

THE CHEMISTRY AND TOXICITY OF SELENIUM COMPOUNDS, WITH SPECIAL REFERENCE TO THE SELENIUM PROBLEM

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I. THE SELENIUM PROBLEM IN AGRICULTURE

A. HISTORICAL

Selenium has found industrial use in photosensitive cells, in rubber, and in pigmented glass. As a laboratory reagent it has been used for dehydrogenation by Ruzicka and as a specific oxidant by Riley. But, owing to the extensive areas in the western Great Plains of the United States, where selenium is present in the soil, the economic losses to agriculture may outweigh the uses to which the element has been put.

As early as the thirteenth century Marco Polo¹ wrote about one section of western China:

Throughout all the mountainous parts of it the most excellent kind of rhubarb is produced in large quantities, and the merchants who come to buy it convey it to all parts of the world. It is a fact that when they take that road, they cannot venture amongst the mountains with any beasts of burden excepting those accustomed to the country, on account of a poisonous plant growing there, which, if eaten by them, has the effect of causing the hoofs of the animals to drop off. Those of the country, however, being aware of its dangerous quality, take care to avoid it.

About six centuries later, stockmen in some sections of the Great Plains described symptoms similar to those related by Marco Polo. Prior to the settlement of the western part of the Great Plains area, Madison (119), a surgeon in the United States Army, described cases of sickness in cavalry horses when they ate native vegetation along the Missouri river near the present boundary between South Dakota and Nebraska.

When stockmen moved into some regions they were unable to graze livestock without losses; later, farmers had the same experience with the grains and forages produced on their farms. At first the reports of a strange malady of livestock received little attention, but after many years the numerous pleas for aid were recognized and several agricultural experiment stations undertook to determine the cause.

The investigations which culminated in the discovery of selenium as the etiological agent were largely carried out by Dr. Kurt W. Franke, who began studies at the South Dakota Agricultural Experiment Station in the fall of 1928. Franke observed the symptoms of poisoning in farm animals and obtained numerous feed samples from those regions where the poisoning was more severe. When he tested these feeds with laboratory animals, he soon showed that the feeds contained a poison and that the observed symptoms of poisoning were not due to the water, as many farmers and stockmen believed. All grains and forages grown on some farms were extremely toxic.

When the problem was called to the attention of the United States Department of Agriculture in 1932, several bureaus became actively interested. Because the symptoms of poisoning indicated a metallic poison, a systematic search for trace elements was made of a sample of grain which Franke had found toxic by bioassay. This led to Robinson's (156) discovery, in 1933, of the presence of selenium.

All of the cereal samples which Franke (57) found to be toxic by bioassay with rats contained selenium. The protein of the cereal grains carried most of the poison (58), and the selenium was confined chiefly to the

¹ *The Travels of Marco Polo*, edited by Manuel Komroff, Chapter 43, page 81. Boni and Liveright, New York (1926).

protein. Selenium was detected in the soil of all known regions where toxic grain grew, but there was still a possibility of the presence of other poisons. In some seleniferous plants Beath, Eppson, and Gilbert (9) found molybdenum and tellurium, and Byers (28) reported chromium, vanadium, and arsenic in a seleniferous soil. The first biological evidence to indicate that selenium was the sole causative agent was the production, by the addition of sodium selenate and sodium selenite to an otherwise normal diet, of symptoms in the rat (Franke and Potter (68)) which appeared identical with those produced by the natural toxicant. By injection of selenium salts into hens' eggs, Franke, Moxon, Poley, and Tully (62) caused the development of monstrosities similar to those previously found in chick embryos (71) from eggs laid by hens which had been fed toxic grains. Franke and Painter (64) removed nearly all of the selenium from a hydrolysate of a seleniferous protein and then found the hydrolysate to be non-toxic when fed.

B. THE TOXICITY OF SELENIUM

1. *General*

Franke, Rice, Johnson, and Schoening (70) have described the symptoms of the chronic selenium poisoning,—usually called "alkali disease",—of farm animals. All animals lose weight and appear emaciated. There is loss of hair from the mane and tail of horses, from the switch of cattle, and from the body of swine. In severe cases there is a discontinuity in the growth of the hoof, which is followed by a sloughing-off of the old hoof. Post-mortem examinations reveal severe lesions at the joints, which probably explain the lameness. Chick embryos are deformed, especially in the upper beak, so that eggs frequently fail to hatch. This chronic type of poisoning rarely causes death except in young animals, but the economic losses to farmers are large.

Symptoms which appear to be due to acute selenium poisoning were described by Draize and Beath (41). This toxicosis is sometimes called "blind staggers," because the animals lose control of their voluntary muscles. There are reports of enormous losses of livestock from a single feeding of toxic vegetation. Although the reviewer is aware that some plants contain large quantities of selenium, observations of the effects of feeding seleniferous feeds to laboratory animals makes it seem incredible that an animal would voluntarily ingest enough at one time to develop acute poisoning.

Presumably the chronic type of poisoning is the result of prolonged ingestion of forages and grains containing from 10 to 30 p.p.m.² of selenium.

² p.p.m. = parts per million.

These are levels often found in grasses (124) and in farm crops in seleniferous areas. A few plants, however (part I, C), may accumulate several thousand p.p.m. of selenium. In some sections in and around the Rocky Mountains, several of these range plants are eaten by livestock. In addition to selenium, some of these plants contain organic poisons which may contribute to the acute symptoms sometimes noted in range stock.

Of the numerous reports on the toxicosis produced by seleniferous diets in experimental animals, Franke and coworkers (57, 64, 66, 67, 68, 69), Munsell, Devaney, and Kennedy (138), and others have studied the effects on the rat. Franke (57) found that diets which contained from 70 to 82 per cent of toxic grains killed about 70 per cent of young rats by the sixtieth day of ingestion. Analysis (66) showed that most of these grains contained from 25 to 30 p.p.m. of selenium. In general, the animals failed to grow, restricted their food intake, frequently became jaundiced, and after several weeks developed anemia (67, 68). Autopsy revealed hemorrhages, lesions at the joints, necrotic livers if the rats were on a seleniferous diet for an extended period, and, frequently, enlarged spleens. Death was often caused by internal hemorrhage.

The results of feeding seleniferous diets to poultry have been reported in numerous papers by Franke and Tully (71, 178) and Poley, Moxon, and Franke (149).

A few papers have appeared on the accumulation, detoxification, and elimination of selenium (138, 130, 136, 165). Large quantities of selenium are found in the liver, kidneys, and spleen with lesser amounts in other organs and tissues. Selenium is eliminated primarily through the kidneys, but some may be exhaled.

2. Different sources of selenium

Selenium from different sources is not equally toxic. When the results of feeding seleniferous diets to rats were summarized, Franke and Painter (66) found the order of toxicity of selenium from several sources to be as follows: wheat > corn > barley > selenate > selenite > selenide > metallic selenium. There was little difference in the toxicity of selenium in cereals, but these were definitely more toxic than inorganic selenium salts. From the data of Smith, Stohman, and Lillie (164) it would appear that selenite is more toxic than selenate. Since the chemical evidence indicates that selenium in plants is in organic forms (part IV), the toxicity of diselenodiacetic acid, selenodiacetic acid, β, β' -diselenodipropionic acid, β -selenodipropionic acid, dibenzyl diselenide, β -seleninopropionic acid, and *n*-propylseleninic acid was compared with that of selenite by Moxon, Anderson, and Painter (130). None of the organic compounds was as toxic as selenite, but they produced similar symptoms of poisoning.

Evidence that selenium in cereals is more toxic than selenite may be found in the data of Schoening (159) and of Miller and Schoening (125), in addition to the results of Franke and Painter (66). Although corn, with 10 p.p.m. of selenium, produced symptoms of poisoning in swine, much larger quantities of selenite were tolerated.

Not all species react similarly to selenium. It has been observed on farms and in the laboratory that adult animals are less susceptible to selenium poisoning than younger animals. Trelease and Trelease (175) presented evidence that some insects are so resistant to the poisonous action of selenium that they thrive on food sources which would be fatal to most animals.

Dudley (46) has found selenium oxychloride to be toxic when applied to the skin. As little as 0.01 ml. caused death in rabbits and third-degree burns when applied to the skin of man. Concentrations of hydrogen selenide in the air as low as 0.02 mg. per liter killed guinea pigs within 25 days (49) on exposure for 60 min. per day.

Franke and Moxon (61) have compared the toxicity of orally ingested selenium as Na_2SeO_3 and Na_2SeO_4 with that of arsenic as Na_2HAsO_3 , of molybdenum as $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, of tellurium as Na_2TeO_3 , and of vanadium as NaVO_3 . When compared at equal weights of the elements, none of the salts was as toxic as selenium. Martin (122) found that tellurium was less toxic than selenium to animals and plants.

3. Injection of lethal doses of selenium

Several papers have appeared on the results of the injection of selenium compounds. These are summarized in table 1.

The results from different laboratories are not exactly comparable, because the terms "minimal lethal dose" or "minimal fatal dose" are often not rigidly defined. In spite of this, the variations in the results on the same species with the same sources of selenium cannot be explained by the different methods of injection, by the difference in the percentage of mortality, or by the time allowed to elapse before death. Regardless of these differences, the data show more clearly than the results of oral feeding that some species of animals are more resistant to selenium poisoning than others and that there is a great difference in the toxicity of different selenium compounds. The high resistance of cattle and swine to selenium is surprising, because on farms both species often show marked symptoms of poisoning. It seems doubtful if the drench method is a true measure of tolerance to selenium in cattle, because absorption from the rumen is slow and in the presence of organic material some reduction of selenite would be likely to occur.

Shortly after the injection of lethal doses of selenium (60), animals

exhale an odoriferous (garlic-like) compound which Hofmeister (89) reports to be methyl selenide; the evidence for its being methyl selenide is

TABLE 1
Toxicity of single doses of selenium

SOURCE OF SELENIUM	ANIMAL USED	FATAL DOSE IN MILLI-GRAMS OF SELENIUM PER KILO-GRAM OF BODY WEIGHT	NUMBER OF ANIMALS	METHOD OF ADMINISTRATION	OBSERVERS	REFERENCE
Na ₂ SeO ₃	Rat	5.7			Muehlbeyer and Schrenk	(137)
Na ₂ SeO ₃	Rat	3.25-3.50	155	Intraperitoneal injection	Franke and Moxon	(60)
Na ₂ SeO ₃	Rat	3.0	45	Intravenous injection	Smith, Stohlman, and Lillie	(164)
Na ₂ SeO ₃	Rabbit	1.5	9	Intravenous injection	Smith, Stohlman, and Lillie	(164)
Na ₂ SeO ₃	Rabbit	0.9			Muehlbeyer and Schrenk	(137)
Na ₂ SeO ₃	Horse	<4.4	5	Stomach tube	Miller and Williams	(126)
Na ₂ SeO ₃	Mule	3.3±	3	Stomach tube	Miller and Williams	
Na ₂ SeO ₃	Cow	11.0±	5	Drench	Miller and Williams	
Na ₂ SeO ₃	Pig	15.0±	5	Drench	Miller and Williams	
Na ₂ SeO ₄	Rat	4.3			Muehlbeyer and Schrenk	(137)
Na ₂ SeO ₄	Rat	5.25-5.75	90	Intraperitoneal injection	Franke and Moxon	(60)
Na ₂ SeO ₄	Rat	3.0	37	Intravenous injection	Smith, Stohlman, and Lillie	(164)
Na ₂ SeO ₄	Rabbit	2.0-2.5	16	Intravenous injection	Smith, Stohlman, and Lillie	
Colloidal selenium...	Rat	6.0			Muehlbeyer and Schrenk	(137)
Organic selenium compounds.	Rat	20-40		Intraperitoneal injection	Moxon, Anderson, and Painter	(130)
Organic selenium compounds.	Rat	>25		Intraperitoneal injection	Moxon	(129)
<i>d,l</i> -Selenocystine.....	Rat	4.0	65	Intraperitoneal injection	Moxon	(129)

far from convincing, as Schultz and Lewis (160) point out. The reviewer cannot distinguish the odor from that of hydrogen selenide. Respiration

becomes increasingly difficult and the animals die gasping for breath. In some cases there is complete anesthesia just before death, but in other cases there is a convulsive struggle. Loss of fluid from the blood into the abdominal and thoracic cavities may cause the hemoglobin level to reach 30 g. per 100 ml. of blood (68).

Franke and Moxon (60) injected the same salts of arsenic, molybdenum, tellurium, and vanadium as were fed orally (61). At equal weights of the element, the salts decreased in toxicity in the following order: tellurite, selenite, vanadate, arsenite, selenate, arsenate, tellurate, and molybdate. There was a much greater difference between the toxicity of tellurite and tellurate and of arsenite and arsenate, than between that of selenite and selenate. Molybdenum, which Beath, Eppson, and Gilbert (9) found to be taken up by plants in quantities which produced symptoms of poisoning in animals, was non-toxic at the levels injected (160 mg. per kilogram of body weight) and when fed (61).

4. *Selenium in human nutrition*

Several papers on the possibility of selenium poisoning in humans have appeared. The discovery of the presence of traces as high as 1 p.p.m. of selenium in the urine of the majority of people living in highly seleniferous areas at first appeared alarming. From the data of the first survey, Smith, Franke, and Westfall (163) found no symptoms which could be considered pathognomonic of selenium poisoning in man; later, Smith and Westfall (166) believed they had evidence that selenium caused gastric or intestinal dysfunction, and possibly hepatic dysfunction, in some sections. These findings are not surprising where locally grown food supplies a large proportion of the diet. Meat, milk, eggs, and vegetables may contain considerable quantities of selenium when produced on farms in the seleniferous areas. Manville (120) emphasizes the potential danger of selenium-bearing foods to public health.

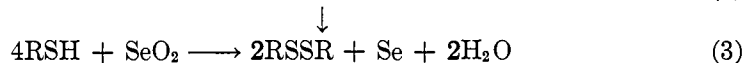
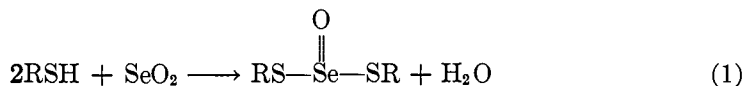
Dudley (47) stresses the potential danger of selenium injury in industrial operations, particularly in copper refining. He describes, as did Hamilton (87), symptoms of poisoning presumably caused by the inhalation of hydrogen selenide, selenium dioxide, and other selenium compounds. The concentration of selenium in the urine of industrial workers showing marked pathological symptoms (44) was less than one-tenth of that of many human urines (163) taken in seleniferous areas. Elimination of inorganic selenium taken into the lungs may be more rapid and follow other pathways than organic selenium taken orally, but these results are difficult to explain in view of the recorded evidence that selenium in food-stuffs (66) is more toxic than inorganic selenium. Hydrogen selenide is volatile and it may be very toxic when inhaled. Franke and Potter (68) found sodium selenide much less toxic to rats than sodium selenate or

sodium selenite when fed, but it is likely that some oxidation of selenide to elemental selenium occurred before absorption.

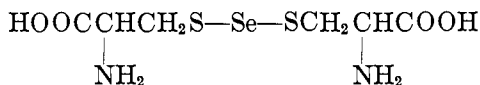
In this connection one might describe the symptoms observed by the reviewer from a single inhalation of hydrogen selenide in high concentration, which passed about 4 in. along the nasal passage. As the vapors traversed the nasal passages there was a metallic sensation, somewhat like that produced by a silver nitrate spray. After a brief sensation of intoxication, no ill effects were felt for about 4 hr. Then a copious discharge of mucous from the nasal passages began. This persisted, with violent sneezing similar to the symptoms of a severe head cold, for 3 or 4 days. No ill effects were noted later. The author has never had "selenium breath," which is rumored to result from working with selenium compounds.

5. Action of selenium in the animal body

Little is known of the mechanism of selenium poisoning. It inhibits carbon dioxide production during yeast fermentation (132), as well as the oxygen uptake of yeast cells (152). Selenite readily oxidizes sulfhydryl compounds, forming disulfide and an unstable RS—Se—SR compound. With sulfhydryl compounds the reaction may take three courses:



The reviewer (unpublished work) has obtained an amino acid from the action of selenite on cysteine; this acid is thought to be



In every preparation isolated, the mole S:Se ratio was slightly greater than 2, owing to the presence of some cystine. Separation of compounds of the type RS—Se—SR from disulfides is difficult, because of the instability of the former. Metallic selenium separates from solution,—but more rapidly from basic than from acidic solutions,—to form the disulfide. Bersin (18), who prepared the compound HOOCCH₂S—Se—SCH₂COOH from thioglycolic acid and selenite, believes that a similar unstable compound forms with glutathione. Seleninic acids will also reduce sulfhydryl compounds to disulfides (unpublished work), but no addition compound has been isolated.

It does not seem improbable that selenite or seleninic acids inhibit certain enzymatic reactions dependent upon reversible sulfhydryl \rightleftharpoons disulfide changes and those systems which require the presence of free sulfhydryl groups, i.e., succinic dehydrogenases. In addition to the chemical studies, Dubois, Rhian, and Moxon (43) find that glutathione when injected will protect rats from doses of selenite which will normally cause death. Selenium in its natural forms in plants must react in a manner different from selenite, because the chemical evidence (part IV) indicates that it is in the reduced form.

The ability of some proteins, when fed at high levels, to counteract the chronic symptoms of selenium poisoning in rats was observed by Moxon and has been further studied by Gortner (84a) and by Lewis, Schultz, and Gortner (118a). This protective action of some proteins cannot at present be ascribed to any particular amino acid of the proteins. Not all "complete proteins" are effective. In some cases methionine,—but not cystine,—supplements (118a) to diets were as effective as high protein, but with other proteins (84a) methionine was not beneficial.

Recently Moxon and Dubois (131) and Dubois, Moxon, and Olson (42) have shown that small amounts of arsenic will alleviate the toxicity of selenium to animals. The exact rôle of arsenic seems very obscure.

C. SELENIUM IN SOILS AND IN PLANTS

Byers (29, 30, 33), Beath and coworkers (8, 11, 12, 111), and Moxon and coworkers (133, 134) have shown that selenium occurs in rocks and soils from the Niobrara, Pierre, Steele, Benton, and other geological formations. The most complete studies of certain formations, those by Moxon *et al.* (133, 134), have shown that the selenium content varies in certain members of these formations, so that highly seleniferous areas may be predicted from the geological formation. It is generally stated that selenium was deposited during the Cretaceous period, but Beath and coworkers (11, 12, 111) find highly selenized soils geologically much older than Cretaceous. After studying the seleniferous soils of Hawaii, Byers, Williams, and Lakin (34) suggested that the selenium in soils is of volcanic origin. Selenium in volcanic emanations may be a primary source of selenium in soils, but the work of Moxon, Olson, and Searight (133) indicates that the selenium in soils of the continental United States is of marine origin.

The surveys conducted by Byers (29, 30, 33) have revealed seleniferous areas extending west from the western Dakotas, Nebraska, Kansas, and Oklahoma to the coast states. One must not gain the impression that the entire area, most of which is marginal agriculturally, is seleniferous. There are, however, sections which are producing seleniferous crops, or are

potential producers, because there is selenium in the soil. As many agricultural sections in this country are underlaid with seleniferous formations which have been covered with glacial drift, it was concluded that glaciated areas were free from seleniferous plants. Recently, Byers and Lakin (32) pointed out a large area in western Canada and in the northwestern United States which was glaciated but which produces highly seleniferous plants. Analysis by Robinson (157) of crops from various parts of the world showed that seleniferous areas are widespread over the surface of the earth.

Rivers and ground waters rarely contain detectable amounts of selenium. Waters of the Colorado river and its tributaries are free from selenium above diversion points (33, 183), but from the points where the drainage from irrigated lands is put back into the river the selenium concentration increases.

Selenium is found in much the same mineral deposits (170) and soil formations as those where sulfur abounds. In this connection the presence of selenium in deep sea deposits (133, 182), in sea water (84), and in meteorites (31, 170) is of interest.

The amount of selenium absorbed by plants is dependent more on the availability of the compounds of selenium than on the selenium content of the soil. The forms of selenium generally considered to be present in soils are as follows: (1) elemental, (2) pyritic or selenide, (3) selenite, (4) selenate, and (5) organic. Very little is in the elemental form (33). In sulfide ores, usually iron pyrites (161), selenium is often present in high concentrations, but the selenium in pyritic concretions of soils is not an important direct source of selenium in plants. Since (1) much of the selenium in seleniferous soils is very insoluble, (2) seleniferous soils are highly ferruginous, and (3) very insoluble compounds of selenium form with selenite and ferric iron (170), Williams and Byers (184) have concluded that a major portion of the selenium in many soils is present as an insoluble basic iron selenite. When they (184) precipitated compounds of ferric selenite from dilute solutions of ferric chloride and sodium selenite, the composition varied with the Se:Fe ratio. Some of their preparations had a composition which could be approximately defined by the formula $\text{Fe}_2(\text{OH})_4\text{SeO}_3$, but in all of their work the Se:Fe ratios were many times greater than the Se:Fe ratios in soils. As supporting evidence for the presence of selenate (probably as CaSeO_4), Williams and Byers (184) found that the water-soluble selenium in soils was reduced to elemental selenium by the same methods which reduce selenate. Olson and Moxon (141) presented data to indicate that a considerable quantity of organic selenium occurs in soils. Humus may contain approximately 40 per cent of the selenium in some soils.

Before selenium can be taken up by plants, it must be present in a available

soil forms. There is a great difference in the availability of different compounds. Moxon, Olson, and Searight (133) grew plants on a non-seleniferous soil to which selenium was added at the rate of 2 p.p.m. from the following sources: Na_2SeO_4 , CaSeO_4 , Na_2SeO_3 , $\text{Fe}(\text{OH})\text{SeO}_3$ (approximate composition), FeSe , and organic selenium (a water extract of *Astragalus*). Selenium was taken up from selenates in large amounts and from selenites in moderate amounts; from iron selenide and from organic selenium there was no absorption of selenium by some cereals and only a very small amount by *Astragalus*. The results with selenites did not support Byers' contention that selenium is relatively non-available in basic ferric selenite. He accounted for the fact that plants growing on moderately to highly seleniferous soils in Hawaii and Puerto Rico have not been found to contain over 3 p.p.m. of selenium (112), by assuming that the selenium is in the form of an exceedingly non-available basic ferric selenite. Obviously the composition of a soil is an important factor in the availability of different compounds of selenium. Selenite may be firmly bound in soils, as Franke and Painter (65) were unable to electro-dialyze all of the selenite added to a suspension of a seleniferous soil. Apparently sufficient time was not allowed by Moxon, Olson, and Searight (133) for bacterial decomposition of the organic selenium compounds, because Beath *et al.* (10) have found these forms of selenium available to plants.

Presumably areas of high rainfall are relatively free of danger from poisonous quantities of selenium in plants, even though the soils are seleniferous. The percolating action of water would remove the more available soluble selenium salts and soluble organic compounds. The selenium would ultimately find its way to the sea. Most soils producing seleniferous crops are immature because they have weathered slowly.

Concomitant with the available selenium in soils is the variable capacity of different plants for absorbing selenium. Cereal grains are moderate absorbers of selenium (124) but rarely contain more than 30 p.p.m. Of the plants in the seleniferous areas, some native grasses absorb the least amount of selenium. A few plants,—notably some species of the genera *Stanleya*, *Oenopsis*, *Astragalus*, and *Xylorrhiza*,—often accumulate several thousand parts of selenium per million. The selenium contents of a group of plants and of the soils upon which the plants were growing are shown in table 2. These results were taken from tables in numerous publications from the laboratories of Franke and Moxon, of Byers, and of Beath. The soils are in the seleniferous areas of the continental United States and range from what may be considered highly seleniferous to mildly seleniferous.

The data show the wide variations in the absorption of selenium by different plants and the variations which occur between the selenium content of the plant and that of the soil upon which the plant was growing.

Presumably the differences were due primarily to different selenium compounds in the soil. The last three species, commonly called indicator plants, have the capacity of absorbing selenium from forms only slightly available to the other plants. Beath, Eppson, and Gilbert (10) found that *Astragalus* would absorb selenium from soils artificially selenized by elemental selenium.

TABLE 2
Selenium content of plants and of soils

VEGETATION	SELENIUM CONTENT							
	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Wheat.....	12.0	40	3.5	40	3.1	25	1.5	4
Corn.....	3.5	10	3.1	23	2.0	1	0.3	0
Barley.....	3.1	22	2.5	10	2.0	12	1.0	1
Alfalfa.....	2.0	12	1.5	60	0.7	1	0.5	25
Sweet clover.....	9.0	50	2.0	3	1.5	25	1.5	1
Western wheat grass.....	13.0	10	5.5	30	0.7	12	0.5	25
Blue gramma.....	6.0	4	3.5	2	2.0	2	0.2	0
Little bluestem.....	9.0	1	4.0	5	3.5	1	1.0	0.5
Mixed native grasses.....	20.4	47	6.0	3	3.0	1	0.5	3.5
Russian thistle.....	3.0	3	2.0	12	1.0	40	0.7	3
Sunflower.....	3.0	12	0.7	7	0.7	4	0.6	1
Wreath aster.....	8.0	130	4.0	7	1.0	210	0.3	1
Woody aster.....	3.5	5390	2.0	200	0.7	120	0.3	6
<i>Stanleya pinnata</i>	20.4	1252	5.0	470	4.0	1070	1.0	20
<i>Oenopsis condensata</i>	20.4	3250	8.1	664	3.5	9120	1.5	850
<i>Astragalus racemosis</i>	27.0	1160	10.0	1690	5.0	5560	2.5	60
<i>Astragalus racemosis</i>	21.0	4100	6.1	2700	5.0	690	0.7	920
<i>Astragalus bisulcatus</i>	20.4	2590	3.0	170	2.0	2120	0.8	3030
<i>Astragalus bisulcatus</i>	8.1	5330	2.5	590	1.5	3140	0.7	100
<i>Astragalus pectinatus</i>	8.0	1330	3.5	4000	2.5	2270	1.5	3890
<i>Astragalus pectinatus</i>	5.0	1980	3.0	2590	2.0	2120	0.2	840

Recently Beath, Eppson, and Gilbert (12) have used the occurrence of selenophilic indicator plants to locate seleniferous soils throughout the country. Sufficient data have been presented to warrant the conclusion that these indicator plants abound on most seleniferous soils. Selenite and selenate (93) are toxic to many plants, but it is doubtful if the concentrations in soils are great enough to explain the absence of these plants on some seleniferous soils. Since Trelease and Trelease (176) find selenium to be a stimulating and possibly an essential element in the metabolism of

some species of *Astragalus*, it may be that the indicator plants compete more successfully with other plants when growing on seleniferous soils.

Indicator plants are potential accumulators of available selenium in soils. Beath *et al.* (10) have shown that, whereas only indicator plants absorb large quantities of selenium from raw shales, the selenium from these plants is readily taken up by other plants, either as soluble organic compounds or after bacterial decomposition and oxidation to inorganic salts. Thus the soils are enriched with available selenium through countless cycles of growth and decay by these converter plants.

D. SELENIUM AND SULFUR IN PLANTS

Because of the similar chemical properties of selenium and sulfur, considerable speculation has appeared in the literature as to whether or not selenium compounds analogous to those of sulfur occur naturally in

TABLE 3
Selenium and sulfur in plants

CROP	SULFUR CONTENT	SELENIUM CONTENT
	<i>per cent</i>	<i>per cent</i>
Cabbage.....	2.99	0.0520
Black mustard.....	1.97	0.0470
Flax.....	1.32	0.0358
Vetch.....	0.77	0.0150
Wheat.....	0.84	0.0225
Soybean.....	0.51	0.0140
Corn.....	0.42	0.0075

seleniferous plants. Aside from the analogous chemical behavior, some studies of plant metabolism have indicated a relationship between these elements.

As early as 1880, Cameron (35) found that plants would absorb selenium and suggested that it might replace sulfur. Hurd-Karrer (94, 96) has found that, in general, plants which absorb large quantities of sulfur absorb large quantities of selenium. A few analyses of young plants reported by Hurd-Karrer (96) are shown in table 3.

From the enormous quantities of selenium absorbed by indicator plants, it would seem that the absorption of selenium is out of proportion to the available sulfur and selenium in the soil, but when grown under experimental conditions *Astragalus bisulcatus*, a wild legume, absorbed selenium and sulfur in a ratio consistent with that found in other plants of the same genus.

The discovery that sulfate or elemental sulfur (93) would diminish selenium injury to plants and reduce the quantity of selenium absorbed

was received with considerable enthusiasm. Hurd-Karrer's (93, 94) results indicated a definite antagonism between the two elements, because the absorption and toxicity was dependent upon the S:Se ratio in the nutrient solution. Martin (121) found less inhibition by sulfur of the toxicity of selenium to plants than did Hurd-Karrer in artificially selenized soils, and Franke and Painter (65) were unable to reduce, by the application of sulfur, the absorption of selenium in crops grown on naturally seleniferous soils. Sulfur was without effect when the source of the selenium in the soil was seleniferous *Astragalus* (10). Most seleniferous soils are abundant in gypsum. Olson and Moxon (141) believe that the forms of selenium in soils have a much greater influence on the absorption of selenium than does the sulfate content.

The divergent results from several laboratories find explanation in later studies by Hurd-Karrer (95, 97), in which she shows that injury by selenate and absorption of selenium decreased progressively with increased sulfate concentrations, but that, with selenite, sulfate was effective over limited changes in concentration. High sulfate concentration did not reduce the selenium in plant tops when selenite was the source of selenium. Thus with analogous compounds,—i.e., selenate and sulfate,—there is a definite relationship in the absorption which depends upon the concentrations. A preferential absorption of sulfur was evident.

In the cereal grains Franke and Painter (63, 145) found most of the selenium in the protein, as is the case with sulfur. The mole S:Se ratios (145) in the whole grain and protein were generally close.

Many of the chemical properties of selenium in plants (part IV) are similar to those of sulfur. If selenium analogs of sulfur compounds are present, the number in most plants is small and confined to a few types. The sulfur compounds in higher plants are of the following types: RCNS (in glucosides), RSR (vinyl and allyl sulfides and in methionine), RSCH₂SR (the cysteine thioacetal of formaldehyde in djenkolic acid), RSH and RSSR (cysteine, cystine, and glutathione). All of these are straight-chain systems. A few other compounds,—a sulfhydryl compound, ergothione, a thiazole derivative, thiamin, sulfonic derivatives, and sulfate esters,—are known. Of these, allyl isothiocyanate, vinyl sulfide, and allyl sulfide are abundant in only a few plant species, whereas cystine, methionine, and sulfates occur generally in higher plants in appreciable amounts.

It can readily be shown that the deposition of selenium does not quantitatively follow that of sulfur and that analogous compounds are not present in the same ratios. In cereals Painter and Franke (145) found the mole S:Se ratios to vary little, as the following illustration will show:

	WHOLE GRAIN	CRUDE GLUTEN	GLIADIN	GLUTENIN
Mole S:Se ratio	159	165	174	145

With *Oönopsis condensata*, an indicator plant, the mole S:Se ratio in the stems and leaves was 6.2, but in the roots it was 2.8. The same plant contained sulfate, but no selenate could be demonstrated (145). The presence of inorganic selenium in plants has not yet been proved, and only metallic selenium has been indicated. Except in the root systems of plants growing in selenized nutrient solutions or soil cultures, the selenium in plants seems to be present in organic forms.

II. METHODS OF ANALYSIS

Selenium could be easily determined where there was enough to weigh, but in plants, soils, and animal products which contained a few parts per million, new methods were essential. Since selenium in biochemical products appears to be in an organic form, oxidation is a necessary step.

TABLE 4
Comparison of methods for the determination of selenium

COMPOUND	SELENIUM CONTENT		
	Parr bomb method	Distillation method	Theory
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Diselenodiacetic acid.....	57.0	57.3	57.2
β, β' -Diselenodipropionic acid.....	52.2	51.9	52.0
Seleninoacetic acid.....	45.2	44.9	46.2
β -Seleninopropionic acid.....	42.4	41.1	42.7
<i>n</i> -Propylseleninic acid + HNO ₃	36.1	36.1	36.2
Benzyl selenide.....	30.1	30.5	30.3
Benzyl diselenide.....	46.4	46.5	46.4
β -Selenodipropionic acid.....	35.1	36.1	35.1

Horn (90) applied the codeine sulfate method to a sulfuric acid digest of plants as a qualitative test, but Martin (121) and Gortner and Lewis (85) report quantities from colorimetric comparisons. The reviewer has found that there is a loss of selenium by oxidation in sulfuric acid; hence the results obtained by this method are likely to be low when applied to materials which are difficult to digest to a clear solution.

The method of Robinson *et al.* (158) obviates the objection just stated, because the digestion is carried out in a closed system with the vapors, which contain some selenium dioxide, passing through a cooled bromine-hydrobromic acid solution. This method of digestion, which is similar to that of Fredga (76), has been widely used in the determination of selenium in plants and soils.

In order to compare the toxicity of selenium in plants with that of inorganic selenium salts, the method for determining selenium in plants must be accurate. Therefore the selenium content of several organic

compounds was determined (Painter, unpublished work) by the distillation method of Robinson *et al.* (158) and by the Parr bomb method, which Shaw and Reid (162) have found to be dependable.

From this comparison it can be assumed that a quantitative recovery of selenium is accomplished by the distillation method. The danger of the loss of volatile selenium bromide from the receiving flask would be greater when determining selenium in cereals, forages, or animal products, because the time required for digestion is much greater than with small samples of organic selenium compounds.

At the temperatures used in the modified methods of Dudley and Byers (48) and of Williams and Lakin (185), which are applicable to biological materials high in water, there is slight danger of loss of oxidized selenium. Dudley (45) has devised a method for the determination of selenium in air-gas-dust mixtures.

The colorimetric comparison of colloidal selenium is not entirely satisfactory, because the probable error in the analysis of plants is large. Franke, Burris, and Hutton (59) have improved the method for smaller quantities than can be compared accurately in solution. With quantities too large for satisfactory comparison of colloidal selenium, the volumetric method of Beath, Eppson, and Gilbert (9) is applicable. Application of the titration of metallic selenium by iodate or bromate and of selenite by thiosulfate, as outlined by Coleman and McCrosky (38), should increase the precision of the determination of selenium in plants.

III. ORGANIC COMPOUNDS OF SELENIUM

It is not in the scope of this review to list all of the known organic compounds of selenium or all methods which have been used in their synthesis. For these, reference is made to the reviews of Bradt and coworkers (20, 21, 22, 23, 24, 25). In the dissertations of Fredga (76) and van Dam (40), special phases of the chemistry of organic selenium compounds are discussed. A few general methods of preparation and a few reactions of each group of organic selenium compounds will be given.

The known types of organic selenium compounds are similar to those of sulfur. Organic selenols and selenides form insoluble complexes with mercury and with some other heavy-metal salts similar to the corresponding sulfur compounds. The general reactions of organic selenium compounds are similar to those of organic sulfur compounds, but selenium exhibits more metallic properties than sulfur. Selenite and selenate, or corresponding organic derivatives, are oxidizing agents, whereas reduced selenium is readily oxidized to the metallic form if inorganic and to diselenide if a selenol.

Metallic selenides or diselenides, selenocyanates, elementary selenium, selenium dioxide, selenium oxychloride, and selenium halides react with appropriate compounds to introduce selenium into organic molecules. Hydrogen selenide, which is necessary in many methods, is conveniently prepared by passing hydrogen through a hot suspension of selenium in a heavy motor oil (86).

A. SELENIDES

The following methods, which involve the direct introduction of selenium, are those usually used in the preparation of selenides:

- (1) $2RX + M_2Se \rightarrow RSeR + 2MX$ (76, 98, 128, 155, 167, 177)
- (2) $RSeM + R'X \rightarrow RSeR' + MX$ (15, 55, 79, 81, 105, 116, 150, 161)
- (3) $RMgX + Se \rightarrow RSeMgX$ (105, 151, 172)
 $2RSeMgX \rightarrow RSeR + Se(MgX)_2$
- (4) $RSeMgX + R'X \rightarrow RSeR' + MgX_2$ (53)
 $RMgBr + R'SeBr \rightarrow RSeR' + MgBr_2$ (13)
- (5) $2RNNX + M_2Se \rightarrow RSeR + N_2 + 2MX$ (113, 116)
 $RNNX + MSeR' \rightarrow RSeR' + N_2 + MX$ (104, 105, 106)
- (6) $RNNX + MSeCN \rightarrow RSeCN + N_2 + MX$ (16, 36, 79, 81, 101,
 105, 106, 114)
 $PhNH_2 + Se_3(CN)_2 \rightarrow p\text{-}H_2NPhSeCN$ (36)
 $RSeCN + R'X + KOH \rightarrow RSeR' + KX + HOCN$ (101)

Aliphatic selenides are readily prepared by method 1, but in method 2 difficulties are encountered, because selenols are readily oxidized to diselenides on exposure to air and some selenols are unstable in alkaline solution. Methods 4 and 6 are limited to compounds of $R'X$ with active halogens, and method 3 is more often applied to the preparation of selenols than of selenides.

Selenoxides and many selenonium compounds give selenides on reduction. Diselenides also give selenides at high temperatures.

Selenides are the most stable class of organic selenium compounds. All but about sixty of more than two hundred known selenides (20) are aryl, heterocyclic, or mixed alkyl-aryl, alkyl-heterocyclic, and aryl-heterocyclic derivatives.

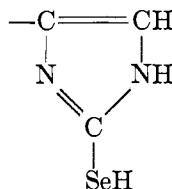
B. SELENOLS AND DISELENIDES

Since selenols and diselenides are reversibly interconvertible, methods for introducing selenium into organic molecules are often identical. The following methods include those generally used:

- (1) $RX + MSeH \rightarrow RSeH + MX$ (79, 123, 177)
 $2RSeH \xrightleftharpoons[2H]{\frac{1}{2}O_2} RSeSeR + H_2O$
- (2) $RSeMgX + HX \rightarrow RSeH + MgX_2$ (55, 105, 114, 172)
- (3) $2RX + M_2Se_2 \rightarrow RSeSeR + 2MX$ (40, 76)
- (4) $RX + MSeCN \rightarrow RSeCN + MX$ (16, 72, 76, 79, 114)
 $RSeCN + MOH$ (or HX) $\rightarrow RSeH + HOCN$
- (5) $2RNNX + M_2Se_2 \rightarrow RSeSeR + N_2 + M_2X$ (116)
- (6) $Se_2X_2 + 2RMgX \rightarrow RSeSeR + 2MX_2$ (169)
- (7) $K_2SeSO_3 + RX \rightarrow KSO_3SeR + KX$
 $2KSO_3SeR \rightarrow RSeSeR + K_2S_2O_6$ (79)

Methods 1, 2, 3, and 4 have found general application when R is aliphatic, and methods 2 and 5 when R is aryl. In method 4 the halogen must be active. There is good evidence that some diselenide of the type $R_2Se=Se$ is formed (76) with $RSeSeR$ in method 3.

Only eight of about thirty known selenols are aliphatic. Two selenols (22) which are imidazole derivatives of the type



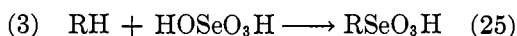
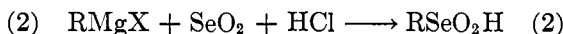
have been described. Mono-, di-, and tri-selenoglycerols have been prepared by Baroni (7), by the use of reactions similar to those in the preparation of the thio derivatives. Wrede (186) has prepared carbohydrates with a hydroxyl group replaced by SeH in the 6-position. Diselenides are more stable than selenols; hence more of them (about fifty) are known. Nearly all are homocyclic, aryl, and heterocyclic derivatives.

Fredga (76) and Backer and van Dam (2) have separated the *d*- and *l*-forms of optically active diselenides. They give surprisingly high values for optical rotation, like those obtained with disulfides.

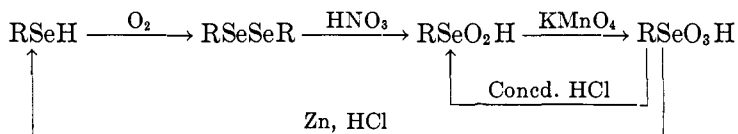
C. ORGANIC SELENIUM ACIDS

The more common members of this class, the seleninic and selenonic acids, are conveniently prepared by the following methods:

- (1) $\left. \begin{array}{l} RSeH \\ RSeSeR \\ RSeCN \end{array} \right\} \xrightarrow[HNO_3]{H_2O_2; KMnO_4; HNO_3} RSeOOH$
 $\xrightarrow{HNO_3} \uparrow$
 $\xrightarrow[(3, 40, 76, 116, 161, 168, 154)]{KMnO_4; Cl_2 \cdot H_2O; 30\% H_2O_2 \text{ in } CH_3COOH; K_2CrO_4} RSeO_3H$



In the stepwise oxidation of selenols, each compound can be isolated, but the reduction is difficult to control.



Bradt and Valkenburgh (25) list twenty seleninic acids and thirteen selenonic acids. In all but seven, the selenium is attached to the benzenoid ring. Several seleninic acids, mostly derivatives of organic acids, were later described by van Dam (40) and by Fredga (76). Banks and Hamilton (6) have described amides of seleninic acids.

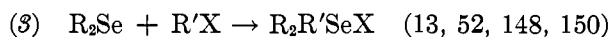
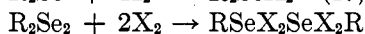
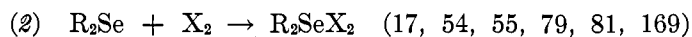
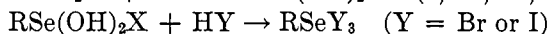
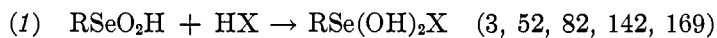
A notable difference in the ease of oxidation of sulfur and of selenium is evident from the methods used to prepare organic sulfur and selenium acids. Reagents which oxidize sulfhydryl groups and disulfides to sulfonic acids do not oxidize selenols and diselenides to selenonic acids, but only to seleninic acids. Seleninic acids are then much easier to prepare than sulfinic acids, but, like sulfinic acids, they are not stable. The acid salts of seleninic acids,—the selenonium compounds,—are more stable than the free acids. Selenonic acids, which require strong oxidizing reagents for their preparation, are more stable than seleninic acids, but both are strong oxidizing agents when compared with sulfur compounds of the same valence.

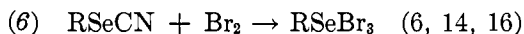
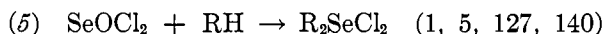
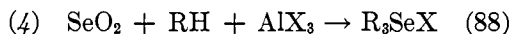
One selenol acid, $\text{C}_6\text{H}_5\text{C}(=\text{O})\text{SeH}$, the acid amides of five seleno acids, $\text{RC}(=\text{Se})\text{OH}$, and the potassium salt of the ethyl ester of selenol carbonic acid (25), potassium selenoxanthogenate, have been described.

D. SELENIUM COMPOUNDS

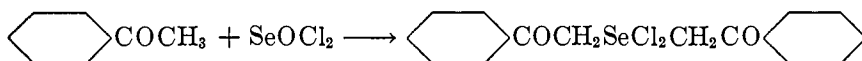
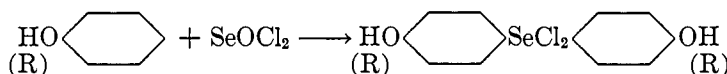
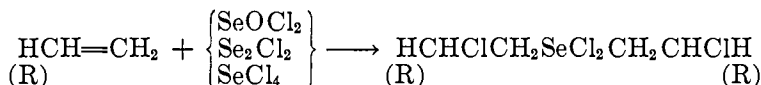
All compounds of the class RSeX_3 , R_2SeX_2 , R_3SeX , and $\text{RSeX}_2\text{SeX}_2\text{R}$ (R = an alkyl, aryl, or heterocyclic group; X = Cl, Br, I, OH, or NO_3) are classified as selenonium compounds. More than three hundred are known, so this is the largest group of organic selenium compounds.

Only a few of the many methods of preparation are listed:

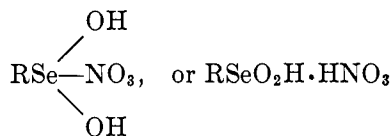




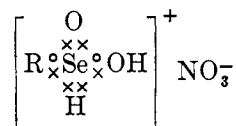
The reactions of SeOCl_2 , Se_2Cl_2 , and SeCl_4 with olefins (17), phenols (127, 140), phenol esters (1), and alkyl-aryl ketones (139) all yield selenonium compounds.



The formation of selenonium compounds from seleninic acids, particularly when excess nitric acid is used to oxidize selenols or diselenide, has been used as an explanation for the failure of nitric acid to oxidize seleninic acids to the selenonic acids (161). The structure usually given for these compounds

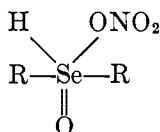


may, when the electronic structure of selenium is considered, be written



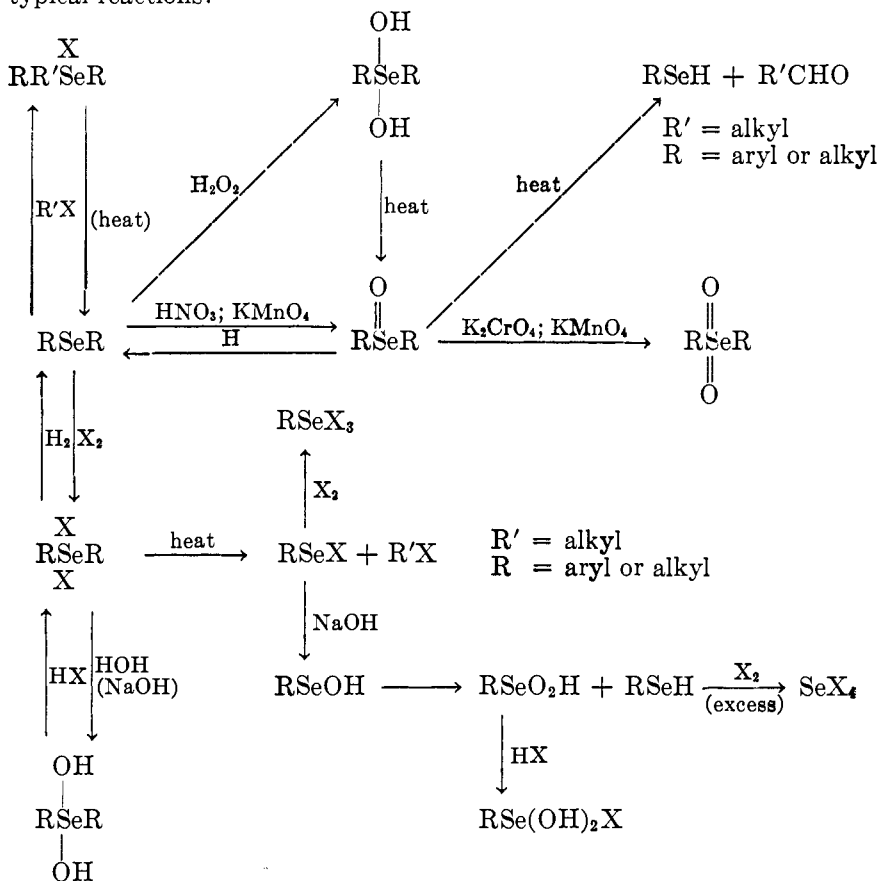
Selenonium compounds possess ionizable groups, and seleninic acids form salts with both acids and bases.

Similarly to its action on selenols or diselenides, nitric acid oxidizes selenides to selenoxides but not to selenones. Foster and Brown (55) suggest that a compound with hexavalent selenium



is formed, which prevents further oxidation. They state that on neutralization the selenoxide may be oxidized to the selenone, but few selenones (81) have been reported. Since these compounds and the dihalides of selenium ethers ionize in solution, it seems that they should be considered salts of a similar type, i.e., as acid addition products of seleninic acids. With halogen acids selenoxides give dihalides.

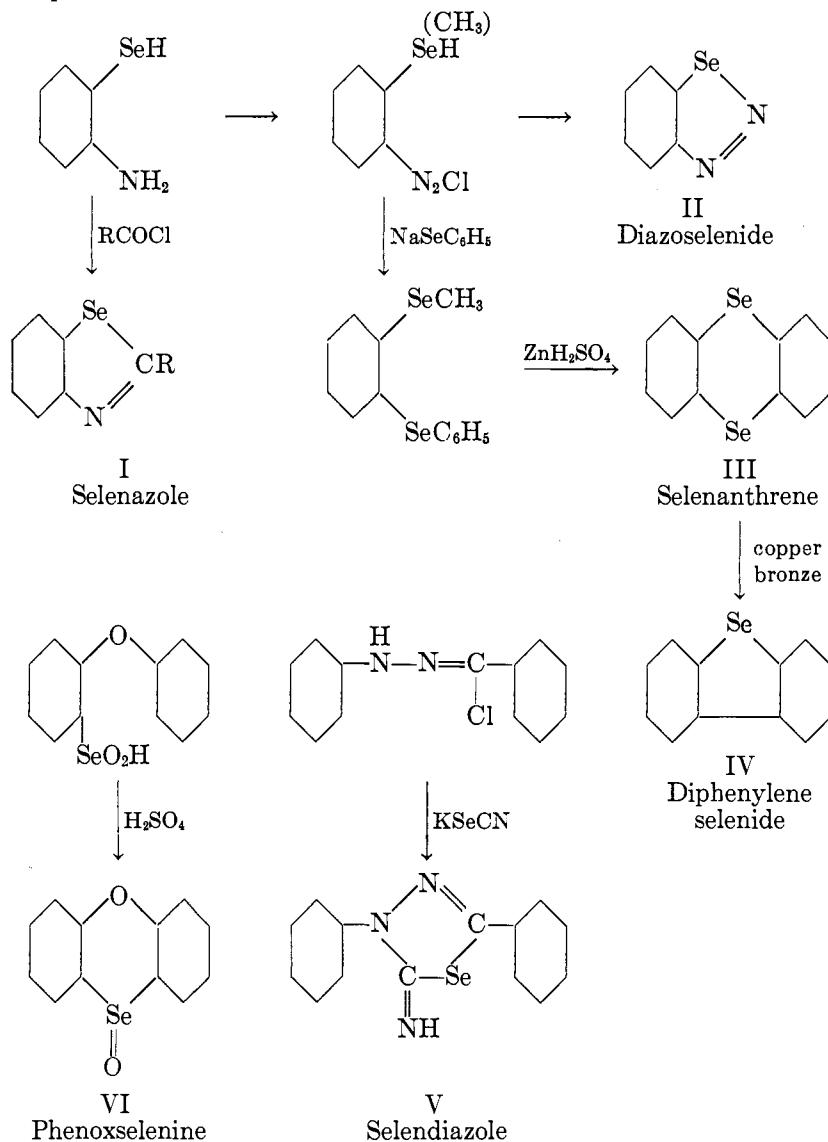
Although the stability of selenonium and related compounds is dependent upon the radical attached to selenium, the following may be considered typical reactions:



If one R is aliphatic, the compounds are less stable (13, 52); hence cleavage to a selenol or monohalide may occur. Selenols or diselenides give similar reactions, but when there is only one organic group attached to the selenium atom there is one more position at which a reagent may be added.

$$\begin{array}{c} \text{O} \\ || \\ \text{R}'\text{SR} \end{array}$$
 Sulfoxides of the type $\text{R}'\text{SR}$ can be resolved, but Gaythwaite, Kenyon,

and Phillips (81, 82) were unable to resolve selenoxides; this indicates that the linkage between selenium and oxygen may not be an unsymmetrical semipolar double bond.



E. COMPOUNDS WITH SELENIUM IN RING SYSTEMS

Many compounds with selenium in ring systems have been prepared. Bradt lists these with selenides, diselenides, or selenonium compounds, and most methods of preparation have been given under these classes.

Selenophene (26, 27, 171, 179) has properties similar to those of thiophene, for both are more stable to reagents than would be expected. A series of cyclic selenides,—cycloselenopropane, cycloselenobutane, cycloselenopentane, and cycloselenohexane and their derivatives,—were described by Morgan and Burstall (128). Substituted compounds of selenazole (I) (12, 37, 51, 108, 107), diazoselenide (II) (100), selenanthrene (III) (39, 100, 102, 103), diphenylene selenide (IV) (39), selendiazole (V) (80), phenoxselenine (VI) (173), selenoxanthene (21), selenoxanthone (56, 116, 173), selenonaphthene (110, 115), phenselenazine, a seleno methylene blue (21), selenothiana (83), 1,4-selenoxane (83), a pelletierine derivative, 3-selen-9-azobicyclo-(3,3,1)-nonane (19), and a spiro compound, 2,6-diseleno-4-spiroheptane (4), are known.

A few diselenides in ring systems have been prepared by Fredga (73, 76) and by Backer and Winter (4).

F. MISCELLANEOUS ORGANIC SELENIUM COMPOUNDS

Many seleno aldehydes and ketones have been prepared (24), but it is doubtful if monomers have been studied, because of the tendency for these compounds to polymerize. The usual method is to add hydrogen selenide to an inert solvent containing an aldehyde or ketone and hydrogen chloride. Shaw and Reid (161) have described several selenomercaptoles.

One selenonium selenol (R_3SeSeH) has been reported (24), but no selenide selenol ($RSeSeH$) is known.

Ethyl derivatives of $-S-Se-S-$ and of $-Se-S-Se-$ and a triselenide, $-Se-Se-Se-$, have been prepared by Levi and Baroni (117) by the reaction between ethyl selenol or ethyl mercaptan and thionyl chloride or selenium oxychloride.

Several selenium compounds which also contain mercury, arsenic, or antimony are of interest because of their possible use in therapeutics (109), and several benzanthrone derivatives (50, 174) have valuable properties as dyes.

IV. THE PROPERTIES OF SELENIUM IN PLANTS AND THEIR RELATION TO KNOWN COMPOUNDS OF SELENIUM AND OF SULFUR

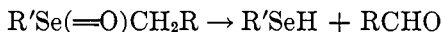
From the properties of selenium in naturally occurring plants it is generally agreed that the selenium is in organic forms. Franke and Painter (63) were unable to extract the selenium from cereals by solvents for inorganic selenium salts or for metallic selenium. Electrodialysis of peptized seleniferous proteins likewise failed to remove selenium. Much of the selenium in the indicator plants is water-soluble but cannot be reduced to metallic selenium by reagents which reduce inorganic selenite or selenate. Not one of the organic selenium compounds studied by Painter, Franke, and Gortner (147) yielded more than a trace of metallic selenium on reduc-

tion. They were reduced instead to selenides, diselenides, or selenols, which are more stable forms than are the oxidized organic selenium compounds.

The only form of inorganic selenium reported in plants is metallic selenium. Several investigators (for references see Hurd-Karrer (97)) grew plants in artificially selenized soils or cultures, using selenite, and reported that metallic selenium was deposited in the growing plant, particularly in the root systems. Levine (118) states that reduction is probably due to microorganisms, but it is well known that many compounds in plants,—i.e., glutathione, ascorbic acid, reducing sugars, etc.,—can reduce selenite to elemental selenium. In view of these facts it may seem surprising that metallic selenium has not been found in naturally occurring seleniferous plants, but we have no proof that selenium is absorbed from naturally seleniferous soils as selenite nor is the evidence for the presence of elemental selenium conclusive. Plants grown in selenite cultures may have an abnormal reddish cast and the selenium may be extracted from the roots by a bromine–hydrobromic acid solution, as Hurd-Karrer (97) found, but that is not proof. Metallic selenium and inorganic selenite and selenate are readily converted by bromine–hydrobromic acid solutions to selenium bromide, which can be distilled, but this reagent also converts many organic selenium compounds to soluble compounds which can be cleaved to inorganic forms of selenium. This property has apparently been overlooked. The results of Westfall and Smith (180), who, after extraction of naturally seleniferous cereals and proteins by dilute bromine in hydrobromic acid or by hydrogen peroxide in trichloroacetic acid and distillation of the extracts in the presence of bromine–hydrobromic acid, were able to reduce selenium in the distillates to the metallic form, can be explained by oxidation and cleavage to inorganic forms and need not be interpreted to indicate the presence of inorganic selenite or selenate, as suggested. Painter (142) found the selenium in *Oenopsis condensata* to be cleaved by bromine–hydrobromic acid solutions to a form reducible by sulfur dioxide and hydroxylamine hydrochloride. The reduction of selenium extracted from indicator plants to the metallic form has not been found to occur without previous oxidation and cleavage.

These results are explained by the reactions of organic selenium compounds. Selenides add bromine to form dibromides, which give dihydroxides in the presence of excess water. Peroxides also convert selenides to dihydroxides. Dihydroxy derivatives of selenium ethers are converted to selenoxides on heating. These selenonium compounds of the type $R'SeX_2R$ [$X_2 = Br_2, Cl_2, I_2, (OH)_2, \text{ or } O$] are easily decomposed if R is aliphatic, as Edwards, Gaythwaite, Kenyon, and Phillips (52) and Be-

haghel and Hofmann (13) have shown. Selenoxides, like sulfoxides, cleave mainly as indicated by the equation



Dihalides and dihydroxides cleave in a similar manner:



With dihydroxides the diselenide (52) was isolated. With some compounds high temperatures were required, but with others the dihalides or dihydroxides decomposed so readily that they have not been isolated. The author (unpublished work) dissolved selenides in bromine-hydrobromic acid solution, distilled until the solution was free of bromine, and recovered inorganic selenium in the distillates. In the presence of excess bromine or peroxide in aqueous solution the cleavage products of each reaction,—RSeH, R'SeX, and R'SeSeR',—would give a seleninic acid. Excess bromine converts each to a tribromide, RSeBr₃, which in aqueous solutions goes to a seleninic acid. Peroxides convert diselenides directly to seleninic acids. Painter (142) and Painter, Franke, and Gortner (147) have shown that seleninic acids cleave to give mostly inorganic selenite. Indeed, the results of Westfall and Smith, when considered with other properties of selenium in cereals, can be better interpreted to indicate the presence of a diselenide or of an easily cleaved selenide.

If elemental selenium sometimes occurs in plants—and this appears likely—it behaves differently from sulfur. No report of elemental sulfur in higher plants is known to the author. We must recognize that when elemental selenium was reported to be deposited, it was from absorbed selenite, which is easily reduced, whereas sulfur is absorbed by plants as sulfate.

Although Painter and Franke (143, 145) found most of the selenium in wheat, corn, and barley to be confined to the protein, Beath, Eppson, and Gilbert (10) found about half of the selenium in a wheat sample to be water-soluble. Whether or not the selenium not accounted for in the proteins (145) isolated,—approximately 20 per cent of the total,—was in a non-peptized or a water-soluble protein which is richer in selenium than the proteins isolated, cannot be stated. The mole N:Se ratio in most of the proteins (145) was higher than the N:Se ratio of the whole grain. This suggests that there is some non-protein selenium present. As previously stated (145), the S:Se ratios in the grain and proteins were fairly constant, indicating that selenium deposition follows that of sulfur more closely than that of nitrogen. The water-soluble selenium in the indicator plants (8, 10) is probably not in proteins, although many of these plants

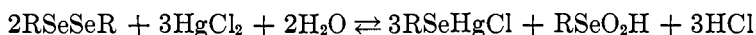
(legumes) contain a large amount of water-soluble protein. In any case there is no good evidence to indicate that the selenium is inorganic.

The fact that the selenium in cereals is in the protein is in itself presumptive evidence of the presence of a nitrogen compound of selenium, probably an amino acid. After the hydrolysis of seleniferous proteins, Painter and Franke (143, 144, 146) and Horn, Nelson, and Jones (92) found, in the hydrolysate, soluble selenium compounds which could not be reduced to elemental form. The humin formed when seleniferous proteins were hydrolyzed (144) by 20 per cent hydrochloric acid or by 33 per cent sulfuric acid always contained selenium. Recently, Schaefer and Moxon (personal communication) boiled the selenium analog of cystine in the same concentration of acids that Painter and Franke used to hydrolyze proteins and noted a slow decomposition accompanied by the separation of selenium from solution. Selenium is less stable than sulfur to acid hydrolysis, because the mole S:Se ratio in the humin (144, 145) is lower than in the protein or hydrolysate. Westfall and Smith (180) cleaved more selenium than sulfur from seleniferous proteins by oxidizing them in acid solutions. The amount of selenium in the humin, as well as of sulfur, could be increased by the addition of carbohydrate or by the use of stronger acids. The observation of Painter and Franke (144), that the hydrolysis of seleniferous proteins in concentrated hydriodic acid and removal of the hydriodic acid by repeated extraction with ether gave a selenium-free hydrolysate, is of interest. Hydriodic acid cleaves ethers and when used to hydrolyze proteins cleaves the methiol group of methionine to form an α -amino γ -thio lactone.

Several attempts have been made to separate selenium compounds from protein hydrolysates. Horn, Nelson, and Jones (92) were able to extract the selenium by means of butyl alcohol. The monoamino monocarboxylic acids are generally considered to be extracted by butyl alcohol, but the amino acids extracted depend largely on the pH of the solution. Only a little selenium was present in the dicarboxylic acid (92) fraction. The hexone bases contained small amounts of selenium (92), but Painter and Franke (143) found considerable selenium in the phosphotungstic acid precipitate of a protein hydrolysate. Mercuric chloride (143) was the most effective of several amino acid precipitants used to remove selenium from protein hydrolysates. Copper salts precipitated some selenium compounds but silver salts, which precipitate histidine and arginine, removed only a trace of selenium. As a result of these studies, it can be concluded that the selenium compounds do not show properties identical with those of any known amino acid. It is of interest to note that every amino acid fraction which contained cystine or methionine also contained selenium, although not in the same S:Se ratio. When Jones, Horn, and Gersdorff

(99) separated partially hydrolyzed products of the enzymatic hydrolysis of seleniferous protein, they found selenium to be in those products which contained cystine.

When the selenium-containing precipitates of mercury and copper salts from a protein hydrolysate were decomposed with hydrogen sulfide, much selenium was in the metallic sulfide. Fredga (74, 75, 76) and Preisler (153) studied the action of metallic salts on diselenides of organic acids. The compounds dismute according to the general scheme:



A seleno mercaptan which is not decomposed by hydrogen sulfide may form when heavy-metal salts are added to a seleniferous protein hydrolysate, or a seleninic acid may form which is cleaved and the selenite may be reduced by hydrogen sulfide.

Like sulfur in proteins, some selenium is cleaved when seleniferous proteins are hydrolyzed in alkaline plumbite. The percentage of total selenium in the lead sulfide was always slightly less than the percentage of total sulfur in the lead sulfide, thereby indicating that selenium is more stable in alkaline solutions than is sulfur. The following comparison from tables by Painter and Franke (146) is typical of several proteins:

PROTEIN	SELENIUM	SULFUR	LEAD SULFIDE PRECIPITATE		FILTRATE FROM LEAD SULFIDE	
			Se	S	Se	S
			per cent	per cent	per cent	per cent
Gluten.....	117	0.74	33.3	52.8	65.0	46.4

Methods of alkaline hydrolysis which produced a higher percentage of lead sulfide also removed a greater percentage of selenium in the lead sulfide precipitate. The cleavage of diselenides in alkaline solutions (147) is similar to that of disulfides. Inorganic selenide, which precipitates as lead selenide in the presence of plumbite, and some selenite form. There is good evidence that some inorganic selenide forms in the absence of lead when seleniferous proteins are hydrolyzed in alkaline solutions (146), but inorganic selenite has not been demonstrated. This may be because the methods are inadequate for detection of the different forms of cleaved inorganic selenium in the dilute solutions. Most of the proteins contained from 100 to 150 p.p.m. of selenium.

The selenium in some of the high absorbers of selenium exhibits a different behavior in alkaline solutions from the selenium in the cereal proteins. Painter (142) dissolved nearly all of the selenium by aqueous sodium hydroxide extraction of *Oönopsis condensata*, but only a trace of

"labile selenium" separated when heated in alkaline plumbite. No selenium was cleaved to selenite. The plant contained 1180 p.p.m. of selenium, so if selenium underwent cleavage, there should have been little difficulty in detecting the different inorganic forms. The organic sulfur content of the plant was 0.17 per cent, but there was only a trace of "labile sulfur."

Additional indication of the probable types of selenium compounds in plants may be gained from the studies of Painter, Franke, and Gortner (147) on organic selenium compounds. Most of the selenium was cleaved in alkaline solutions from diselenodiacetic acid and β, β' -diselenodipropionic acid. Benzyl and *n*-propyl diselenides were partially cleaved. Selenium ethers were stable, with the exception of β -selenodipropionic acid which gave lead selenide almost quantitatively in alkaline plumbite. Seleninic acids, except *n*-propylseleninic acid, which was stable, gave mostly inorganic selenite. Since most seleninic acids, especially derivatives of organic acids, are unstable and are cleaved in acidic as well as in basic solutions, it is improbable that they occur in plants.

From the properties of selenium in cereals it would appear that the selenium is present in stable compounds. If the selenium is an integral part of the protein molecule, it would be expected to undergo few changes without hydrolyzing the protein. There is a general belief among farmers that seleniferous plants become less toxic upon long storage. Franke and Painter (66) found that a wheat sample decreased in toxicity after several years of storage. Moxon and Rhian (135) repeated the determination of selenium in some cereals after an interval of two to three years and found a consistent decrease of approximately 30 per cent in the selenium content. If selenium were slowly oxidized, there would probably be cleavage, but organic selenium compounds are not oxidized as readily as organic sulfur compounds. If a dismutation of diselenides took place, slow loss of selenium would occur, but this seems doubtful in cereal grains under dry storage conditions. The organic selenium compounds prepared by Painter (142) differ greatly in their stability, but few are as stable as selenium compounds in plants. Diselenodiacetic acid was much less stable than other diselenides. It deposited metallic selenium rapidly when exposed to air. Seleninic acids decomposed more readily than the acid salts,—the selenonium compounds. No decomposition of selenium ethers has been noted after more than three years' storage.

In the group of indicator plants which accumulate large amounts of selenium, volatile compounds of selenium are present. A large percentage of selenium (10) may be lost on drying these plants when they are collected green. These volatile selenium compounds may impart a disagreeable odor to some plants, and seleniferous *Astragali* may be detected from the

indicator plants where as high as 15,000 p.p.m. of selenium has been reported, the S:Se ratio must be less than 1 in some samples. The advantage of working with these plants is obvious, but, since little is known about the chemistry of these plants, wholly different compounds from those in cereals may be present. Studies of the properties of the selenium analogs of the sulfur-containing amino acids should answer a fundamental question regarding selenium in cereal grains. Selenocystine is the only selenium analog of the naturally occurring organic sulfur compounds which has been prepared. Fredga (77) studied the reaction between the methyl ester of α -amino- β -chloropropionic acid (from serine ester) and potassium selenide, and Painter (142) treated the chloro ester with sodium hydroselenide and oxidized the selenol to the diselenide. After hydrolysis, selenocystine precipitated when the solution was neutralized. As expected, selenocystine gave "labile selenium" in alkaline solution.

In addition to supplementing the chemical evidence that the selenium in plants is organic, animal experimentations also indicate that selenium- and sulfur-containing amino acids are related in metabolism. The toxicity of *d,l*-selenocystine (table 1) was much greater than that of any other organic selenium compound when injected. It was nearly as toxic as inorganic selenite. Elemental selenium, reported to be in plants, was virtually non-toxic when fed to rats (68), and the selenium in cereals was more toxic than any inorganic form of selenium studied. Both the *d*- and the *l*-forms of the selenium analog of cystine (separated by Fredga (78)) have been fed to rats. Moxon (129, and personal communication) found the toxicity of selenocystine to be in the same range as the selenium in cereal grains and the *l*-form more toxic than the *d*-form. This seems significant when it is recalled that the naturally occurring amino acids are of the *l*-configuration and that *l*-cystine is more efficient in supporting growth than is its optical isomer.

Further similarity in the behavior of selenium and sulfur in the animal body has recently been discovered by Moxon *et al.* (136). Bromobenzene lowered the selenium content in the blood and greatly increased its urinary excretion. Bromobenzene is known to become conjugated with tissue cystine and methionine and to be excreted in the urine in this form.

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