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Chemical Form and Distribution of Mercury and Selenium in Eggs from Chickens Fed Mercury-Contaminated Grain

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Interactions between dietary mercury and selenium have been observed in chickens whereby significant amounts of both elements are deposited in the eggs (KIWIMAE et al. 1969; SELL et al. 1974; MAGAT & SELL 1979). Dietary transfer into eggs is influenced by the level and chemical form of both elements in the diet (LATSHAW, 1975; EMERICK et al., 1976; MAGAT & SELL 1979). We have previously examined the chemical form and distribution of mercury and selenium in the edible muscle of marine and freshwater fish and in human tissue (CAPPON & SMITH 1981a,b). Information is reported here on (1) the level of specific mercury and selenium chemical forms in egg white and egg yolk, and (2) the distribution of these forms in specific protein fractions of these egg components.

EXPERIMENTAL

Samples. The two whole egg samples used in this study came from domestic chickens accidentally fed, for a 2-mo period, seed grain treated with a mercurial fungicide (ENGLENDER et al. 1980). Total mercury concentration in the grain was 13 ppm. Eggs were shipped refrigerated to the laboratory and stored at 3°C until analyzed. Raw egg whites were separated from the yolks, and appropriate portions of each component were taken for protein extraction and analysis for mercury and selenium.

Protein extraction. A 1.0 g portion of accurately weighed sample was fractionated according to the procedure of CAPPON & SMITH (1978). The resulting sample fractions -- solid residue, aqueous extract, TCA precipitate -- were individually analyzed for mercury and selenium.

Analysis. The entire solid residue and TCA precipitate, and a 2-5 mL portion of the aqueous extract were used for analysis. An equal amount (1.0 g) of whole sample was also analyzed. Analysis for organic and inorganic mercury and for Se II and Se IV, and Se VI performed by gas chromatography (CAPPON & SMITH 1977, 1978).

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RESULTS AND DISCUSSION

Mercury and Selenium Content and Form. Data on the mercury and selenium content for the white and yolk components are given in Table 1. Excessively high total mercury levels were present in each component, especially the white. The levels were 100 - 1000 times higher than those commonly found in market eggs (MAGGI et al. 1979), reflecting the consumption of mercury-contaminated grain. However, the levels in the whites were comparable to those previously reported for chickens fed experimental mercury-containing diets (SCHAFER et al. 1976). Although the predominant mercury contaminant in the feed grain was phenylmercury (ENGLENDER et al. 1980), methylmercury was the only organic form identified in the eggs in the present study. This may be due to (1) partial breakdown of phenylmercury to inorganic mercury during digestion, followed by rapid renal excretion in the chicken, and (2) partial transformation of phenylmercury into methylmercury within the chicken (KIWIMAE et al. 1969) and subsequent deposition in the eggs. The egg whites contained higher concentrations of total and methylmercury, as confirmed previously by MAGAT & SELL (1979). Inorganic mercury was the predominant form in the yolk. The excessively high mercury levels in the yolks are of interest. Apparently, the chemical form of ingested mercury influences its deposition in and distribution among egg components. TAKABE et al. (1972) noted that mercury was more concentrated in yolks rather than whites of eggs from hens fed phenylmercury acetate, as was the situation with the present samples.

Although the total selenium content in the whites and yolks was far less than that of mercury, it was much higher than that commonly found in market eggs (MORRIS & LEVANDER 1970). LATSHAW (1975) observed similarly high selenium levels in egg whites and yolks from hens fed diets containing 0.10 - 0.42 ppm Se as either natural or selenite-selenium. Since the grain fed to the chickens contained normal selenium concentrations (< 0.1 ppm), the elevated selenium egg levels were probably caused by the increased dietary intake of mercury. Like mercury, selenium was more concentrated in the yolk, as confirmed by LATSHAW (1975) and MAGAT & SELL (1979). Higher percentages of total selenium as Se VI were also present in the yolk.

Mercury and Selenium Distribution. The extraction procedures used to separate the specific sample protein fractions is outlined in Figure 1. The general types of chemical components present in each fraction which may bind mercury and selenium are also listed.

The distribution of specific chemical forms of mercury and selenium in the white yolk protein fractions is presented in Table 2. Only 35 - 50% of the total yolk mercury content was water-extractable, while > 95% was extractable from the white. On a percentage basis, methylmercury was more extractable than

TABLE 1. Mercury and Selenium Content and Chemical Form in Eggs.[a]

Sample	Mercury Residues (ng/g)				Selenium Residues (ng/g)				
	Methyl[b]	Inorganic	Total[c]	%Methyl	-II, IV	VI[d]	Total	%VI	Molar Hg:Se[e]
Egg #1									
white	4800	310	5110	94	240	20	260	7.7	7.7
yolk	490	2600	3090	16	1500	400	1900	21	0.6
Egg #2									
white	6700	310	7010	96	150	20	170	12	16
yolk	480	4500	4980	9.6	1300	500	1800	28	1.1

[a] Results are the means of duplicate analyses.

[b] Methylmercury content expressed as ng/g Hg.

[c] Total mercury values represent the sum of the corresponding methyl and inorganic values.

[d] Se VI values represent the difference between the corresponding total and -II,IV values.

[e] nanomoles total Hg/nanomoles total Se.

TABLE 2. Mercury and selenium chemical form and distribution in egg fractions.[a]

	Protein Fraction:	Solid Residue			Aqueous Extract			TCA Precipitate		
A. Mercury	% of total sample Hg:	T[b]	M[c]	I[d]	T	M	I	T	M	I
	Sample									
	Egg #1									
	white	2	2	16	59	59	53	39	39	31
	yolk	66	51	69	32	49	29	2	-	2
	Egg #2									
	white	1	1	-	67	68	45	32	31	55
	yolk	51	33	53	48	64	46	1	3	1
B. Selenium	% total sample Se:	T[e]	-II,IV	VI	T	-II,IV	VI	T	-II,IV	VI
	Egg #1									
	white	9	9	21	63	63	67	28	28	12
	yolk	52	42	84	45	55	8	3	2	8
	Egg #2									
	white	13	14	5	72	71	79	15	15	16
	yolk	49	44	59	49	53	29	2	3	12

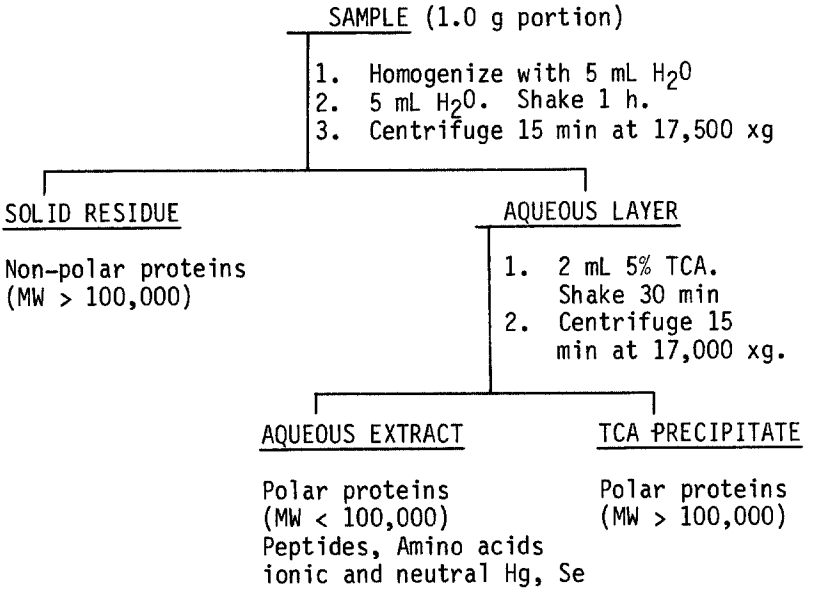
[a]Values represent averages of duplicate analyses. For each chemical form, the sum of the percentages for the corresponding protein fractions is 100%

[b]Total mercury. [c]Methylmercury. [d]Inorganic mercury. [e]Total selenium.

inorganic mercury for both components. A significant portion of total mercury in the white, averaging 35%, was present in the TCA fraction, where both methyl- and inorganic mercury were equally present on a percentage basis. Very little mercury from the yolk (~1%) was precipitated by TCA.

The selenium distribution pattern in both egg component was similar to that for mercury. In the white, Se VI was more water-extractable than Se II and Se IV, on a percentage basis. The opposite was true for the yolk.

FIGURE 1. Protein Extraction Procedure.



The partition of specific mercury and selenium forms between egg white and yolk is due to differential protein binding. This may result from different metabolic and transport processes for both elements in tissue organs (e.g., liver and kidney) and body fluids (e.g., blood), and subsequent selective accumulation of specific chemical forms within the egg. LATSHAW (1975) suggested that the different affinity of selenium for egg white and yolk is due to the origin of the corresponding proteins from the oviduct and liver, respectively. The lower concentration in the former tissue could explain the lower selenium concentration in the white. Since the majority of egg white mercury and selenium in the present samples was water-extractable, both elements may be either unbound (i.e., neutral and ionic) or bound to polar constituents such as simple amino acids, peptides, and low molecular weight proteins (MW < 100,000). However, the existence of unbound mercury as CH_3Hg^+, Hg^{2+}) and selenium as SeO_3^{2-}, SeO_4^{2-})

species is highly unlikely, since these species have a strong affinity for sulfhydryl (-SH) binding sites in tissue. Therefore, the association of both elements with water-soluble (polar) proteins is most likely in egg white. MAGAT & SELL (1979) demonstrated the preferential binding of inorganic mercury by ovalbumin and of selenite-selenium by globulin in egg white. The isolation and characterization of specific mercury and selenium-binding proteins from egg yolk has not been reported. The present results suggest that both elements have a slightly greater affinity for higher molecular weight (non-polar) constituents (e.g., lipoproteins, phospholipids) in the yolk, more so for inorganic mercury and Se VI.

The exact forms and chemical bonding sequence of mercury and selenium in animal tissue proteins has not been established. BURK et al. (1974) isolated a Hg-Se-protein complex from rat plasma which contained both elements in a 1:1 atomic ratio. It was postulated that selenium was attached to the protein-SH groups and that mercury was attached to the selenium. Mercury-binding selenoproteins probably originate in tissues such as liver and kidneys (KOMSTA-SZOMSKA & CHMIELNICKA 1977) and may be subsequently transferred to eggs and other tissues. Future protein isolation experiments are clearly needed to characterize the possible Hg-Se complexes in eggs.

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