turnover. It is possible that the increased glucose oxidation in selenium deficiency could be a reflection of impaired mitochondrial substrate regulation.

Selenium has long been recognized as a constituent of sperm and essential for spermatogenesis (39, 82). Calvin et al presented evidence that the element is present in a protein of the capsule surrounding the sperm mitochondria and they postulated a structural function for it (22). This could explain its essentiality for spermatogenesis, but would be the first described structural role for selenium.

EFFECTS OF SELENIUM IN WHOLE ANIMALS

Pure Selenium Deficiency

The early descriptions of severe manifestations of selenium deficiency—such as dietary liver necrosis in the rat (69) and exudative diathesis in chicks (68)—were reports of combined selenium-vitamin E deficiency. The production of a clinically manifest pure selenium deficiency proved difficult, but was first accomplished in the late 1960s (58, 76).

Male rats fed a selenium-deficient Torula yeast diet with adequate vitamin E from weaning weigh approximately 10% less than controls by six months of age. They maintain this weight difference throughout life. In addition, they may show a thin coat and spots of complete hair loss. Some selenium-deficient rats develop aspermatogenesis and atrophy of the semeniferous tubules by a year of age. The selenium-deficient rats seem as vigorous and hardy as the controls and have a similar life span.

More severe selenium deficiency can be produced by further depleting pups from mothers fed a selenium-deficient diet. Under these conditions, severe growth retardation occurs, and hair may never emerge unless selenium is provided in the diet (74). The males never produce sperm, and some develop cataracts (74). The life span of second-generation rats deficient in selenium has not been reported.

Selenium-deficient rats consume less food than controls. This is the major reason they gain less weight. However, selenium-deficient rats also have a lower weight-gain/food-intake ratio than controls, indicating poorer food utilization (32). The cause of appetite suppression in selenium deficiency is not known, but it has also been observed in chicks (8). It can be reversed within hours by selenium administration. The degree of appetite suppression appears to be related to the severity of the selenium deficiency. Second-generation selenium-deficient rats are severely affected; therefore, experiments with them should employ pair feeding.

The lens contains selenium-dependent glutathione peroxidase. Loss of this enzyme in selenium deficiency has been hypothesized to be the cause of cataract formation in selenium deficiency (53). The rather heavy incorporation of the element into the testis and into sperm—described from several laboratories (3, 6)—seems related to the aspermatogenesis of selenium deficiency. More specifically, it could be related to the sperm protein described by Calvin et al (22).

Effects of Selenium Deficiency on Responses to Drugs and Other Chemicals

Moderate selenium deficiency, such as that caused by feeding a weanling rat a selenium-deficient diet for four to eight weeks, produces a mild clinical

condition, described above. However, the metabolic effects of this deficiency markedly alter responses of the animal to a number of stresses. Some of these altered responses can be explained by effects of selenium discussed in previous sections. Other responses point to undiscovered biochemical functions of the element.

COMPOUNDS DETOXIFIED BY GLUTATHIONE AND GLUTATHIONE S-TRANSFERASES Many hepatotoxic compounds and metabolites are detoxified either by binding to glutathione S-transferases or by conjugation with glutathione by the actions of the transferases. As discussed above, selenium deficiency increases these processes in the rat liver.

Iodipamide is a hepatotoxic drug that is a nonsubstrate ligand of glutathione S-transferase (55). Selenium deficiency markedly diminishes its hepatotoxicity (13). The increased glutathione S-transferase of selenium deficiency could be sequestering iodipamide by binding it.

Acetaminophen metabolism by the hepatic microsomal cytochrome P-450 system produces a toxic reactive metabolite that is detoxified by conjugation with glutathione. In the rat, selenium deficiency protects against acetaminophen hepatotoxicity (13). This was demonstrated under conditions wherein the selenium deficiency did not affect cytochrome P-450 level. Therefore, the protective effect of the deficiency appears to reside in the increased glutathione-conjugating capability. In the mouse, selenium deficiency worsens acetaminophen toxicity (79). Present research cannot explain this phenomenon.

Aflatoxin B_1 is another compound that is activated by the cytochrome P-450 system and detoxified by glutathione conjugation. Chen et al reported that selenium deficiency inhibits covalent binding of aflatoxin B_1 in rat liver (26). The hepatotoxicity of aflatoxin B_1 is also markedly attenuated by selenium deficiency (14). Thus, in this case too, increased glutathione conjugation appears to explain the effect of selenium deficiency. From these three examples, we can predict that selenium may reduce the toxic effects of other compounds that act through intermediates that bind to glutathione S-transferases or that are conjugated with glutathione.

REDOX-CYCLING COMPOUNDS A number of drugs and chemicals in common use have been shown to undergo redox cycling. In the process, a molecule accepts an electron from an electron-transfer enzyme and then donates it to a substrate such as O_2. This produces $O_2^{\cdot-}$ and regenerates the original compound so it can repeat the process. The bipyridylium herbicides, paraquat and diquat, react in this way.

Redox cycling is thought to underlie the toxicity to animals of both diquat and paraquat. Their redox cycling depletes NADPH (the electron donor) and produces $O_2^{\cdot-}$ in the cell. The bipyridylium radical acts as the intermediate in this process. Paraquat and diquat are many times more toxic to selenium-deficient rats than to controls (16, 21). Doses of diquat that cause no injury to controls prove fatal to selenium-deficient animals within hours (16). A high rate of ethane production accompanying the toxicity indicates the occurrence of lipid peroxidation. Injection of selenium into selenium-deficient rats reverses the susceptibility to diquat-induced lipid peroxidation and mortality—before selenium-dependent glutathione peroxidase increases (16). This indicates that selenium protects by a mechanism other than glutathione peroxidase.

Nitrofurantoin, another redox-cycling compound, is a commonly used antibiotic. It is more toxic to selenium-deficient chicks than to controls (62). Selenium-deficient rats develop renal tubular necrosis after modest doses of nitrofurantoin that are not toxic to controls (14). Evidence of lipid peroxidation accompanies the renal injury. The toxicity of other redox-cycling compounds in use may be enhanced by selenium-deficiency. It is apparent that exposure of selenium-deficient animal or human populations to agents of this class should be approached with caution.

MERCURY Selenium-deficient animals are more susceptible than controls to poisoning by methylmercury and by inorganic mercury (37). The major hypotheses of selenium protection against mercury toxicity were discussed above.

Effects of Selenium Deficiency on Carcinogenesis

Selenium as an anti-carcinogenic agent has received much attention (38). Most of the work done involves administration of the element to animals already replete in selenium. Since the mechanisms involved in such "chemoprevention" are not likely to be physiological functions of selenium, they are not discussed further in this review. However, this does not deny their potential importance.

In most respects, chemical carcinogenesis presents an analogy to toxicity. Reactive metabolites are formed and detoxified. If detoxification is inadequate, or if metabolite formation is excessive, the reactive metabolite may cause cell necrosis or transformation to malignancy. Thus, we can expect selenium deficiency, by altering xenobiotic metabolism, to affect carcinogenesis.

Cohen's group examined the effects of selenium on the metabolism of the carcinogen, 2-acetylaminofluorene (4). Selenium deficiency increases the carcinogenicity of this compound (41). In rats fed the selenium-deficient diet for 3–4 weeks, they found increased urinary excretion of N-OH-acetylaminofluorene. This suggests that selenium may prevent the production of this

carcinogenic metabolite or promote its detoxification. Further, Ip & Sinha demonstrated that selenium deficiency increases the carcinogenicity of dimethylbenz[a]anthracene in rats (45). However, they did not examine its metabolism.

Selenium deficiency may decrease the carcinogenicity of some compounds as it diminishes some toxicities. Aflatoxin B_1 covalent binding decreases in selenium deficiency, and it seems likely that its carcinogenicity may also be decreased. Future work will examine this possibility.

Apparently, selenium protects against cancer induction by some agents. Because the theoretical possibility exists that it may actually promote carcinogenesis under other conditions, caution should be used in recommending its use in human beings.

Effects of Selenium in Man

Research efforts have identified groups of people with low selenium intakes and presumed selenium deficiency based on blood selenium levels lower than control values. The causes of reduced selenium intake have been reviewed elsewhere (9, 54).

In North America and most of Europe, blood selenium levels range from 15–25 μg/dl. Inhabitants of New Zealand and low-selenium regions of Scandinavia generally show blood selenium levels between 5–10 μg/dl owing to their low dietary intake of the element (77). The most severe selenium deficiencies in free-living humans have been reported in China. People from regions in China with low soil selenium content show blood selenium levels below 3 μ/dl (27). In other parts of the world, researchers observed similarly low selenium status in patients treated with either therapeutic diets low in selenium (56) or with parenteral alimentation (78).

Keshan Disease, a cardiomyopathy of children and young women in China, was first recognized in 1935 (27). As shown in recent years, Keshan Disease occurs exclusively in regions where selenium levels are extremely low, and prophylactic selenium supplementation can eradicate the disease (50). Thus, Keshan Disease is the first human disease shown to be related to selenium deficiency. Other factors may be involved in the disease, but selenium deficiency clearly plays a major role.

Several cases of cardiomyopathy associated with low selenium levels have been reported in patients nourished parenterally in the United States (35, 47). None have appeared from New Zealand yet, but one patient there developed leg muscle weakness and pain as a consequence of selenium deficiency after continued parenteral feeding (78). The biochemical function of selenium associated with the cardiomyopathy is unknown.

By analogy with the animal responses to selenium deficiency, it might be expected that people of low selenium status would have altered sensitivities to

the toxic and carcinogenic effects of certain drugs and chemicals. No evidence for this has yet been presented. However, comparison of side effects and toxicity of substances such as nitrofurantoin and paraquat in populations of different selenium status could be rewarding. The significance of selenium in human health and disease is just beginning to be understood.

CONCLUSIONS

Selenium has a variety of biological effects. Deficiency of the element, if very severe, may impair normal development and physiological functions. The major effect of selenium deficiency, however, appears to be alteration of the animal's response to chemical stresses, manifest under conditions of moderate selenium deficiency. A few stresses, such as liver injury due to aflatoxin B_1, are ameliorated by selenium deficiency. Others, such as paraquat toxicity and vitamin E deficiency, change in character and are markedly worsened in selenium deficiency. Recent research reveals that alterations in glutathione metabolism and in the activities of xenobiotic-metabolizing enzymes in selenium deficiency may be responsible for some of the changed responses.

ACKNOWLEDGEMENTS

The author is grateful to Mrs. Rebecca E. Ortiz for typing the manuscript.

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