

Amount and Chemical Form of Selenium in Vegetable Plants

JOHN W. HAMILTON and
O. A. BEATH

Division of Biochemistry,
University of Wyoming,
Laramie, Wyo.

Eighteen common garden vegetables were grown on soil containing variable levels of added selenium supplied in either organic or selenate form. All of the vegetables studied, when grown on soil containing available selenium, were capable of absorbing, metabolizing, and storing in their tissues variable amounts of selenium. Vegetables varied in their selenium uptake from soils containing 20 p.p.m. organic or 3 p.p.m. selenate selenium. In the majority of the vegetables studied, selenium uptake was greater when they were grown on soils containing the organic selenium. Cabbage plants contained the highest levels of selenium in their tissues. In most instances, the generally considered inedible portions of the plants contained the highest levels of selenium. All of the plants possessed the ability to convert a portion of the absorbed selenate selenium into an organic form. The vegetables studied were capable of absorbing some of the selenium added to the soil as organic compounds, converting at least a portion of it into other compounds and storing it in their tissues both as organic and inorganic compounds.

THE PRESENCE of selenium in vegetable plants grown in some seleniferous areas has been observed and reported by numerous workers. Abnormal conditions or ill health of humans, believed to be the direct result of consumption of selenium-containing vegetables, cereal grains, and dairy products, has been reported by physicians, public health workers, and agricultural extension personnel. Hurd-Karrer (3, 4), Knight and Beath (6), and others have reported that most plants possess the ability to absorb, metabolize, and store selenium in their tissues when grown on soil containing available selenium. The selenium contents of certain soils and the selenium contents of numerous plants grown on these soils were extensively studied (7). Evidence that significant quantitative differences in the amount of selenium absorbed do occur among plant species growing on the same soil was first presented by Hurd-Karrer (3). She studied the problem under uniform conditions in the greenhouse and reported that selenium added to the soil as selenate is more toxic to plants than selenite. Toxic symptoms exhibited by wheat plants were chlorosis, stunting, and yellowing of the leaves. Hurd-Karrer (4) reported the total amounts of selenium found in cabbage, cauliflower, onion, broccoli, lettuce, pea, spinach, etc., but made no attempt to determine the chemical nature of the selenium compounds present. She found that all of the vegetable plants studied contained selenium when grown on soil containing available selenium. She also found that

the selenium-absorbing ability of the plant closely paralleled its sulfur-absorbing ability when selenate was present in the soil. Cruciferae were among the most efficient selenium-absorbing families of plants.

Beath and Eppson (7) studied the total selenium contents, the relative amounts of organic and inorganic selenium present, and the amounts of hot-water-soluble selenium compounds present in numerous plants. These investigators found that some plants contain only organic compounds of selenium, while other plants contain variable amounts of both organic and inorganic compounds of selenium. Organic compounds of selenium or selenium homologs of sulfur-containing amino acids have been isolated from *Astragalus bisulcatus* by Trelease *et al.* (10) and from *A. pectinatus* by Horn and Jones (2). The chemical nature of the selenium compounds and the amount of selenium found in wheat grain was investigated by Jones *et al.* (5) and by Thorvaldson and Johnson (8).

This study was initiated to determine the ability of some commonly grown vegetable plants to absorb available selenium from the soil. The amounts, water solubility, and chemical nature of the selenium compounds found in the plant tissues were determined.

Experimental

All of the plants were grown in the greenhouse from seed planted in a soil mixture contained in redwood boxes. The soil was a mixture of one part of

black soil, high in organic matter, from a forest location and two parts finely divided shale from the Chugwater formation. This mixture contained less than 0.1 p.p.m. selenium. In all research reported in this paper, levels of 3 p.p.m. inorganic and 20 p.p.m. organic selenium were added to the soil with thorough mixing. Inorganic selenium was added as a water solution of potassium selenate. Organic selenium was supplied as a finely powdered mixture of air-dry *A. bisulcatus* and *A. preussii* plants containing 765 p.p.m. selenium. Chemical examination of the dry plant material did not indicate the presence of selenate or selenite selenium. No organic matter was added to the soil receiving the inorganic selenium. The vegetables grew at a satisfactory rate, and no evidences of selenium toxicity were detected. Water, fertilizer, and insecticides were applied as needed. The plants were harvested at the stage of growth when the edible portions of the plant are normally harvested. The edible portion of the plant and the remainder of the plant, other than root portion, were analyzed for moisture and selenium contents. The moisture contents of the freshly harvested and air-dry plants were determined by heating in a vacuum oven at 72° C. for 8 hours. Moisture contents of the freshly harvested plant materials are expressed as percentages of the fresh weights. Total, total water-soluble, and total water-soluble inorganic selenium contents of the finely powdered air-dry plant material were determined using the methods of Beath and Eppson (7) or

Table I. Average Amounts of Various Forms of Selenium in the Edible Portion of Certain Vegetables

Vegetable	Variety	Number of Samples	Portion of Plant	Soil Selenium	Moisture (Fresh), %	Selenium (Oven-Dry Basis), P.P.M.				
						Total	Total soluble	Soluble organic	Soluble selenate	In-soluble
Bean	Giant	2	Green pod, fruit	Organic	88.1	95.5	59.5	49.0	10.5	36.0
		2	Green pod, fruit	Selenate	88.2	72.5	46.5	35.5	11.0	26.0
	1	Mature seed	Organic	15.3	51.0	34.0	9.0	25.0	17.0	
Beet	Detroit Red	1	Mature seed	Selenate	14.2	47.0	34.0	11.0	23.0	13.0
		2	Root	Organic	80.6	28.0	17.5	9.5	8.0	10.5
Broccoli	Italian Green	2	Root	Selenate	80.5	33.0	21.5	11.0	10.5	11.5
		3	Bud, stem	Organic	79.6	81.7	59.7	22.3	37.4	22.0
Cabbage	Copenhagen	3	Bud, stem	Selenate	78.7	155.0	130.3	41.7	88.6	24.7
		3	Head	Organic	78.0	150.3	127.0	77.3	49.7	23.3
Carrot	Imperator	3	Head	Selenate	76.6	159.7	121.4	77.4	44.0	38.3
		1	Root	Organic	87.0	32.0	25.0	16.0	9.0	7.0
Cucumber	Chicago Pickling	1	Root	Selenate	87.2	23.0	12.0	6.0	6.0	11.0
		1	Fruit	Organic	92.4	41.0	32.0	21.0	11.0	9.0
Eggplant	Black Beauty	1	Fruit	Selenate	93.1	18.0	12.0	7.0	5.0	6.0
		2	Fruit	Organic	90.7	45.0	38.0	19.0	19.0	7.0
Lettuce	Improved Hanson	2	Fruit	Selenate	90.5	32.0	29.0	10.5	18.5	3.0
		2	Leaf	Organic	89.4	48.0	31.5	16.5	15.0	16.5
Okra	Dwarf Green	2	Leaf	Selenate	88.6	23.5	16.0	7.5	8.5	7.5
		2	Fruit	Organic	81.6	73.0	37.0	18.0	19.0	36.0
Onion	Yellow Globe	2	Fruit	Selenate	81.0	50.0	29.0	10.0	19.0	21.0
		2	Bulb	Organic	87.0	103.0	87.0	40.5	46.5	16.0
Parsnip	Improved Hollow Crown	2	Bulb	Selenate	87.1	58.0	50.5	11.5	39.0	7.5
		1	Root	Organic	73.9	21.0	16.0	9.0	7.0	5.0
Pea	Laxton's Progress	1	Root	Selenate	74.8	35.0	28.0	8.0	20.0	7.0
		2	Immature seed	Organic	65.5	58.0	46.0	12.5	33.5	12.0
Pea	Little Marvel	2	Immature seed	Selenate	65.8	52.5	37.0	11.0	26.0	15.5
		2	Mature seed	Organic	20.8	101.0	71.0	44.0	27.0	30.0
Potato	Bliss Triumph	2	Mature seed	Selenate	21.7	46.5	29.0	14.5	14.5	17.5
		1	Tuber	Organic	80.3	35.0	27.0	14.0	13.0	8.0
Radish	White Icicle	1	Tuber	Selenate	79.4	19.0	14.0	7.0	7.0	5.0
		1	Root	Organic	89.4	93.0	79.0	23.0	56.0	14.0
Rutabaga	American Purple	1	Root	Selenate	88.9	36.0	27.0	13.0	14.0	9.0
		2	Root	Organic	80.9	43.0	30.5	15.0	15.5	12.5
Spinach	Viking	2	Root	Selenate	80.1	37.0	27.5	15.5	12.0	9.5
		2	Leaf	Organic	87.0	114.0	86.0	34.0	52.0	28.0
Swiss Chard	Lucullus	2	Leaf	Selenate	85.6	89.0	61.0	29.0	32.0	28.0
		2	Leaf	Organic	89.0	100.0	63.0	49.0	14.0	37.0
Tomato	Bison	2	Leaf	Selenate	88.6	76.0	45.0	25.0	20.0	31.0
		2	Fruit	Organic	89.0	40.0	28.5	16.5	12.0	11.5
		2	Fruit	Selenate	89.2	36.0	28.5	10.5	18.0	7.5

Trelease and Beath (9). Proportions of 1 part of air-dry plant and 10 or 20 parts of 90° C. water were utilized in preparing the water extracts. No selenite selenium was detected in the water extracts, and all soluble inorganic selenium present was assumed to be selenate. The amounts of soluble organic and insoluble selenium were determined as differences. The levels of selenium present in the soils at the beginning and end of this study were determined by the method reported by Trelease and Beath (9). In all instances, the soil selenium levels at the end of the experimental period were nearly equal, less than 0.5 p.p.m. lower, to the levels present at the beginning. The possibility exists that microbial action or other conditions in the soil may transform the organic compounds of selenium added in the plant material, to other organic compounds or perhaps inorganic compounds, prior to absorption by the plant. The chemical nature of selenium in the soil at the end of the experiment was not determined.

Results and Discussion

The results of the analyses are shown in Table I. The values given represent a

single sample, or means of two or three samples. Similar data except that the selenium values represent single samples for the generally considered inedible portions of the vegetable plants are presented in Table II.

The authors assumed, as did Beath and Eppson (7), that nearly all of the hot water-insoluble selenium remaining in the plant dregs was organic. Peterson and Butler (7) reported evidence to indicate the existence of a small amount of elemental selenium in the tissues of plants supplied Se⁷⁵ in culture solutions. In the present study, the vegetables were not examined for the presence of elemental selenium.

Beans readily absorbed organic and inorganic selenium from the soil, and the green pods and immature seeds contained much higher levels of total, soluble organic, and insoluble selenium than did the mature seeds or the mature leaves and stems. The mature seeds and mature plants contained much higher levels of inorganic selenium and consequently a higher proportion of soluble selenium than did the immature pods and seeds. The mature seeds contained more than twice as much in-

organic as soluble organic selenium. This is in marked contrast to the low levels of selenate and the relatively large amounts of soluble selenium present in the green beans.

The edible portions of beets, carrots, cucumbers, egg plant, lettuce, parsnips, potatoes, rutabagas, and tomatoes contained lower levels of selenium than did the inedible portions of other vegetables. The inedible portions of most of these vegetables, including beet and rutabaga leaves which are occasionally eaten, contained considerably higher levels of total selenium. The amounts of organic and selenate selenium found in the edible portions were nearly equal in most of these vegetables. It appears that eating these vegetables would pose a serious health hazard when grown on soils containing levels of selenium comparable to or somewhat less than those used in this study. In all the vegetables except eggplant, 20 to 40% of the selenium present in the edible portion was not readily soluble in hot water.

The levels of selenium found in the edible and inedible portions of four members of the Cruciferae (mustard family) were studied. The selenium

Table II. Amounts of Several Forms of Selenium in Generally Considered Inedible Portions of Certain Vegetables

Vegetable	Soil Selenium	Partion of Plant	Mois- ture (Fresh), %	Selenium (Oven-Dry Basis), P.P.M.				
				Total	Total soluble	Saluble organic	Soluble selenate	In-soluble
Bean	Organic	Leaf, pod, stem	42.6	67	50	21	29	17
	Selenate	Leaf, pod, stem	43.4	63	47	12	35	16
Beet	Organic	Top	81.0	46	31	17	14	15
	Selenate	Top	82.4	40	32	14	18	8
Broccoli	Organic	Leaf, stem	77.8	93	69	32	37	24
	Selenate	Leaf, stem	77.4	260	239	52	187	21
Cabbage	Organic	Leaf, stem	74.2	300	259	138	121	41
	Selenate	Leaf, stem	75.8	378	326	150	176	52
Carrot	Organic	Top	78.0	73	49	23	26	24
	Selenate	Top	78.6	58	42	16	26	16
Cucumber	Organic	Leaf, stem	83.2	177	153	61	92	24
	Selenate	Leaf, stem	84.0	88	69	19	50	19
Eggplant	Organic	Leaf, stem	79.4	108	93	48	45	15
	Selenate	Leaf, stem	77.8	69	58	19	39	11
Okra	Organic	Leaf, pod, stem	75.7	103	85	44	41	18
	Selenate	Leaf, pod, stem	74.8	105	84	29	55	21
Onion	Organic	Mature top	82.4	108	104	68	36	4
	Selenate	Mature top	82.0	78	72	21	51	6
Parsnip	Organic	Top	80.2	50	32	18	14	18
	Selenate	Top	80.8	41	32	8	24	9
Pea ^a	Organic	Leaf, pod, stem	78.0	80	72	28	44	8
	Selenate	Leaf, pod, stem	77.3	55	40	12	28	15
Pea ^b	Organic	Leaf, pod, stem	72.6	139	99	34	65	40
	Selenate	Leaf, pod, stem	74.0	67	43	16	27	24
Potato	Organic	Leaf, stem	84.5	48	43	19	24	5
	Selenate	Leaf, stem	84.5	35	30	13	17	5
Potato	Organic	Peel	74.0	46	38	16	22	8
	Selenate	Peel	73.9	27	19	6	13	8
Radish	Organic	Top	81.3	121	101	39	62	20
	Selenate	Top	81.0	95	82	19	63	13
Rutabaga	Organic	Top	84.3	100	85	36	49	15
	Selenate	Top	84.0	125	109	46	63	16
Tomato	Organic	Leaf, stem	80.9	119	101	24	77	18
	Selenate	Leaf, stem	81.4	86	70	17	53	16

^a Laxton's Progress Variety. ^b Little Marvel Variety.

levels in the edible portions of these plants varied within rather wide limits with cabbage being highest, broccoli next, followed by radish and rutabaga. In all instances, except broccoli and radish grown on soil with added organic selenium, the inedible portion contained much higher levels of selenium than did the edible portion. The amounts of selenium found in the radish root sample grown in soil containing selenate selenium and samples of rutabaga roots grown in soil containing organic or inorganic selenium represented only a small fraction of the selenium present in the entire plants. Hurd-Karrer (4) showed that cabbage and broccoli contain high levels of sulfur. On the basis of her study of the relative uptakes of sulfur and selenium by these members of the Cruciferae and other plants, she concluded that the sulfur requirement of the plant determines its tendency to absorb selenium. This parallel absorption of sulfur and selenium may be partially explained by the generally accepted concept that selenium partially replaces sulfur in some of the sulfur-containing compounds found in plants. More than 70% of the total selenium present in the

inedible portions of these four plants was water soluble. The edible portions of the broccoli samples and radish samples supplied with organic selenium contained considerably more selenate than soluble organic selenium.

The okra fruits contained levels of selenium higher than found in the edible portions of 10 other vegetables. More than 40% of the total selenium present in these fruits was insoluble in water.

The selenium uptake by onions was quite high, and the major portion of the selenium compounds were water soluble. The high selenium content of onions is apparently related to their comparatively high sulfur content. The relative proportions of soluble organic and inorganic selenium compounds in the onion were apparently strongly influenced by the form of selenium present in the soil.

Two varieties of peas varied in respect to the selenium contents of the green and mature seeds and their responses to the different forms of soil selenium. Immature Laxton's Progress peas contained 49% or more of the selenium in the selenate form as compared to 31% or more in the Little Marvel mature peas. The influence of variety and stage of

Table III. Analysis of Variance of Selenium Uptake by Vegetables

Variable	Degrees of Freedom	Sum of Squares	Mean Squares
Selenium	1	1,530.89	1530.89 ^a
Vegetables	19	102,708.35	5504.70 ^a
Selenium- vegetable interaction	19	16,912.03	890.11 ^a
Experimen- tal error	32	1,336.51	40.52
Total	71	122,487.78	

^a Significant at 1% level.

maturity upon the chemical nature of the selenium content of these peas is unknown.

The entire potato tubers contained relatively low levels of selenium. Peeling of potatoes will markedly reduce the selenium content of the edible portion since the peels from some of the potatoes contained considerably higher levels of selenium than did the peeled tubers. The vines of the potato plants contained nearly the same levels of selenium as did the peels.

Spinach and Swiss chard are relatively efficient selenium absorbers. Approximately 25 to 40% of the total selenium in the edible portion was not readily soluble in hot water. Swiss chard is a close relative of beets, but it absorbed considerably larger amounts of selenium than did beets. Swiss chard tops contained, on an average, more than twice the amount of selenium found in beet tops. The results of using different varieties of peas and different species such as Swiss chard and beets are further evidence that different species and varieties of plant vary in their ability to absorb and metabolize selenium.

Statistical treatment of the mean total selenium contents of the edible portions of vegetables grown on soil containing 20 p.p.m. organic selenium, and 3 p.p.m. selenate selenium is shown in Table III. The statistical study reveals that the 72 samples differed significantly in their response to the presence of organic and selenate selenium in the soil at the levels provided. The significant interaction term indicates that the response to the presence of organic and selenate selenium was not the same for all of the vegetables included in the study.

Use of Duncan's multiple range test indicates which selenium content values are significantly greater at the 5% level. The results of this statistical treatment are shown in Table IV.

All of the vegetables studied, when grown on a soil containing available selenium, were capable of absorbing, metabolizing, and storing selenium in their tissues. The plants were able to absorb selenate from the soil and convert

Table IV. Results of Use of Duncan's Multiple Range Test of Significance

Vegetable	Form of Soil Selenium	Mean ^a Selenium Content, P.P.M.	Significance	Vegetable	Form of Soil Selenium	Mean ^a Selenium Content, P.P.M.	Significance
Cabbage	Selenate	159.7	a	Bean (dry)	Selenate	47.0	f g h i j
Broccoli	Selenate	155.0	a	Pea (L.M.)	Selenate	46.5	f g h i j
Cabbage	Organic	150.3	a	Eggplant	Organic	45.0	f g h i j
Spinach	Organic	114.0	b	Rutabaga	Organic	43.0	h i j
Onion	Organic	103.0	b c	Cucumber	Organic	41.0	g h i j k
Pea (L.M.)	Organic	101.0	b c	Tomato	Organic	40.0	g h i j k
Swiss chard	Organic	100.0	b c	Rutabaga	Selenate	37.0	g h i j k l
Bean (green)	Organic	95.5	c	Tomato	Selenate	36.0	h i j k l
Radish	Organic	93.0	c	Radish	Selenate	36.0	h i j k l
Spinach	Selenate	89.0	c d	Potatoes	Organic	35.0	h i j k l
Broccoli	Organic	81.7	d e	Parsnip	Selenate	35.0	h i j k l
Swiss chard	Selenate	76.0	d e	Beets	Selenate	33.0	i j k l
Okra	Organic	73.0	e	Eggplant	Selenate	32.0	i j k l
Bean (green)	Selenate	72.5	e	Carrots	Organic	32.0	i j k l
Onion	Selenate	58.0	f	Beets	Organic	28.0	j k l
Pea (L.P.)	Organic	58.0	f	Lettuce	Selenate	23.5	k l
	Selenate	52.5	f g	Carrots	Selenate	23.0	k l
Beans (dry)	Organic	51.0	f g h	Parsnip	Organic	21.0	k l
Okra	Selenate	50.0	f g h i	Potatoes	Selenate	19.0	l
Lettuce	Organic	48.0	f g h i	Cucumbers	Selenate	18.0	l

^a Any means indicated by the same letter are not significantly different ($P < 0.05$).

it into organic selenium compounds. They appear capable of absorbing selenium that was a part of organic compounds when added to the soil. The exact chemical nature of the selenium at the time of absorption is not known; however, selenium is readily absorbed, metabolized, and stored in the plant tissues as a part of organic and inorganic compounds. The ability of vegetables to absorb, metabolize, and store selenium in their tissues emphasizes the need for location of the vegetable garden or field on soil that is free of available selenium.

Acknowledgment

The authors are grateful to R. G. Sackett for technical help and supervision

of the greenhouse. They are also indebted to Constance Tyndall and Shirley Norman for analytical assistance, and to Glenn P. Roehrkasse for assistance with the statistical analysis of the data.

Literature Cited

- (1) Beath, O. A., Eppson, H. F., *Wyoming Agr. Expt. Sta. Bull.* **278**, 2, (1947).
- (2) Horn, M. J., Jones, D. B., *J. Biol. Chem.* **139**, 649, (1941).
- (3) Hurd-Karrer, A. M., *J. Agr. Res.* **50**, 413, (1935).
- (4) *Ibid.*, **54**, 601, (1937).
- (5) Jones, D. B., Horn, M. J., Gersdorf, C. E. F., *Cereal Chem.* **14**, 130, (1937).
- (6) Knight, S. H., Beath, O. A., *Wyoming Agr. Expt. Sta. Bull.* **221**, 29, (1937).

- (7) Peterson, P. J., Butler, G. W., *Australian J. Biol. Sci.* **15**, 126, (1962).
- (8) Thorvaldson, T., Johnson, L. R., *Canadian J. Res. B.* **18**, 138, (1940).
- (9) Trelease, S. F., Beath, O. A., "Selenium, its Geological Occurrence and its Biological Effects in Relation to Botany, Chemistry, Agriculture, Nutrition, and Medicine," p. 248, Champlain Printers, Burlington, Vt., 1949.
- (10) Trelease, S. F., Di Somma, A. A., Jacobs, A. L., *Science* **132**, 3427, (1960).
- (11) Williams, K. T., Lakin, H. W., Byers, H. G., *U. S. Dept. Agr. Tech. Bull.* **758**, 41, (1941).

Received for review July 31, 1963. Accepted December 23, 1963. Published with the approval of the Director, Wyoming Agricultural Experiment Station, as Journal Paper No. 208.

GAME ANIMALS AS MEAT SOURCES

Vitamin Content and Amino Acid Composition of Some African Game Animals

IGNACY MANN

Animal Industry Section, Department of Veterinary Services, Ministry of Agriculture and Animal Husbandry, Kenya, East Africa

DURING the symposium on the Conservation of Nature and Natural Resources in Modern African States held in Arusha in September, 1961, great interest was shown in the possibility of game ranching and cropping as a source of valuable protein for human nutrition. Many papers gave figures on the efficiency of wild life compared to domestic animals in the utilization of vegetation in the semiarid, un-

improved areas. Quoted yields of meat per beast and the ratio of meat to fat and moisture were given much thought (4-7, 12). In view of the possibility of game becoming a marketable meat supply, research work was organized to determine the amino acid and vitamin composition of the four species of animals likely to be cropped in East Africa. These are the Elephant (*Loxodonta Africana*), Wildebeeste (*Gorgon taurinus*),

Zebra (*Equus burchelli*), and Kongoni (*Alcelaphus cokei*). For purposes of comparison, an analysis of the indigenous African zebu, grazing in the same area as the wild animals, was also made.

Experimental Procedure

Collection of Specimens. To ensure accurate results, almost all animals were