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Distribution of Mercury and Selenium in Egg Components and Egg-White Proteins (40573)[1]

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Previous research has shown that the transfer of mercury (Hg) and selenium (Se) from the diet into eggs of chickens was influenced by both the level and the chemical form of Hg and Se in the diet (1–4). Interactions between dietary Hg and Se also have been observed whereby amounts of Hg or Se deposited in eggs were changed (1, 2, 5–7). When Hg was given to chickens in the form of methyl Hg, 80 to 94% of the total egg Hg was deposited in the white (2, 5, 6) and seemed to be associated primarily with the egg-white protein, ovalbumin (5). In contrast, Se was deposited mainly in egg yolks of chickens fed Se (3, 4), but the manner in which it was bound in egg yolk or white was not determined.

Although it has been shown that dietary Se increased Hg deposition in eggs, and vice versa, the influence of dietary Se and Hg on the distribution of Hg and Se among egg-white proteins has not been described. The research reported herein was conducted to determine the effects of dietary Hg and Se on the distribution of Hg and Se in egg-yolk and egg-white components and on the preferential binding of Hg and Se to specific proteins of egg white of chickens.

Materials and methods. Each of two experiments involved 16 White Leghorn laying hens that were individually caged and maintained on a practical diet (6). Four hens were assigned to each of the four dietary treatments, which consisted of the following combinations of Hg (as CH_3HgCl) and Se (as Na_2SeO_3) in ppm: 0-0, 20-0, 0-8, and 20-8. After 3 days on the treatments, the administration of oral doses of ^{203}Hg and ^{75}Se was started and eggs were collected daily. The practical diet contained approximately 0.03 ppm of Hg and 0.25 ppm Se by previous analysis (6).

In experiment 1, all hens received six daily doses of 3 μCi $CH_3{}^{203}HgCl$ (New England Nuclear, 3.0 mCi/mg = 1.0 μg/hen daily) and 4 μCi ^{75}Se as selenious acid (83.0 mCi/mg = 0.048 μg/hen daily) each, administered by oral capsule. Dietary treatments were given to each group over a 20-day test period, and feed consumption was recorded daily. Eggs were collected and cooked in boiling water. The yolk and whites were separated and were homogenized individually. One-gram samples of white and of yolk were counted for ^{203}Hg and ^{75}Se radioactivity by using a Nuclear–Chicago gamma counter Model 1426 equipped with a NaI (thallium-activated) crystal.

Experiment 2 was designed to determine the distribution of ^{203}Hg and ^{75}Se in egg-white proteins. Each hen received a daily oral dose of 6 μCi $CH_3{}^{203}HgCl$ (1.3 mCi/mg = 4.6 μg/hen daily) and 6 μCi $Na_2{}^{75}SeO_3$ (85.6 mCi/mg = 0.070 μg/hen daily) for 2 days. All eggs were collected, the raw egg whites were separated from the yolks, and samples of raw egg whites were assayed for ^{203}Hg and ^{75}Se activity. The egg whites from eggs representing the third day after the start of the oral dose, contained the highest radioactivity, and were fractionated.

Egg-white proteins were fractionated (8, 9) by column chromatography with carboxymethyl cellulose (7 meq/g, Sigma Chemical Co.). The column measured 2.0 × 42 or 1.6 × 80 cm, and the flow rate was 40 ml/hr. A gradient elution method was employed (8), with HAc–NH_4OH (0.1 *M* with respect to acetate) buffer system from pH 4.0 to 10.0. The absorbance of the effluent at 280 nm was monitored by a spectrophotometer equipped with a flow-through cell and recorder. The 7-ml fractions were collected and counted for

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^{203}Hg and ^{75}Se as previously described. The fractions corresponding to the protein peaks were pooled and concentrated.

The protein fractions were identified by electrophoresis on 7% acrylamide (Bio-Rad) according to the procedure described by Smith (10). Tris–glycine, pH 8.3, buffer was used, and 3 mA/tube was applied. Electrophoresis time was 1 hr and 45 min. The gels were stained with Coomassie brilliant blue G-250 (0.25% w/v in 3.5% $HClO_4$) (11). The purified standards consisted of ovalbumin, conalbumin, egg globulins, and ovomucoid (Sigma Chemical Co.).

Total selenium content of the egg-white proteins was determined by the fluorometric method according to Olson *et al.* (12). Total mercury determinations in the egg-white proteins were performed by the atomic absorption spectrophotometric method, "cold vapor" technique (13) with the following modifications: (i) Acid digestion was done with H_2SO_4–HNO_3 (5:1) at 85°C for 2 hr. (ii) Reducing mixture consisted of 53.2 g $SnCl_2$, 30.0 g hydroxylamine·HCl, 10.0 g NaCl, and 100 ml H_2SO_4 diluted to 1 liter with distilled H_2O. A Perkin–Elmer atomic absorption spectrophotometer, Model 460, with a mercury hollow-cathode lamp and recorder (Model 165) was used. Protein determination of egg-white fractions was done by the Lowry method (14).

Results. Most of the ^{203}Hg found in eggs produced by chickens during the 20-day test period of experiment 1 was present in the egg white (Table I). This was true irrespective of dietary levels of Hg or Se. The 8 ppm dietary Se significantly increased the ^{203}Hg content of egg white, seemingly at the expense of ^{203}Hg in the egg yolk. Levels of dietary Hg did not influence ^{203}Hg in egg white, and no interaction was observed between Hg and Se for ^{203}Hg in white or yolks. Hg at 20 ppm significantly increased ^{75}Se in egg white and reduced ^{75}Se content of the yolk. Dietary Se reduced ^{75}Se in both the egg white and egg yolk. A significant interaction between dietary Hg and Se was observed for ^{75}Se in the egg white. In this instance, dietary Hg increased ^{75}Se in egg white markedly when fed alone, but had little influence on ^{75}Se in egg white when fed to chickens together with Se.

The egg-white proteins from egg whites in experiment 2, possessing the highest radioactivity were fractionated (Fig. 1) to reduce counting error. Fractions were identified by gel electrophoresis, and ^{203}Hg and ^{75}Se activities of each protein fraction were determined. More than 98% of the ^{203}Hg in egg white was associated with the ovalbumin (Table II). Highest concentrations of total Hg per milligram of protein were also observed in ovalbumin when 20 ppm were fed to chickens, however, Hg was most concentrated in the globulin fraction when Hg was not added to the diet.

TABLE I. INFLUENCE OF DIETARY METHYL MERCURY AND SELENIUM ON ^{203}Hg AND ^{75}Se DEPOSITION IN EGGS, EXPERIMENT 1

Dietary Hg-Se (ppm)[b]	Percentage of ^{203}Hg and ^{75}Se dose[a]					
	White		Yolk		Whole egg	
	^{203}Hg	^{75}Se	^{203}Hg	^{75}Se	^{203}Hg	^{75}Se
0-0	58.82	5.93	9.01	29.97	67.83	35.90
0-8	65.96	6.02	7.99	7.96	73.95	13.98
20-0	59.04	13.09	12.24	24.78	71.28	37.87
20-8	63.68	8.98	9.52	4.96	73.20	13.94
SEM[c]	3.06	0.24	0.93	0.78	2.65	0.72
	Components of variance[d]					
Hg	NS[e]	0.01	0.05	0.01	NS	NS
Se	0.05	0.05	0.05	0.05	NS	0.01
Hg × Se	NS	0.05	NS	NS	NS	NS

[a] Averages of four hens per ration treatment including approximately all eggs produced in 20 days.
[b] Daily feed intake averaged 108 g/hen and was not affected by dietary treatment.
[c] Standard error of the mean.
[d] Indicates independent or interaction effects of dietary Hg and (or) Se level.
[e] Indicates no significant effects (NS) or the level of probability of statistically significant differences.

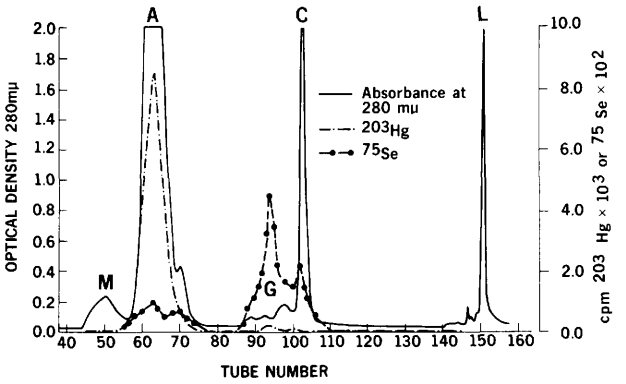

FIG. 1. Fractionation of egg white on carboxymethyl cellulose. Column measured 1.6 × 80 cm. Eluting buffer was 0.1 *M* HAc–NH_4OH (pH 4–10). Flow rate was 40 ml/hr, and fraction size was 7 ml. Letters on the peaks correspond to the proteins: ovomucoid (M), ovalbumin (A), globulin (G), conalbumin (C), and lysozyme (L).

TABLE II. DISTRIBUTION OF ^{203}Hg AND STABLE Hg AMONG PROTEIN FRACTIONS OF EGG WHITE, EXPERIMENT 2

Dietary Hg-Se (ppm)	Protein fraction	Percentage of total[a] in egg white		Total Hg in egg white	
		^{203}Hg[b]	Stable Hg	Protein (ng/mg)[c]	White (μg)
0-0	Ovalbumin	99.0	66.3	0.4	1.48
	Globulin	ND[d]	23.7	2.1	0.53
	Conalbumin	1.0	10.0	0.2	0.22
0-8	Ovalbumin	99.6	70.8	5.9	13.53
	Globulin	0.4	16.1	23.0	1.37
	Conalbumin	ND	13.1	2.4	1.39
20-0	Ovalbumin	100	97.9	215.0	385.0
	Globulin	ND	1.4	75.0	4.75
	Conalbumin	ND	0.7	7.0	3.04
20-8	Ovalbumin	98.2	98.9	135.8	241.0
	Globulin	1.8	0.6	17.0	1.44
	Conalbumin	ND	0.5	5.6	1.40
Standard error of mean					
	Ovalbumin	1.8	5.6	25.4	60
	Globulin	0.8	1.8	17.6	1.0
	Conalbumin	—	1.0	2.5	1.1

[a] Averages of three or four hens per dietary treatment.
[b] Small amounts of ^{203}Hg were observed occasionally in the ovomucoid fraction. These values are not included.
[c] There were no treatment effects on protein content of egg whites. The average protein content of ovalbumin, globulin, and conalbumin fractions were 1765, 94, 308 mg per egg white, respectively.
[d] Not detectable.

The inclusion of 20 ppm Hg in the diet did not change the distribution of ^{203}Hg among egg-white proteins. Added dietary Hg, however, significantly increased the proportion and concentration of total Hg in the proteins of egg whites and especially in ovalbumin. The proportion of total egg white Hg in ovalbumin exceeded 97%. This increase was not affected by the addition of dietary Se. Even though the total Hg concentration was increased in the ovalbumin by 20 ppm dietary Hg, the proportions of total egg-white Hg in the globulin and conalbumin were reduced.

The largest percentage of ^{75}Se dose was detected in the globulin and conalbumin fractions of egg white (Table III), and this distribution was not changed significantly when dietary Hg was given to chickens (Table IV). The pattern of distribution of total Se was somewhat different. Most of the Se was as-

sociated with ovalbumin. This was due, as was the case with total Hg, to the large amount of ovalbumin present in egg white together with a moderate concentration of Se in this protein fraction. The highest concentration of total Se was found in globulin, especially when 8 ppm Se were fed to chickens. Dietary Hg levels did not significantly affect stable Se concentrations nor Se distribution among egg-white proteins, but 8 ppm dietary Se increased stable Se in the three protein fractions. The magnitude of increases was most notable in the globulin fraction.

Discussion. The observation that higher concentrations of ^{203}Hg were deposited in egg whites than in yolks agrees with previous reports (2, 5, 6). Our data also showed that dietary levels of Hg and Se influenced deposition of ^{203}Hg in eggs. The 20 ppm Hg increased ^{203}Hg in yolks while 8 ppm Se en-

TABLE III. DISTRIBUTION OF ^{75}Se AND STABLE Se AMONG PROTEIN FRACTIONS OF EGG WHITE, EXPERIMENT 2

Dietary Hg-Se (ppm)	Protein fraction	Percentage of total[a] in egg white		Total Se in egg white	
		^{75}Se[b]	Stable Se	Protein (ng/mg)[c]	White (μg)
0-0	Ovalbumin	ND[d]	86.0	3.5	6.28
	Globulin	52.9	5.8	5.6	0.34
	Conalbumin	45.8	8.2	1.5	0.58
0-8	Ovalbumin	ND	45.9	4.2	7.23
	Globulin	73.2	41.7	91.2	6.62
	Conalbumin	24.4	12.4	7.5	1.90
20-0	Ovalbumin	12.8	76.7	2.9	4.73
	Globulin	66.2	12.5	8.8	0.74
	Conalbumin	21.0	10.8	2.3	0.63
20-8	Ovalbumin	ND	46.7	5.5	10.73
	Globulin	74.2	40.1	93.0	9.16
	Conalbumin	18.5	13.2	6.9	2.25
Standard error of mean					
	Ovalbumin	—	0.2	0.4	3.8
	Globulin	3.7	0.2	7.3	1.8
	Conalbumin	3.4	0.1	0.6	0.8

[a] Averages of three or four hens per dietary treatment.
[b] ^{75}Se was detected in small and variable amounts in the lysozyme fraction. The values constitute the remaining fraction of ^{75}Se not shown.
[c, d] See corresponding footnotes, Table II.

TABLE IV. THE COMPONENTS OF VARIANCE SHOWING THE INDEPENDENT AND INTERACTION EFFECTS OF DIETARY Hg AND Se ON Hg AND Se IN EGG WHITE PROTEINS, EXPERIMENT 2

Components of variance	Percentage of total in egg white		Total Hg in egg white		Percentage of total in egg white		Total Hg in egg white	
	^{203}Hg	Stable Hg	Protein (ng/mg)	White (μg)	^{75}Se	Stable Se	Protein (ng/mg)	White (μg)
Ovalbumin								
Hg	NS[a]	0.05	0.01	0.01	NS	NS	NS	NS
Se	NS	NS	NS	NS	NS	0.01	0.01	0.01
Hg × Se	NS	NS	NS	NS	NS	NS	NS	NS
Globulin								
Hg	NS	0.05	0.05	0.01	NS	NS	NS	NS
Se	NS	NS	0.05	0.05	0.05	0.01	0.01	0.01
Hg × Se	NS	NS	0.05	NS	NS	NS	NS	NS
Conalbumin								
Hg	NS	0.05	0.05	NS	NS	NS	NS	NS
Se	NS	NS	NS	NS	0.05	NS	0.01	0.01
Hg × Se	NS	NS	NS	NS	NS	NS	NS	NS

[a] NS indicates no significant effect; the numerical values indicate level of probability for significant independent or interaction effects ($P \leq 0.05$ or $P < 0.01$).

hanced ^{203}Hg deposition in whites. These effects of Hg and Se were most evident when each was fed to chickens separately. Se in the diet did not change the pattern of binding of ^{203}Hg to proteins. Nearly all ^{203}Hg was associated with ovalbumin, as suggested previously (5). This was true irrespective of dietary Hg levels. However, Se increased ^{203}Hg deposition in egg white but, in doing so, did not diminish the preferential binding of ^{203}Hg to ovalbumin.

Dietary Se produced different patterns for deposition of ^{203}Hg in experiment 1 and total Hg in egg white in experiment 2. Whereas Se increased ^{203}Hg in egg whites in experiment 1, total Hg in egg-white proteins produced by hens fed 20 ppm Hg was reduced by Se in experiment 2. This discrepancy may be related to the fact that two separate experiments were involved and that the egg samples of each experiment represented different sampling times. The ^{203}Hg data in experiment 1 were obtained on all eggs produced during a 20-day period, and the total Hg data in experiment 2 were derived from egg whites produced on the third day of a similarly designed experiment. The discrepancy, regardless of origin, does not change the primary observations that ^{203}Hg and total Hg were associated almost entirely with ovalbumin, and dietary Se altered the pattern of Hg distribution among egg-white proteins only to a minor extent.

More ^{75}Se was deposited in egg yolk than in egg white. This finding agrees with Latshaw's (3, 4) observations with various forms of Se in the feed provided to laying hens. The inclusion of 8 ppm Se in the diet markedly reduced ^{75}Se found in the eggs. This may have been due to the decreased absorption of ^{75}Se associated with feeding 8 ppm Se, a "dilution-effect" of unlabeled Se on ^{75}Se deposited in eggs, or both.

Dietary Hg modified ^{75}Se deposition in eggs, and more ^{75}Se was found in egg white and less in egg yolk than was observed when no Hg was added to the diet. Fractionation of egg-white proteins showed that, when no Hg or Se was added to the diet, ^{75}Se was found primarily in the globulin and conalbumin fractions. The inclusion of Hg and (or) Se in the diet slightly increased the proportion of ^{75}Se associated with globulin at the expense of ^{75}Se bound to conalbumin.

In general, the pattern of total Se concentration in egg-white proteins was similar to that of ^{75}Se. The percentage distribution of total Se among proteins of egg white, however, was different. Whereas ^{75}Se was found almost exclusively in globulin or conalbumin fractions, total Se was found in all three proteins of egg white. The total Se concentration was relatively low in ovalbumin, but, since ovalbumin constituted a large portion of the egg-white protein, a very large proportion of total Se in egg white was associated with this protein. The concentration of total Se in the globulin fraction was increased markedly by 8 ppm dietary Se while that of total Se in ovalbumin was changed only slightly. Consequently, the proportion of egg-white ^{75}Se in globulin increased, and that in ovalbumin decreased for this treatment group.

These data illustrate that egg-white globulin has a definite affinity for Se, especially when a large amount of Se enters the metabolic pathways. Similarly, ovalbumin has an affinity for Hg, and this becomes most obvious when large quantities of Hg are metabolized. This relatively independent binding affinity of Hg and Se seems to explain the virtual absence of interaction effects between the two elements with respect to distribution among egg-white proteins, even though dietary Se level altered Hg content of egg white and vice versa.

Summary. Methylmercuric chloride (Hg) at 20 ppm and sodium selenite (Se) at 8 ppm were fed, separately and in combination, to laying hens. Oral doses of $CH_3{}^{203}HgCl$ and $H_2{}^{75}SeO_3$ also were given to all hens. The concentration of ^{203}Hg and ^{75}Se in egg white and egg yolk, and the distribution of the radioisotopes among proteins of egg white, were determined. The highest concentrations of ^{203}Hg were observed in egg white. The 8 ppm of dietary Se significantly increased the concentrations of ^{203}Hg in egg white when compared with a diet containing no added Se. At the same time, the addition of Se to the diet reduced ^{203}Hg in the egg yolk. ^{75}Se was found primarily in egg yolk, but 20 ppm dietary Hg significantly decreased ^{75}Se in the egg yolk and increased it in the egg whites. A significant Hg × Se interaction was observed

for deposition of ^{75}Se in egg white. When dietary Hg was fed separately from dietary Se, ^{75}Se deposition was increased in the egg whites.

More than 97% of the total ^{203}Hg in egg white was associated with ovalbumin. Similarly, total Hg was found in greatest quantities in ovalbumin, irrespective of the addition of dietary Hg or Se. The largest proportion of total ^{75}Se dose and the highest concentration of total Se per unit of protein occurred in globulin, especially when 8 ppm Se were fed. The data illustrate preferential binding of Hg by ovalbumin and of Se by globulin as compared with other major proteins of egg white.

1. Emerick, R. J., Palmer, I. S., Carlson, C. W., and Nelson, R. A., Fed. Proc. **35,** 577 (1976).
2. Kiwimae, A., Swenson, A., Ulfvarson, U., and Westoo, G., J. Agric. Food Chem. **17,** 1014 (1969).
3. Latshaw, J. D., Proc. Md. Nutr. Conf. Feed Manuf. **25,** 72 (1978).
4. Latshaw, J. D., J. Nutr. **105,** 32 (1975).
5. Sell, J. L., Guenter, W., and Sifri, M., J. Agric. Food Chem. **22,** 248 (1974).
6. Sell, J. L., Poult. Sci. **56,** 939 (1977).
7. Stoewsand, G. S., Bache, C. A., and Lisk, D. J., Bull. Environ. Contam. Toxicol. **11,** 152 (1974).
8. Rhodes, M. B., Azari, P. R., and Feeney, R. E., J. Biol. Chem. **230,** 399 (1958).
9. Peterson, E. A., and Sober, H. A., J. Amer. Chem. Soc. **78,** 756 (1956).
10. Smith, I., "Chromatographic and Electrophoretic Techniques," Vol. 2, p. 210. Yearbook Med. Publishers, Chicago (1976).
11. Reisner, A. H., Nemes, P., and Bucholtz, C., Anal. Chem. **64,** 509 (1975).
12. Olson, O. E., Palmer, I. S., and Cary, E. E., J. Ass. Offic. Anal. Chem. **58,** 117 (1975).
13. Deitz, F. D., Sell, J. L., and Bristol, D., J. Ass. Offic. Anal. Chem. **56,** 378 (1973).
14. Lowry, D. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).

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