# SELENIUM IN BIOLOGY

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## INTRODUCTION

On the basis of much fundamental research in nutrition (1–4), the value of combinations of sodium selenite with alphatocopherol in veterinary medicine was established in the early 1960s. The roles of Vitamin E and selenium (Se) in disease of animals have been clearly documented. A long history of confusion and controversy on the role of Vitamin E in nutrition (5–8) and in human medicine (9–12) is widely publicized. Although the clinical value of Vitamin E against intermittent claudication is accepted by many, the public use of supplementary Vitamin E has been deplored (12).

The fact that Se was suspect as a carcinogen in the United States until recently (13–15) delayed its application in combination with Vitamin E in human medicine along the lines developed by veterinarians (3, 5, 16). The clinical study of a suitable combination for control of human coronary disease followed logically from the successful application of various Vitamin E-Se products in veterinary medicine (16–18). The outcome of some of this experimentation will be discussed. The absolute essentiality of Se, independent of Vitamin E, was shown (19, 20). Nevertheless, their roles are interdependent (1, 3, 14).

Changes in attitudes toward Se are evident in the three editions of Underwood's classic book (21). From attention to only toxic aspects in the first edition, progress led to more attention to Se as a nutrient in the third edition than to any other element, save copper. Now more papers are appearing on Se in biology than on any other trace element. The role of Se and Vitamin E in biochemistry now suggests that their deficiency underlies various chronic human diseases (13, 14, 22). This appears consistent with the discovery that Se is the active grouping of important enzymes in animals (23, 24) and microorganisms (25). This discovery also appears consistent with findings that a high level of coenzyme $Q_{10}$ appears to be needed for host defense (26) and for cardiac function (27). Although the need for Vitamin E for ubiquinone biosynthesis was controversial for years, the need for Se for this function appears

well established (21, 28, 29). The cloud over Se has effectively inhibited investigation of the Se - Vitamin E - ubiquinone interrelationships.

Although Se was first feared because it could be taken up by plants at levels that could be toxic to animals (30, 31), it is now clear that Se functions more importantly in animals and microbes than in plants. Indeed, this difference may prove to be a major distinction between the animal and vegetable kingdoms (14). Animal tissues have far more avidity for Se than do plant tissues. The uptake of Se in the ecosystem was reviewed (32).

The proton and electron transfer properties of Se appear to be such that its biogeochemical function, supported by that of Vitamin E, appears to be intermediary between controlled dehydrogenations of metabolites in the respiratory chain and in avoiding adverse oxidations, particularly of unsaturated lipids (33). Evidence (34) suggests that Se is involved somehow in the still little understood coupling of oxidative phosphorylations via the cytochromes and SH compounds.

Discovery that Se may be nature's antidote to heavy metal toxicities ($Cd^{2+}$, $Hg^{2+}$, $Ag^{+}$, and possibly $Pb^{2+}$) has attracted much interest. The phenomenon was reviewed (14, 35) and is the subject of many current studies.

Recent discoveries that Se is involved in immune mechanisms (36, 37), although immediately tending to complicate the picture, may finally help to unravel its many diverse roles in homeostasis, as well as metabolism. The finding of Se in many functional proteins (38) and recently in selenouridine in tRNA (39) further bespeaks the versatility of this remarkable element.

The Se cycle deserves study in relation to the sulfur cycle (14, 22). Evidence suggests that selenium inadequacy may be increasing, particularly in the already Se-low areas. Unknowns in the Se cycle will be discussed.

Although Se has been impugned (40) at high levels as a cause of caries, this is not thought to occur at nutrient levels (41). There is some question whether Se inadequacy may increase susceptibility to periodontal disease in range animals (4, 42). Theoretically, this could result from diminished biosynthesis of coenzyme $Q_{10}$, recently postulated as essential to gingival integrity (43).

Selenium is known as the least plentiful but most toxic trace element. Its safe uses were established in veterinary medicine (44, 45) and for feed additive use in New Zealand (42) about 15 years ago. The need to clarify the safe uses of selenicals in human medicine is clear (4, 22). This review approaches many aspects of the Se problem, presents data on the satisfactory tolerance to selenite-tocopherol combinations in monkeys and man, and notes some of the clinical experience with this combination in heart disease. It bears briefly on the beneficial role of Se-tocopherol in arthritis.

## SELENIUM CHEMISTRY AND BIOCHEMISTRY

How the proton and electron transfer properties of Se influence its catalytic role in biochemistry appears as a primary question. The relation of Se to S is something like that of As to P. In both cases the trace elements catalyze the biochemical reactions of their chemical congeners. The rate of nucleophilic reactions for Se far

exceeds that of S or the transfer of carbon to carbon bonds. The involvement of Se in enzymes, in muscle proteins, and in tRNA, connotes its general catalytic role. Almost a third of the Se in the body appears in muscle. McConnell (38) determined these Se contents in μg/g for crystalline proteins: myosin, 25.9; aldolase, 12.6; cytochrome, 0.8; and hemoglobin, 0.49. The high Se content of myosin, the contractile protein of muscle, suggests that it may be involved in the release of chemical energy from high-energy bonds. Heart muscle has even more Se than other musculature. Although the role of Se in the heart has yet to be learned, its involvement in oxygen utilization seems assured.

The presence of Se in a cytochrome-like protein was confirmed by Whanger et al (47). This protein is diminished in muscle of Se-deficient lambs with white muscle disease, but restored by Se injections. The half-life of $^{75}Se$ in cytochrome of young female rats was reported to be only about 14 hr (38a). As noted by the authors, such rapid turnover reflects Schoenheimer's steady-state dynamism (48). Aerobic cells are known to synthesize their own chromoproteins independently, and cytochromes act as adaptive enzymes. The role of Se in the biosynthesis of ubiquinones may be interdependent with these biosynthetic mechanisms. Burk (49) failed to find Se in some of the cytochromes, of which there are many.

Whether Se forms transient persulfide links, S–SeH, in proteins, or transient selenotrisulfide links, –S–Se–S–, or both, remains to be learned. Ganther (50, 50a) supplied evidence for both possibilities. Burk (51) offered evidence against the involvement of selenotrisulfide. Levander et al (34) indicated that selenopersulfides fit the known reactions and that Se appears to amplify the role of S compounds in biology. Possible effects of Se and S compounds in relation to disease and aging were reviewed (52).

The ability of inorganic Se compounds to fulfill needs for the element (53) and the ability of animal cells to transform selenicals from a valance of +6 to –2 requires Vitamin E (33, 54). As noted elsewhere (14), the toxicity of selenicals appears to be due primarily to accumulation of selenite ions. Too high a concentration of these may disrupt SH metabolism. Removal of the excess selenite or its reduction to the selenide reduces the toxicity. Although $Se^{2-}$ is one of the most active and toxic chemicals known, animal cells employ it as a catalyst. The union of hydrogen to oxygen, the final step in the respiratory chain, is accomplished by $Se^{2-}$-dependent enzymes, glutathione peroxidase for one (23, 24).

There is little of the biology of metal selenides in Russian (55, 56) or English scientific literature (57). The importance of Se as a factor in minimizing heavy metal toxicities is relatively recent (35, 24), however, and this attribute of the biochemistry of Se was hardly predictable. There is a need to learn much more about the solubilities and properties of the metal selenides and selenites in biological fluids and in cells (55). It seems likely that the variable affinity of $Se^{2-}$ and $S^{2-}$ compounds for metal ions will be found to be the basis for many homeostatic mechanisms. Little is known as to the relative affinity of the various ligands for metal ions as against their affinity for Se and S compounds. Undoubtedly, many reactions previously assigned to reactions with SH compounds will have to be reconsidered in terms of reactions with SeH compounds, or perhaps, –S–SeH compounds.

The evolutionary background of energy mechanisms in biogeochemistry (58) notes that iron in specialized structures, such as the porphyrins and nonheme iron-containing proteins, played a key role in oxygen transport and metabolism. The new found role of Se in energy mechanisms and in cytochrome-like enzymes and glutathione peroxidase may throw new light on evolutionary developments. The Se findings are so new and the antipathy toward it has been so great that reference to Se was omitted from most reviews dealing with mechanisms of energy transduction in biological systems (59). In such reviews, the role of the ubiquinones is described. Again, however, little attention has been paid to its nutritional antecedents, or to the early association (38, 38a) of Se with muscle proteins, or with the cytochromes. The recent findings relating Se-dependent enzymes to microbial metabolism (25) and to energy mechanisms in animals (23, 33, 34, 54) call for the inclusion of Se-dependent enzymes in the consideration of most biological energy transformations. From the standpoint of evolution one may ask how nature developed its marvelous controls involving Se. Does Se act in some way as a catalyst with Fe-dependent and Cu-dependent enzymes? Are the proton and electron transfer properties of Se-dependent enzymes the keys to many little understood steps in metabolism?

The tendency to view Se and S biochemistry similarly came naturally, so much so that a major treatise was devoted to organic Se compounds (46). As noted by Martin (53), however, Se as selenite or selenate fulfills nutrient needs for the element. Due to its extreme reactivity, most organic compounds of Se are unstable. Some reactions, such as transmethylations, may proceed more rapidly for selenomethionine than for methionine (3, 25). There appears little evidence, however, that organic selenicals play an essential role in nutrition. The organic selenides can apparently (53) be converted in the animal to the selenite form. This can account for the common pathway of selenite accumulation to above tolerated levels for both inorganic selenicals and the variety of organic selenides made by plants (14).

Although organic selenicals of relatively low toxicity have been described (153), none are known to be commercially available. To the authors' knowledge, none have been tested comparatively in farm animals or in humans against selenite or selenate. Besides the question of cost, there is the critical question of stability. Organic selenicals tend to be unstable and their stability in feeds or in pharmaceutical preparations would have to be assured. This is not intended to discourage such research. On the other hand, as recently noted by the progenitor of all this (154), toxicity is a relative matter. Selenite-tocopherol combinations have been used safely in veterinary medicine for over a decade and there is recent evidence for their safety in human medicine.

## THE SELENIUM CYCLE

Although Se and S have many common properties, their cycles in nature differ markedly (14). Selenium dioxide, an oxidant, is promptly reduced in the atmosphere to elemental selenium, $Se^0$. It apparently falls out rapidly as particulate $Se^0$ or may be carried very long distances in the vapor state. Some evidence (60) indicated that the Se content of glacial ice in Greenland had remained constant for centuries, while

the content of S had increased in recent decades. Only 4 of 123 samples of Spanish moss were found to contain measurable Se and that at very low level (61). Thus Se appeared to act quite differently from As, Cd, Cr, Co, Cu, Pb, Ni, and Vd, which were all found in Spanish moss samples where high rates of vehicular and industrial emissions occurred. This finding conforms to a report from England (62) noting that Se was found in the atmosphere at lower concentrations than any other essential element, except possibly iodine. It had the lowest washout factor of any element suggesting its transport in air for only short distances, or perhaps its transport as vapor in the upper atmosphere. It was surprising in light of the above, therefore, to find evidence that Se and other volatile elements appear to be enriched in the atmosphere at the South Pole (63).

The finding of up to 7 ppm of Se in zooplankton in Lake Michigan near Chicago led to its designation as an unknown pollutant (64). The accumulation of Se in plankton as part of the food chain from water to land animals has been described (32). Thus, there seems little evidence to view Se as a pollutant, even though large amounts of Se emanate from the burning of fossil fuels (65, 66). It may be noted that in the last-named study Se was not included in the table of particulate emissions. Government sources (67) reveal that about one thousand tons of Se are released from all industrial sources, including fossil fuel burning. This is about the same amount of Se obtained industrially from the refining of metals.

Contrary to viewing Se as a pollutant, the concept was expressed (14, 22). that nutritional inadequacy of Se may be resulting from losses of available Se in the Se cycle. This concept began in part from consideration of a discovery in New Zealand (68) that less that 1% of $^{75}Se$ selenite fed to sheep was excreted in a form available to plants. In more recent work (69) it was noted that whereas S compounds were excreted via the urine, the Se was excreted via the feces as $Se^0$. The authors stated, "It has been suggested that there is a selenium cycle in Nature, but there is no evidence for some parts of this cycle, particularly where grasslands are concerned." The authors also cited the observation that, whereas colloidal Se would be oxidized by the atmosphere in alkaline or mildly acid conditions to selenite, $Se^{4+}$, further oxidation to selenate, $Se^{6+}$, would not occur. Selenite is adsorbed onto iron oxide and in this form is relatively unavailable to plants. Lakin (31, 76b) noted the high levels of Se in the high-Fe, acidic soils of Hawaii, but the relatively low levels of Se in the plants there.

Selenate is readily leached from alkaline soils. A great deal of Se is carried from land to sea from the so-called seleniferous areas. Fortunately, the leaching of selenite is negligible (70). In a study of the ecological effects of adding selenite in fertilizer to field crops (71), it was concluded that there would be little or no hazard to any species. Most plants take up only very low levels of Se; so Se fertilization of soils is wasteful. Such use is not in line with the view of Se as a critically essential element, now in short supply. The question whether sufficient Se is taken up by plants in Se-low areas and whether Se uptake by plants is adversely affected by sulfate accumulation (14, 22) seems more pertinent to the overall problem.

Evidence that the Se levels in plants varies widely (72) provided a workable basis to compare the incidence of certain diseases in man (13, 14, 22, 45). The Se deficiency in feed grains and soy bean meal in most eastern states also accounts for the

relatively low levels of Se in swine muscle fed home-grown rations (73). The muscle of swine fed South Dakota rations, and the rations themselves, had ten times as much Se as did the rations or swine muscle from many eastern states (45, 73, 101). The study by Ku et al (73) clearly established striking differences in Se levels in animal tissues dependent on the relative availability of the ambient sources of Se. Although the solubilities and redox behavior of various selenicals in soils is known to some degree (74), such knowledge falls short of answering the question whether Se inadequacy via the soil-plant-animal cycle represents a mounting problem. The fact that zoo animals in metropolitan areas of the eastern United States have appeared to develop Vitamin E-Se deficiency signs in many species (75) invited investigation of the reality of the Se cycle.

The $SO_2$ fallout problem and acid rains (75a) have increased markedly in the last 15 years, the period in which the Se inadequacy problem developed. The amount of S that emanates from all man-made sources was reported to surpass that from all known volcanoes (76). On the positive side, the sulfate fallout fulfills the need for sulfur for the synthesis of the various sulfur compounds needed by plants. Nevertheless, sulfated fertilizers are widely used, further contributing to competition between S and Se for uptake by plants (76a). Plants have no known requirement for Se, even though they have a clear need for S (76b).

Contrary to expectations, more Se was found in soils east of the 97th meridian (central Iowa) than west of it (76c). The far greater availability of Se to plants in much of the western United States is due to the greater soils alkalinity there. This difference in Se availability may be aggravated by the prevailing west-to-east transport of $SO_2$ in air. Evidence suggests that diminished Se availability to plants may be worldwide. This would result logically from dominance of the S cycle in the form of $SO_2$ and a diminished or nonexistent Se cycle (14). The question remains (76d) how Se is returned to the selenite or selenate forms, the only forms taken up sparingly or readily by plants. Such questions have significance for animal production and for health.

## SELENIUM IN NUTRITION

Although the clear need for Se in animal nutrition has been known for many years and about 40 species demonstrate Se-responsive diseases, Se is not yet recognized as an essential nutrient for humans by the Food and Nutrition Board. Studies in children (3, 4) have indicated such a need. Study of Se tissue content and Se excretion in humans led to the conclusion that Se is clearly essential (77). Monkeys were shown to need Se as a nutrient (94).

The role of Se in nutrition has been well documented. This discussion is limited to recent work that may throw light on directions for further research on the role of Se in health. The truly remarkable list of Se-deficiency symptoms assigned by 1961 (1) (Table 1) was later expanded (Table 2) to include the elevation in blood of some enzymes, SGOT, and the reduction in tissues of others, glutathione peroxidase (14). The finding (78) of cataracts in rats with advanced Se deficiency, but receiving high levels of Vitamin E, spotlights the need for Se to maintain integrity

**Table 1** Selenium responsive diseases reported by 1961[a]

| | Growth deficit | Necrosis Liver | Kidney | Heart | Muscular dystrophy | Calcifications | Paradentosis | Lung hemor. | Pancreas atrophy | Serum protein | Exudative diathesis |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rat | (+) | + | + | (–) | (+) | + | + | (+) | (+) | + | – |
| Mouse | (+) | + | + | + | + | | | (–) | + | + | |
| Rabbit | | + | | | | | | + | | | |
| Hamster | + | | | | + | | | | | | (+) |
| Mink | | | | + | + | | | | | | |
| Hog | | + | | | + | | | | | | (+) |
| Sheep | (+) | | | + | + | + | + | | | | |
| Cattle | | | | + | + | + | | | | | |
| Chicken | + | | | | (+) | | | | | + | + |
| Turkey | + | | | | (+) | | | | | + | + |
| Horse | | | | | + | | | | | | |
| Trout | ? | + | | | ? | | | | | | |

[a] +, Pronounced pathological changes; (+), not always detectable; (–), occasionally found, –, no pathological changes; ?, possible, but not clearly documented; open spaces, not investigated or not involved.

of the lens throughout life. Severe signs of Se deficiency were noted in the lens and in the testicles. These symptoms were associated with vascular hypoplasia, and with decreased RNA and SH groups in proteins. A need for adequate Se for reproduction in males was suggested by the finding (79) that Se appeared to be needed for the motility of sperm. The $^{75}Se$ appeared in sperm mitochondria. It was diminished along with sperm motility by Se deficiency (79a).

After rats fed Se-low diets developed abnormal electrocardiograms (80), further study (81) indicated that Se-deficient lambs also had abnormal electrocardiograms accompanied by blood pressure changes and histopathology. In piglets born of Se and Vitamin E-deprived sows, progressive vascular damage was reported. Lesions were noted in connective tissues of the heart. This change appears to characterize mulberry heart disease of swine and may result in sudden death.

Avitaminosis E and/or Se deficiency in swine with resultant mulberry heart disease in Ontario and the northern US was not a problem (83) until recently. Because the hemorrhages seen resembled scurvy, the ability of deficient swine to

**Table 2** Selenium - Vitamin E deficiency states reported by 1972[a]

| Symptom | Species | Symptom | Species |
|---|---|---|---|
| Liver necrosis | Rat | Nonmotile sperm | Rat |
| Growth depression | Rat | Infertility | Sheep |
| Exudative diathesis | Chick | Gizzard myopathy | Chick |
| Muscle dystrophy | Sheep, cattle | Yellow fat disease | Horse |
| Elevated SGOT | Sheep, cattle | Paradentosis | Rat, sheep |
| Decreased $GSH_{Px}$ | Rat, chick, pig | Kwashiorkor | Human |
| Hepatosis dietetica | Pig | Pancreatic lesions | Chick |
| Mulberry heart | Pig | Alopecia | Rat, monkey |
| Sudden death | Sheep, pig | Cataracts | Rat |
| Hemorrhage | Rabbit | Muscle calcification | Rat, sheep, cattle |

[a] In humans, angina pectoris, bursitis, and arthritis were reported to respond to Vitamin E - selenite therapy.

synthesize adequate ascorbic acid was investigated. These studies led to the conclusion that swine could synthesize ascorbate even when Se and Vitamin E deficiency prevailed. Vitamin E is needed for the hydroxylation of proline and lysine and for the integrity of collagen in the visco-elastic system. Subsequent studies in ducks (84) revealed that early phases of muscular dystrophy in this species, due to Se deficiency, may be due to a secondary ascorbic acid deficiency. Other studies at Guelph (85) had established that mycotoxins in high-moisture grain led to exudative diathesis and other signs of Se - Vitamin E deficiency. These studies appear to bring the roles of Vitamin E, Se, and ascorbic acid together to some extent in terms of collagen metabolism. Because collagen is the major protein of the body and its integrity is essential for avoidance of cardiovascular disease, these interrelationships call for more research (82).

The fact that sudden death of Se-deficient animals may occur appeared to point to the sudden infant death syndrome (SIDS) in human infants (4, 6, 42, 86, 87). Studies in the US (88–89), however, have indicated no correlation with Se or Vitamin E inadequacy in sudden infant deaths. This work dealt only with blood and plasma levels of Se and/or Vitamin E. Neither study considered other tissues such as liver, or other parameters such as ubiquinone tissue levels. These results may seem contrary to an earlier report (90) that the neonatal death rate in the US was inverse to Se distribution in herbage (72). Neonatal deaths include only the first month, whereas the SIDS may include deaths up to a year of age or more.

It is known that the newborn arrive free from antibody immunoglobulins (91). Their immunologic competency depends not only upon antibodies obtained from the mother but on the ability to build immunoglobulins. Although the addition of Vitamin E to cow's milk formulas is now practiced in New Zealand, the addition of Se is forbidden. Nevertheless, studies showed (92) that Se levels, but not tocopherol levels, differed considerably in pasteurized cow's milk from different areas of New Zealand. Further experiments must be carried out in order to settle all questions regarding the hypothesis advanced by Money (86) that deficiency of Se and/or Vitamin E is the cause of sudden infant deaths in many species, including humans.

It was discovered more than a decade ago (93) that excised liver tissue from rats deficient in Se and Vitamin E rapidly lost the ability to take up oxygen. This so-called respiratory decline characterized the prenecrotic phase in Se-deficient rats. The deficiency of these factors results in more rapid respiratory decline of liver slices or liver homogenates in the Warburg respirometer than deficiency of other nutrients known to be involved in cellular respiration. The decline was prevented by dietary Se or tocopherol, or by injections of tocopherol shortly before death. It was also noted that traces of $Hg^{2+}$, $Cd^{2+}$, or arsenite hastened the respiratory decline in mitochondria. This was accompanied by loss of titratable SH. Of the trace elements studied, only Se and $Mn^{2+}$ protected against respiratory decline. Tocopherol and reduced glutathione also protected. Recently, the finding was reported that liver mitochondria from Se-deficient rats required both SH groups and selenite for swelling. As in the case of respiratory decline, the same elements that hastened respiratory decline reduced induction of mitochondrial swelling by glutathione and

selenite. On the basis of this and other evidence, it was deduced that Se functions in oxidative phosphorylations as selenopersulfide, and that this facilitates the transfer of electrons from SH compounds to cytochrome *c* (34).

The optimum nutrient intake of Se for any species at any age is unknown. It may be as high as 1–2 ppm to counteract carcinogenesis (95), to stimulate immunoglobulin formation (37), or for maximum growth (96). The rate at which Se may be inactivated, as by heavy metals or other stresses, is certain to vary. The need for Se is interdependent with that for Vitamin E and for antioxidants that spare the need for Vitamin E. At the chronically toxic level of 3 ppm Se as sodium selenate, starting in the first year at 2 ppm, the element was reported to increase life span significantly in rats (97). The authors made little of this finding, but tended to stress their belief that the selenate feeding had caused cancer in some of the rats. The dilemma created by such negative attitudes toward the element has been noted (14, 98) along with the observation that this work failed to prove the carcinogenicity of selenate. It is perhaps little wonder that Se has been termed "the most maddening, frustrating nutritional element to examine in the entire table of elements" (99).

Although the need to add Se to some animal feeds is now officially sanctioned, in the US, the possibility that humans may need Se over and above that provided by diets has yet to receive serious attention. The rationale for further epidemiologic studies relating the distribution and adequacy of Se to various human disease rates seems clear enough. Selenium appears to be fully as critical as any of the vitamins in terms of nutrient need. Like iodine and fluorine, its distribution and adequacy varies with the ambient circumstances. The striking differences in Se content of corn from different midwestern states (100) and the even more dramatic variances in Se content of swine muscle from home-grown rations of eastern and western states (73) revealed the situation for animals. Allaway (101) noted that these muscle Se values agreed with the map of regional distribution of Se in crops (72). Finally, it was shown (102) that, even after selenite supplementation, swine on a Michigan ration had less Se in their tissues than swine on home-grown, but unsupplemented, South Dakota rations. Thus, the effects of regional Se availability are seen in farm animals. Tissues of wild animals were found to contain high Se levels (45), indicating avidity of such tissues for the element. Sea animals have more Se than land animals. This appears to be due in part at least to accumulation of heavy metal selenides in sea animals, presumably a detoxication mechanism.

Whether human diets contain enough Se for optimum health or to minimize certain diseases is a critical question requiring much more study. Some evidence, noted here, suggests that Se inadequacy is chronic and is worsening. This concept is contrary to general expectation, and the question of Se adequacy has received no official attention. Some studies have noted the Se content of representative foods (4), but a survey treating Se as an essential nutrient has yet to be made.

In 1973, based on the wealth of biochemical and animal evidence, Se was included among the five elements to be studied in relation to coronary heart disease (CHD) by the World Health Organization and the International Atomic Energy Agency. Based on animal evidence and the relation to ubiquinone biosynthesis, the rationale to test combinations of Se with Vitamin E against various aspects of human heart

disease seem strong indeed. Clinical studies in Mexico and elsewhere, backed by careful toxicologic evaluations, with selenium-tocopherol capsules have been made. Part of this experience will be noted here. The trial of Se-tocopherol capsules in humans grew out of the veterinary experience with this combination in degenerative muscle disease in sheep, cattle, and horses, and arthritis in dogs and cats. Various considerations led to a revision of the formula used since about 1964 to treat lameness and arthritis in small animals. Thus the selenium-tocopherol capsules registered in Mexico for the relief of some aspects of heart disease contain per capsule: 0.5 mg selenite Se and 100 IU *d*-alphatocopherol succinate. This is half the level of Se in the veterinary capsules, but about half again as much tocopherol.



## SELENIUM AND THE UBIQUINONES (COENZYMES Q)

The apparent need for Se for ubiquinone biosynthesis in animals was recently reviewed (14, 21, 123). An early facet of the ubiquinone (coenzyme Q) story was the polemic whether Vitamin E is needed for ubiquinone biosynthesis. Moore (124) and Morton (125) reported that dietary lack of Vitamin E did not alter ubiquinone tissue levels in rats or guinea pigs. Diplock et al (126) noted, however, "that the synthesis of ubiquinone is, contrary to the findings of others, significantly affected by vitamin E." Significantly, it was the discovery by Green, Diplock and others (127) which led to the realization that Se is needed for ubiquinone biosynthesis. In the interim, the need for Se in this context was repeatedly confirmed in many species. But the need for Vitamin E for ubiquinone biosynthesis remained unconfirmed. As reviewed (14), by 1966 there was apparent agreement (8, 128) that when adequate Se was fed, the deficiency of Vitamin E had no measurable effect on tissue ubiquinone levels. On the other hand, the addition of selenite to the diets of normal stock animals was reported to cause an increase in the content of Se in the hearts, though not the livers, of the treated animals. The possible involvement of Se in heart function involving coenzyme $Q_{10}$ biosynthesis was confirmed in calves suffering from nutritional muscular dystrophy (29).

It is highly important to clarify any biosynthetic relationship between Se, Vitamin E, and the ubiquinones (14, 123). To the reported value of coenzyme $Q_{10}$ against infectious diseases and cancer (26), heart disease (27), and periodontal disease (43), one may now add experimental hypertension in rats and dogs (129). The implications seem clear. The prevention, or treatment, of such disease may be found to have a basis in the adequacy of Se and Vitamin E intake to support ubiquinone biosynthesis.

Much evidence (130, 132) indicates that $GSH_{Px}$ activity in tissues increases with increasing dietary levels of Se. The findings cited for ubiquinones in relation to health, plus the finding that Se-dependent enzymes protected against oxidative damage (23), may stimulate investigation of this interrelationship. It bears directly on research on heart disease and cancer, both for prevention and treatment. Unfortunately, perhaps, the rationale in nutrition-biochemistry to test selenium-tocopherol capsules against human diseases became very involved. Some of the uncertainties regarding Vitamin E were noted in the introduction. Unfortunately

also, the medical and public controversy about possible health effects of high levels of Vitamin E has taken place almost as though Se were not involved. Nor have the reported disease-control findings with unbiquinones been related to the nutritional adequacy of Se or Vitamin E (26, 27). The value of Vitamin E therapy to relieve intermittent claudication in controlled studies in humans (8, 11), although accepted by some (7, 11), was not cited by the National Academy of Science-National Research Council (12).

The need to test the value of selenium-tocopherol capsules in conditions in which ubiquinones were reported effective seems clear. For instance, lowering of blood pressure in animals by coenzyme $Q_{10}$ (129) may relate to the similar effect of Se-tocopherol therapy in angina. Much time and research will be needed to quantify such relationships. The safety of Se-tocopherol capsules for humans is well established (see Section, Selenium-Tocopherol Formulations for Humans). This follows logically from the fact that Se and E are essential nutrients. Nevertheless, because misunderstandings about Se and Vitamin E prevail, their adequate clinical trials deserve government sponsorship. This is especially true in view of the timeliness of such investigation in the programs now under way on heart disease and cancer.

## SELENIUM AND IMMUNE MECHANISMS

The recent discovery that Se and Vitamin E stimulated antibody formation in rabbits (36) parallels evidence that Se enhanced the immune response in mice (37). Spallholz et al (37) reported greatest antibody production against injection of sheep red blood cells at the 1–3 ppm dietary level. Two weeks after sensitization, the level of immunoglobulin (IgG) was markedly raised at dietary levels of 1–3 ppm Se, less so at higher or lower levels. Measurements at 4 or 7 days after sensitization showed some increase in both immunoglobulin G and immunoglobulin M titers. Only the IgG titer remained high after 14 days. This behavior seems to fit what is known of the role of Se in specific blood protein formation.

The very rapid appearance of $^{75}$Se in blood proteins reported originally by McConnell (38a), was confirmed (51). The specificity of protein formation and the fact that Se enters many proteins in significant amounts was shown (38). The rapid turnover of $^{75}$Se in cytochrome *c* was viewed in terms of Schoenheimer's dynamic steady-state concept (38a, 48). This appears true for the relatively rapid transformations of Se in immunoglobulin (37).

The specific attraction between antigens and antibodies may be due to the properties conferred on antibodies by Se. The group at Colorado State University proposed that vaccines be supplemented with selenium to increase their effectiveness. Supplementation of a Vitamin E- and Se-low diet with Se was reported to decrease mortality from graded injections of *Salmonella enteriditis* (163). The conclusion was, "These results are consistent with a role for Se in phagocyte integrity and cellular immune response."

Such evidence suggests that Se is a critical factor in host defense. The fact that a high nutrient level, 1–3 ppm Se, conferred the greatest immune response in mice (37) suggests that the optimum Se input should be sought for various species. Do

these findings relate to those of the New England Institute with the ubiquinones (coenzymes Q) (26, 26a, 123)?

Susceptibility to infection is postulated to depend on availability to the invading microorganisms of $Fe^{2+}$ and $Zn^{2+}$ (143). Conversely, "nutritional immunity" is thought to result from minimal availability to microorganisms of these metal ions. How the Se nutriture of the host and the roles of Se and the ubiquinones may fit with these hypotheses can only be learned by further research.

## SELENIUM IN HOMEOSTASIS

The fact that Se is at the hub of homeostasis seems to be borne out by the following examples of some of its roles in biology:

1. Maintenance of muscle and erythrocyte integrity.
2. Function in DNA-RNA.
3. Function in essential enzymes in microorganisms and in animals.
4. Inactivation of physiological excesses of $Ag^{2+}$, $Hg^{2+}$, $MeHg^{+}$, $Cd^{2+}$, and $AsO_2^{-}$.
5. Prevention of mercury and methylmercury toxicity and induced Cd hypertension.
6. Control in part of ion fluxes across cell membranes.
7. Maintenance of vigor and motility of sperm.
8. Integrity of keratins, avoidance of hyperkeratosis and cataracts.
9. Maintenance of pancreatic function to insure lipid absorption.
10. Stimulation of antibody (immune globulin) synthesis.
11. Stimulation of the biosynthesis of ubiquinones (Coenzyme Q) in animals.

The intuition of unity amid diversity which impels science fits the Se story. From an element still largely banned by society, Se is now emerging as the key to many problems in biology. The unique photoelectric and electron, or proton, transfer capabilities of Se in applied physics, as described in leading encyclopedias, have yet to be realized in medicine.

In the metabolism of foodstuffs, oxidation denotes both hydrogen removal and oxygen addition. Evidence suggests that Se may aid both. In briefly reviewing what is known of Se in terms of electron theory in organic chemistry (113), one finds its electron transfer capability by some criteria below that of oxygen or sulfur. By other criteria, it is higher. The rate of scission of S–S bonds is known to be far more rapid than that of C–C bonds (114). It is also known that Se–Se bonds are cleaved far more rapidly than S–S bonds (115). The rate of scission of S–Se bonds may provide the key to how Se helps control the redox potential of cells.

As noted (114), selenoxides can add water rapidly and reversibly to form the $> Se(OH)_2$ group. Selenoxides, $Se^{4+}$, and their apparently ready and reversible conversion in vivo to selenides, $Se^{2-}$, connotes the role of Se to maintain oxidation-reduction equilibria in cells. The poising of redox potential within cells and the maintenance of hydrogen ion concentration, pH, are among life's greatest accomplishments and mysteries. A great deal of evidence now suggests that enzyme-

catalyzed selenical transformations between $Se^{2-}$ and $Se^{4+}$ in vivo play a major role in redox poising (116). The roles of $GSH_{Px}$ in sulfhydryl-disulfide interchanges, of Vitamins E and C, and of the ubiquinones had been recognized for some time (116a). Despite its complexity, the fitting of trace elements, particularly Se, into metabolic schemes may help get us through the maze.

It was noted in the 1930s (117) that there was "the possibility that the injurious effects (of selenite) are due to the removal of sulfhydryl groups essential for the oxidative mechanism." It was postulated then (118) that dehydrogenase inhibition might be due to excess selenite, a hypothesis which seems supportable now (14). In the 1930s, however, the intricate changes that selenicals undergo in metabolism (33, 54) were unknown. One may wonder whether the catalytic roles of Se to transfer both electrons and protons, possibly to facilitate the generation and handling of $H_2O$ by cells, does not exceed the expectation of selenophiles. In any case, the toxicity of selenicals appears to be predictable, rapidly reversible, and readily avoided. This knowledge was not available in the 1930s.

The rapid appearance of Se in DNA and RNA (119) and its association with tRNA (39) will in time help explain its role in protein synthesis and organization. The great avidity of many animal tissues for Se was noted (3, 14, 52). Its jealous retention by testes and brain (79) denotes its critical need in specialized tissues. The same appears true for the heart. Protection against harmful oxidations, as by glutathione peroxidase (23), represents a phase of respiratory metabolism in animals that may be subject to environmental hazards. These appear to include carcinogenesis, excesses of certain heavy metals, and excessive intakes of polyunsaturated fatty acids (PUFAs). In each, an adequate dietary level of Se and Vitamin E are needed for best protection.

Hydrogen peroxide and superoxide ions must be disposed of efficiently by tissues in the following type reactions:

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + \tfrac{1}{2}O_2$$

$$RH_2 + H_2O_2 \xrightarrow{\text{Peroxidases}} 2H_2O + R$$

The roles of iron- and copper-dependent enzymes in controlled biological oxidations and in biochemical evolution were outlined (58, 121). The new found role of Se has yet to be fitted into such considerations. Little is yet known about what makes for the efficient coupling of high-energy phosphate bonds as in ATP, or how to minimize the uncoupling of such bonds (122). Evidence suggests that, in ways not yet understood, both As and Se are needed at critical levels for optimum energy flow. Interrelations between compounds of Se, As, S, and P are clearly at the heart of energy metabolism and are also basic to homeostasis.

## SELENIUM AND TOXIC TRACE METALS

The ability of Se and/or Vitamin E to reduce heavy metal toxicities was discovered years ago (3, 4, 14, 35). This is one of the most active fields of research. Arsenicals

were found in the 1930s to counteract Se toxicity (3, 30) and the interrelationship between Se and As (52) has yet to be clarified. Silver nitrate was used to enhance experimental Vitamin E deficiencies. But not until the role of Se as active selenide in essential enzymes had been discovered was this phenomenon understood (14, 35). Similarly, counteraction of $Cd^{2+}$ testicular injury by a half molar equivalent of Se (3) was partly clarified by recent research (35). The finding that traces of $Ag^{+}$, $Cd^{2+}$, $Hg^{2+}$, or arsenite hasten respiratory decline of excised tissues (1, 93) is clearly related to interference with the function of Se and tocopherol. Overall, the labeling of Se as nature's antidote to heavy metal toxicities fits well. Yet it is clear that this is an accomplishment of the total metabolism involving S compounds, enzymes, and Vitamin E, or other antioxidant. Whether Se binds metals entirely as inert selenides or in some cases as the selenites has yet to be shown.

One of the more dramatic discoveries was the revelation that less than 20% of tuna added to an otherwise Se-deficient diet protected against methylmercury toxicity (35a). Tuna contains about 3 ppm Se, of which only about a third is biologically available. A large part of the Se in tuna is bound, possibly as metal selenides or selenites. At appropriate levels, reciprocal detoxication was shown between Se and Hg (133, 134), Se and Cu(134), and Se and As (135).

Chronic cadmium toxicity resulting from Cd pollution has been vigorously represented as a possible cause of human hypertension. Recently, it was reported that a half molar equivalent of Se protected against $Cd^{2+}$–induced hypertension in rats (136). Although there is little agreement that Cd exposure represents a hypertension-causing hazard for humans, such findings may stimulate clinical study of the role of selenium-tocopherol capsules in hypertension in humans.

The mechanisms by which selenicals inactivate metal toxicants still leave many questions. In his extensive studies of mercury and cadmium detoxification by Se, Parizek (146) noted that dimethylselenide could actually enhance methylmercury toxicity. How this detoxification may occur is the subject for much investigation, only one being cited here (147). The presence of added sulfur amino acid was found to enhance the effect of Se to detoxify mercury, thus suggesting that SH metabolism is also involved (148). Similar disturbances in keratin protein metabolism, reported in Se and Zn deficiencies (149), tend to further relate the total metal ion balance to that of Se and the metabolism of sulfur amino acids. As noted elsewhere (14), present evidence suggests that the keratoses resulting from overmedication with trivalent arsenicals, such as Fowler's solution, may have been due to the inactivation of $Se^{2-}$–dependent enzymes needed for keratin integrity.

Careful study of trace element history will reveal to any who spend the needed effort that governmental misunderstandings about trace elements tend to get frozen into inept laws and regulations. Such regulations may continue to cost vast amounts without providing the public health protection intended. This seems quite clear for the medicolegal histories involving As and Se. Under certain conditions, arsenical toxicity was found to be enhanced by the simultaneous administration of Se (150). How the ingestion of a clearly toxic level of Se and a relatively nontoxic level of As affected the people in England in 1900 is still an open question. Much evidence reveals quite clearly that there was enough Se present in the beer, brewing sugar,

and $H_2SO_4$ used to invert the brewing sugar, to cause the poisoning reported in the British beer poisoning epidemic of 1900 (14, 52). The setting of and persistence of unrealistic tolerances for As, costing billions of dollars, plus the unfounded apprehension about safe uses of arsenicals throughout this century merely illustrates the power of arsenophobia. It also illustrates the futility of laws and tolerances in matters not sufficiently understood. On the positive side, because Se is an absolutely essential though very toxic trace element, its history offers great educational value.

The banning of swordfish containing more than 0.5 ppm of mercury in the US just prior to the discoveries (35, 35a) that sea animals naturally contain relatively high levels of Hg, presumably as nontoxic HgSe complexes, also illustrates how legal interpretations may get ahead of the science. The livers of sea animals were found to contain Se and Hg in a 1:1 ratio (151) while the fish on which they feed had at least 40 times more Se than Hg. Fresh water fish also have relatively high levels of Se and Hg in their tissues (152).

Although there is a modicum of evidence that Se may minimize lead toxicity, the theoretical reasons to suspect this may be so, together with the concern about Pb toxicity, suggest that more attention be devoted to this question. The safe uses of Se and Vitamin E clearly offer one of the most effective ways to protect against the damaging effects of certain heavy metal pollutants. The possibilities to minimize personnel hazards from overexposure to toxic metals remain to be explored. This applies also for the antidotal potential in cases of frank toxicity. Theoretically, selenium-tocopherol injections or capsules should have therapeutic advantages over BAL or penicillamine against some heavy metal toxicities.

## SELENIUM AND ARTHRITIS

A Se-tocopherol capsule product used in dogs and cats for relief of clinical signs of arthritis contains the following per capsule: sodium selenite Se, 1 mg with 68 IU of Vitamin E as *d*-alphatocopherol acid succinate. The value of this product, which has been in use about 12 years, is documented in veterinary literature (16, 17). The anti-arthritic effects are described (18) as drug effects. Search for an explanation led some investigators (104) to investigate lysosome stabilizing effects of Vitamin E, of Se, and the combination. Lysosome leakage is thought to lead to hemolytic, pyrogenic, and inflammatory disturbances. Membrane stabilizers, such as cortisol or aspirin, are thought to minimize damage caused by the enzymes released (105). It was found (104) that Se, Vitamin E, or both together did stabilize membranes of the lysosomes isolated from rat livers. Low doses of Se potentiated the effect of Vitamin E, but high doses antagonized. As reviewed (52), it was held that protection afforded by Se and Vitamin E against carcinogenesis may involve avoidance of lysosomal membrane degradation. Vitamin E and Se were reported to stabilize serum albumin to heat-induced aggregation (106–107).

Selenium, as sodium selenite or as organoselenium compounds, was reported to have anti-inflammatory value in the granuloma pouch test in rats (108). This is a standard test for anti-inflammatory steroids. Further evidence indicated that the Se effect was enhanced by Vitamin E (109). It has been theorized that Se binds to

proteins in a manner that protects them from oxidative inactivation (110). The catalysis by Se of sulfhydryl ⇌ disulfide interchange involving protein synthesis may be critical to prevent rheumatoid arthritis. Deficiency of serum-SH groups characterizes arthritis (111) and also some types of cancer, and is a biochemical feature of aging (52). Aberrant sulfur metabolism was reported in polyarthritic rats (112).

Due to the extreme complexity of arthritis, only continued research can clarify the role of selenium-tocopherol capsules in humans. The established effectiveness of this therapy in animals provides needed background for its clinical trial in humans. Such trials need not be delayed by the evidence that other apparently unrelated materials may also prove effective. Many metabolic defects may lead to arthritis. Any fault in collagen synthesis or repair is surely involved. Thus many types of treatment may be found effective for its prevention or relief.



## SELENIUM AND CANCER

The struggle to learn the truth about Se in relation to cancer began with the mistaken view that selenicals might cause cancer. This concept was questioned for 15 years (3, 4, 14, 45, 52). The fact that selenical feed additives do not pose a carcinogen hazard for humans was accepted recently (15). Nevertheless, the evidence that selenate at a chronically toxic level increased lifespan in rats, the longest lived rats ever reported, failed to alter Schroeder's view of Se as a potential cause of cancer (97, 97a).

The possible anticancer value of Se, a constructive outgrowth from the above conflict in attitudes toward the element, although reported initially seven years ago (120), was so contrary to popular belief that follow-up of the question has been meager (14). The reality of the reported inverse relation between ambient Se availability and human cancer mortality (13, 13a) has yet to be confirmed or rejected by the National Cancer Institute or by oncologists generally. The same is true as to whether Se inadequacy increases susceptibility to cancer; conversely, whether supplemental Se and Vitamin E decrease susceptibility to experimental carcinogenesis (13b, 95). In that Se and Vitamin E function together, the combination should be considered in the light of the reported anticancer value of ubiquinones (26, 26a) and antioxidants (13c–13e).

The irreversible changes in cell replication, which spell cancer, may stem from damage to many metabolic targets. The role of Se in glutathione peroxidase may be such a target; so may the apparent involvement of Se in tRNA and in immunoglobulin synthesis.

The possible roles of S compounds and of aberrant oxidations in the genesis of cancers, as discussed by Szent-Gyorgyi (138), may help explain the anticancer value of Se. Proteins were viewed as semiconductors, oxygen as the most general electron acceptor of the biosphere, and glutathione as one of the most widespread of all tissue constituents. Cancer was viewed as one disease, i.e. "many roads to Rome but they all may have to pass through the same gate." Szent-Gyorgyi traced evolution as the development of systems capable of using $O_2$ safely (see also 58, 121). He noted that,

when dividing, cells must dismantle their oxidative machinery and rely on fermentation mechanisms. This was likened to a shift to the earlier undifferentiated, proliferative state of anaerobic forms of life. After dividing, the new animal cells have to resume their oxidative states. If deranged by free radical damage at this crucial stage, uncontrolled cell proliferation might result. Aberrant aldehyde reactions were viewed as damaging free radicals. Szent-Gyorgyi's search for enzymes or prosthetic groups able to control such damaging aldehyde reactions remained incomplete (138).

The need for Se and Vitamin E to prevent the accumulation of malonyldialdehyde was recently voiced by Hoekstra (139). The need for Se and Vitamin E to minimize chromosome damage as part of experimental carcinogenesis, noted by Shamberger et al (13d, 140), is accentuated by the indication that such active aldehydes may themselves induce cancer. Their generation from polyunsaturated fats may be in line with observations that such diets may increase susceptibility to cancer (14, 141).

Underfeeding proved to be about the only way to minimize the incidence or severity of experimental cancer (142). But trace element deficiencies were not anticipated as critical. Furthermore, Se was viewed as a probable cause of cancer. This situation has been reversed. Evidence now suggests that nutrient inadequacy of Se, perhaps coupled with inadequacy of Vitamin E (or other antioxidant), and coupled also with GSH metabolism may hold the key to mechanisms by which cancer is initiated. Evidence further suggests that avoidance of carcinogenic damage is a quantitative matter, i.e. that inadequacy may increase susceptibility, but that a relatively high nutrient level of Se is needed to ward off cancer induction (13, 95). As noted (14, 52), this hypothesis can be tested in many ways, both experimentally and epidemiologically. Evidence for the anticancer value of some organic selenicals is reviewed elsewhere (13, 143), as is the evidence that the basic question deserves consideration (144).

The strategic plan for cancer research (145) stated, "Increased efforts will be directed toward studies of protein synthesis in normal and cancer cells and in their extracts, with specific attention to regulation and the translation of RNA, whether this regulation is altered in cancer and how such alterations can be prevented." From the evidence at hand, impaired protein synthesis and regulation would be expected to occur in Se deficiency, also impaired ability to handle oxygen safely, particularly as regards lipid metabolism.

## SELENIUM SULFIDE SUSPENSION

Until recently, selenium sulfide shampoo for use against exfoliative and seborrheic dermatites was restricted to sale via prescription. After the findings (155, 156) that $SeS_2$ is relatively nontoxic, a less concentrated product was sold without prescription. Forms of $SeS_2$ suspension have been used to control blepharitis and tinea versicolor. The slight urinary Se excretion noted following prolonged application of $SeS_2$ suspension over much of the body surface was well below day-to-day Se excretion by healthy people in areas of high Se availability (117, 137). Garlic breath from dimethylselenide exhalation was apparently not a problem.

## REGULATORY PROBLEMS

The evidence that laws and regulations regarding the safe uses of arsenicals and selenicals have been well ahead of scientific knowledge and out of tune with nature was repeatedly advanced (14, 45, 52). The complete taboo and no-residue regulations for Se in agricultural products in the US were recently revised to permit limited feed additive uses of sodium selenite and selenate (15). The US and Canadian regulations, unlike those in many other countries, continue to reflect regulatory concern about the generation of Se residues in animal tissues. Such concern has yet to take the following fully into account, particularly as regards human health:

1. The Se level in animal tissues reflects the ambient availability of Se. Tissues have great avidity for Se in essential enzymes, but dietary excesses of Se are readily excreted. The food animal would show Se toxicity signs well before its tissues would contain high enough levels of Se to cause damage to human consumers. There is actually no evidence that such has ever occurred.
2. Animal products are among the best sources of Se for humans.
3. Selenium meets all of the criteria of an essential nutrient. Evidence indicates that it is only unwarranted apprehension about Se toxicity and ignorance of its many roles in health maintenance which continues to deter its expanded applications in agriculture and in medicine.

The regulatory bind on Se stems mainly from its unfounded association with cause of cancer (52). It remains to be seen how long it will take WHO, Food and Agriculture Organization, (FAO), and the Codex Alimentarius Commission to recognize fully not only the noncarcinogenicity of Se but also its apparent anticancer value. An example of the confusion caused by this error in judgment is found in the EPA limit for Se in public drinking water, i.e. 0.01 ppm. The WHO limit was lowered in 1962 from 0.05 ppm because of the alleged association of Se with cause of cancer. The US Public Health Service followed suit, followed by the EPA in 1971.

Many wells in Colorado have recently been found to have more than 0.01 ppm Se. So does human milk. The average urinary excretion of Se in healthy people in seleniferous areas in the US ranges to 10–30 times this limit (117).

In Venezuela, high urinary excretion of Se is the rule and the high Se intake in foods and water is not considered a public health hazard (137).

What seems to be needed are critical evaluations of the health status of people in such high-Se areas versus the health and chronic disease status in the Se-low areas. Perhaps this will be accomplished in WHO/International Atomic Energy Agency studies (103). It may be noted that the coastal plain of Georgia and the Carolinas, an area of low Se availability to plants (72, 76c), is an area of high chronic disease mortality as compared to the Piedmont. The same applies to many other selected sections of the US and of many other countries. The question seems to be how soon the regulatory climate can adjust to realization that Se is a critically important element, unavailability of which may adversely affect people.

## SELENIUM—TOCOPHEROL FORMULATIONS FOR HUMANS

Favorable experience in treating animals for arthritic conditions and certain theoretical considerations led one drug company to prepare and test a selenium-tocopherol preparation for humans (157). The encapsulated formulation contains 0.5 mg selenite-selenium and 100 IU of Vitamin E as *d*-alpha tocopherol acid succinate. Preliminary to human trial, among other testing, was a 1-year chronic toxicity study in monkeys. Selenium-tocopherol was administered orally 7 days per week to groups of 5 male and 5 female Rhesus monkeys at dosage levels of 20, 64, and 200 μg/kg per day in terms of selenium. A control group received no drug. After 55 weeks of dosage 3 males and 2 females from each treated group and the control group were sacrificed. Selenium-tocopherol was withdrawn from the surviving treated monkeys at week 58, and terminal sacrifice was performed during week 70. Using the following as criteria it was concluded that no signs of toxicity were seen that would be attributable to the administration of selenium-tocopherol: daily observations; physical examinations and behavior survival and body weight gain; electrocardiograms (lead II); blood pressure determinations and heart rates; gross necropsy observations; absolute and relative organ weights; histological observations; ophthalmological examinations; hemograms; coagulation times and thrombocyte counts; clinical chemistry evaluations including plasma urea nitrogen, serum alkaline phosphatase, serum glutamic pyruvic and oxalacetic transaminase; serum creatinine; albumin globulin ratios; serum electrophoresis; and qualitative urinalysis. Table 3 shows selenium contents of liver tissue for a chronic toxicity as well as for a subacute study in monkeys. There was less accumulation in the liver with the longer term administration and the values tended to diminish after 3 months off the drug.

Safety studies in human volunteers (157) were undertaken at two different institutions. The studies were double-blind. Both studies employed 7 subjects on drug treatment and 7 on placebo. The period of evaluation was 18 weeks. Each subject was given 1 week of placebo therapy during which baseline control observations were made. This was followed by 10 continuous weeks of selenium-tocopherol treatment in increasing dosage (0.5 mg Se/day for 3 weeks, 1.0 mg Se/day for 3 weeks, and 2.0 mg Se/day for 4 weeks). Each subject in the test group received a total of 87.5 mg Se during a 70 day period. Weekly observations were made by the physician throughout the study. A battery of laboratory safety studies designed to monitor vital organ functions were performed during the evaluation period and every 2 weeks thereafter. The conducting physicians, assisting personnel, and all subjects were unable to distinguish between drug and placebo. Laboratory studies showed no variations attributable to the drug. Blood and urine levels of selenium were measured on weeks 0, 4, 11, 14, and 18, the latter two being 3 and 7 weeks respectively after receiving the drug. Blood levels of selenium rose only slightly, but especially while the subjects were taking the 2 mg/day dosage. However, urine levels reached as high as 14 times that of control levels. All levels returned to normal 3 weeks after withdrawal of drug.

**Table 3** Selenium contents of liver tissue as revealed by a 1 year chronic toxicity study and a 13 week subacute study in monkeys[a]

| Interval | Dietary selenium level (μg/kg/day) | Mean value (ppm) |
|---|---|---|
| 1. Chronic toxicity study[a] | | |
| 12 months | Control | 0.701 |
| | 200 | 1.165 |
| 15 months | Control | 0.874 |
| (off drug 3 months) | 200 | 0.986 |
| | 64 | 1.025 |
| | 20 | 0.858 |
| 2. Subacute study | | |
| 13 weeks | Controls | 0.891 ± 0.068 |
| 13 weeks | 160 | 1.512 ± 0.086 |

[a]Mean values of 5 animals per group are given.



Satisfied with the demonstration of the safety margin of selenium in humans, spurred by the observations that Vitamin E has an oxygen sparing effect, realizing that selenium increases the efficiency of cardiac muscle and that the two work synergistically (157, 158), Ramirez and his co-workers (158) undertook a study of selenium-tocopherol in coronary heart disease in the human (157, 158). Thirteen patients started the study, after suitable control observations, on selenium-tocopherol capsules and 9 patients started on placebo capsules. After 5 weeks the medications were switched. Two patients received selenium-tocopherol only. The conclusions were that selenium-tocopherol reduced the incidence of anginal pain in 22 of 24 patients. In 5 of 24 patients there was some favorable effect of placebo. The administration of placebo either prior to or after the drug did not reduce the frequency or intensity of the anginal pain in the remaining 15 subjects. Other studies of selenium-tocopherol in angina pectoris are under way.

## CRITIQUE

The tendency toward misjudgment about the toxic trace elements, which characterizes man's history, still prevails. This tendency for people and their governments to overestimate possible hazards and underestimate potential benefits includes the tendency by some to employ this human failing to advantage. This political trend was made possible by scientists and lawmakers who failed to fully recognize our dependence on being right about the elements. This failure is exemplified in the Honorable James J. Delaney's opening remarks on *Chemicals Guilty Until Proven Innocent* (159a), "Chemicals do not have rights. People do. Chemicals generally are highly toxic, and should be considered guilty of being hazardous until proven innocent." Clearly, man's ability to survive among the elements is apt to depend on how correctly he judges, or misjudges, them. The question whether selenophobia, or arsenophobia, or the unreasoning fear of any other element, will paralyze man's ability to solve critical problems needed to be asked (14).

Outspoken lawyers, environmentalists, scientists, and congressmen, who have had the greatest impact in formulating trace element regulations, have emphasized only the risks. This failure to place the risk/benefit ratio in perspective permitted the passage of laws and regulations anathema to food production and even to the advance of medical science and the improvement of public health. This situation is perhaps highlighted best by the developing knowledge about Se. This sequence is told in part elsewhere (3, 4, 13, 14, 22, 45, 52, 98, 99).

The synergism and interdependence between the biochemical roles of Se and tocopherol has been a source of confusion regarding their safe medical applications. Fortunately, their veterinary applications have gone forward. Justice cannot be done to the wealth of information on Se-Vitamin E amassed over the last 18 years by research workers in animal agriculture and in veterinary science in many countries. Suffice it to note that in 1962 Salisbury and others (159) clearly described the common occurrence of uncomplicated exudative diathesis and white muscle disease in poultry in New Zealand. These chickens, raised on South Island-grown grains and well supplemented with tocopherol, recovered when given about 0.1 ppm of Se as sodium selenate in water or feed. By 1970, the safe uses of Se in agriculture was reported as one of the two most important advances there in the last quarter century.



## SUMMARY

The role of Se in biology appears from the evidence now at hand to be as a catalyst par excellence. As unique prosthetic group of a variety of enzymes, presumably as $Se^{2-}$, Se functions with tocopherol to protect cell and organelle membranes from oxidative damage, to facilitate the union between oxygen and hydrogen at the end of the metabolic chain, and to transfer ions across cell membranes, in protein synthesis in erythrocytes and in liver organelles, in immunoglobulin synthesis, and in ubiquinone syntheses. As perhaps the most versatile and rapid nucleophile, Se is thought to amplify and orient SH⇌ –S–S–interactions involving glutathione and proteins. Its toxicity appears to be due to overaccumulation of selenite ions, which act as oxidants to inhibit SH interactions. Such toxicity is readily avoided or reversed in many ways.

Although not yet recognized as essential for man, Se is clearly essential for many animal species and some microorganisms. As the active selenide, Se emerged as the target for many heavy metal toxicities; contrariwise, as a specific antidote against heavy metal toxicities. Despite all this, its unusual toxicity and the many preconceived notions about Se continue to confuse attitudes toward the safe uses of selenicals. From a suspected cause of cancer, Se metamorphosed, via evidence over many years, into something of possible anticancer value. Interrelations between Se, Vitamin E, the ubiquinones, and various chronic diseases appear as beckoning research areas.

The reported veterinary values of Se-tocopherol combinations in animals, together with clinical evidence, plus human and animal evidence for safety, offer promise for intensive medical investigation. The historical confusion and misunderstandings regarding Se must be corrected, however, before advantage can be taken of its potential for human welfare. The many misjudgments about Se, ever since 1900

and more obviously since the 1930s, have involved other trace elements. Unrealistic regulations stemming from these misunderstandings prevail worldwide.

Evidence suggests that, once the nutrition biochemistry and toxicology of Se is sufficiently understood and appreciated, major breakthroughs in agriculture, medicine, and public health can result. Much has been accomplished along these lines in New Zealand in animal agriculture, in the US and other countries in veterinary medicine, and in Mexico in human medicine.

## ADDENDUM

Disclosures of positive roles of Se in biology continue. Hoekstra and others reviewed research, particularly its role in glutathione peroxidase, in a major book on trace element metabolism (160). Schrauzer, Rhead & Evans have offered support for the concept that cancer mortality is inverse to ambient Se availability (161). They stated that "the diminished plasma Se levels observed in malignant disease supports the suggestion that Se is a cancer-protecting agent; statistical analysis demonstrated that those states in the United States with elevated Se levels in forage crops and grains have lower cancer mortality."

An inverse relation between human birthrate and ambient Se availability was reported (162), again fitting the concept that Se inadequacy may be a human health problem (14, 22, 45).

Following the lead of Spallholz and others (37) and indicating stimulation of the immune response by Se, Ganther's group at Wisconsin reported that selenium-deficient rats injected with *S. enteriditis* suffered greater mortality than similar groups receiving supplementary Se (163). They concluded: "These results are consistent with the role for Se in phagocyte integrity and cellular immune response." In work at Colorado State University, somewhat similar protection of Vitamin E- deficient chicks infected with *E. coli* resulted from administration of large doses of Vitamin E (164). The similar effects of supplementary Se and Vitamin E in combating infectious diseases fit their roles in coenzyme $Q_{10}$ biosynthesis. This relationship was outlined above and elsewhere (14, 123).

McConnell's group at the University of Louisville established the form of Se as selenouridine in TtRNA (165).

Evidence for the combined roles of Se and Vitamin E in muscle integrity, particularly in heart muscle (167), added further weight to the hypothesis outlined in this review that human heart disease is in part due to the dietary inadequacy of both elements.

Long-term Se deficiency was found to lead to cataracts in second generation rats in studies at Oregon State University (78). Follow-up studies by Hoekstra's group at Wisconsin showed that glutathione peroxidase activity in the lens of second generation Se-deficient rats was greatly reduced (168). The possibility was advanced that depletion of glutathione peroxidase in the lens could lead to aberrant SH protein metabolism resulting in cataracts.

In confirmation of work recorded elsewhere (14), a very low level of Se was found in paper pulps from different parts of Canada and the US (169).

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