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Distribution of Mercury among Components of Eggs following the Administration of Methylmercuric Chloride to Chickens

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The mercury concentration of eggs produced by hens fed 10 ppm of CH_3HgCl for 10 days increased sharply through 12 days of the experiment and then declined slowly for the subsequent 58 days. Maximum concentrations of more than 10 and 5 ppm of Hg were attained in whites and yolks of eggs, respectively. A similar pattern of response was observed with regard to radioactivity in eggs after hens were given 25 μCi of $CH_3{}^{203}HgCl$ by a single i.p. injection. During the 70-day trial, hens fed CH_3HgCl deposited 55% of the total Hg consumed in eggs, while eggs produced by hens given $CH_3{}^{203}HgCl$ contained 65% of the total radioactivity administered. Mercury was found in whites, yolks, and shells of eggs, with more than 80% of the total egg Hg occurring in the white. Additional research showed that the majority of the Hg in egg white was associated with the protein, ovalbumin, rather than with ovotransferrin, ovoglobulin, or ovomucoid.

Considerable information has been accumulated about the toxicity of various mercury-containing compounds for animals of the Aves class (Swensson and Ulfvarson, 1969; Fimreite and Karstad, 1971; Gardiner, 1972; Gardiner *et al.*, 1971; and Spann *et al.*, 1972). Concurrently, data have been presented describing the distribution of mercury among tissues of birds following the administration of mercury (Hg) in various chemical forms (Swensson and Ulfvarson, 1969; Gardiner *et al.*, 1971; Hough and Zabik, 1972; and Wright *et al.*, 1973). It has also been reported that mercury was transferred readily from the diet into eggs, particularly when alkylmercury compounds were fed (Tejning and Vesterberg, 1964; Kiwimäe *et al.*, 1969; Westöö, 1969; Fimreite *et al.*, 1970; Campbell *et al.*, 1971; and Spann *et al.*, 1972).

The data presented by Smart and Lloyd (1963), Kiwimäe *et al.* (1969), and Campbell *et al.* (1971) indicated that the majority of mercury in eggs was present in the white when alkylmercury compounds were fed. The research described herein was conducted to determine the distribution of mercury among the white, yolk, and shell of eggs following the administration of methylmercuric chloride to chickens. Research was also conducted to determine the protein fractions of egg white with which mercury was predominately associated.

REAGENTS

Reagent grade methylmercuric chloride was obtained from Alfa Inorganics Ventron, Beverly, Mass. [^{203}Hg]Methylmercuric chloride (specific activity, 130 mCi/g; radiopurity, in excess of 95%) was purchased from Amersham-Searle Corp., Arlington Heights, Ill. The purified proteins of egg white, ovalbumin, ovotransferrin, ovoglobulins, and ovomucoid were obtained from Sigma Chemical Co., St. Louis, Mo., and the Sephadex G-10 from Pharmacia Fine Chemicals Inc., Piscataway, N. J.

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APPARATUS

A Model 305 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.) was used in the determination of mercury. Radioactivity was determined with a Model 1085 deep-well γ counter equipped with a NaI (thallium activated) crystal (Nuclear-Chicago, Chicago, Ill.). Ultrafiltration was performed with a Model 12 filtration cell (Amicon Corp., Lexington, Mass.). A Gilson Model MF fraction collector (Gilson Medical Electronics, Middleton, Wis.) was used during gel filtration, and electrophoresis was done using a Canalco Model 6 apparatus (Canalco, Rockville, Md.).

PROCEDURE

Five Single Comb White Leghorn hens, approximately 68 weeks of age and kept in individual metabolism cages, were used. Three hens were fed a practical ration in which methylmercuric chloride (CH_3HgCl) was included at a level of 10 ppm. This level of CH_3HgCl was shown by analysis to correspond to about 8 ppm of Hg. The CH_3HgCl was dissolved in 95% ethanol prior to mixing in the ration. The mercury-containing ration was fed *ad libitum* for 10 consecutive days. The hens were then fed a ration which contained no added mercury for the remainder of the 70-day trial.

Each of the remaining two hens, also fed the practical laying hen ration, were given a single, intraperitoneal injection of 25 μCi of radioactivity as [^{203}Hg]methylmercuric chloride. The carrier solution was 0.1 ml of ethanol.

Eggs were collected from all hens during the 70-day trial and were weighed. The eggs were broken and the white, yolk, and shell were separated. The whites and yolks from eggs produced by hens fed 10 ppm of CH_3HgCl were analyzed for total Hg by atomic absorption spectrophotometry according to the method of Deitz *et al.* (1973). Radioactivity of the components of eggs obtained from the hens injected with $CH_3{}^{203}HgCl$ was determined using the deep-well, γ counter. The volume and geometry of the samples were standardized to 1.0 cm^3 and compared with

Table I. Levels of Total Hg and Radioactivity in Egg Components of Hens Fed CH_3HgCl and Given an Injection of $CH_3{}^{203}HgCl$, Respectively

After start of expt.		Part A						
		Mercury, ppm			Total Hg in egg, %			No. of eggs represented
Day	Egg No.	White	Yolk	Shell[a]	White	Yolk	Shell[a]	
4	2	0.178	0.024	n.d.	92.8	7.2		2
6	4	4.200	0.342		92.2	7.8		3
9	6	7.953	2.033		88.3	11.7		2
12	8	10.704	5.167		81.5	18.5		2
19	13	8.298	4.570		78.6	21.4		2
32	22	3.542	1.616		79.6	20.4		3
48	31	1.495	0.620		80.9	19.1		2
70	44	0.226	0.059		88.5	11.5		1
		Part B						
		dpm per g			Total radioact. in egg, %			
3	2	28724	1543	2023	96.1	2.6	0.3	2
4	4	68280	10959	2891	90.8	8.3	0.9	2
8	6	87525	16670	1855	90.2	9.3	0.4	2
10	8	65769	20937	1923	84.0	15.3	0.7	2
15	13	36271	14760	1190	80.8	18.6	0.6	2
32	27	11421	4697	380	83.4	16.0	0.6	1
48	42	1852	606		84.1	15.9		1
70	64	656	290		80.3	19.7		1

[a] Includes shell and membranes. Hg content of eggshells from hens fed 10 ppm of CH_3HgCl was not determined.

appropriate standards. All data on radioactivity were corrected for natural decay.

In order to determine the distribution of mercury among proteins of egg white, samples of this egg component obtained from hens treated with $CH_3{}^{203}HgCl$ were subjected to dialysis, ultrafiltration, gel filtration, and electrophoresis. Preparatory for dialysis, 1 ml of egg white was mixed with 4 ml of distilled water. One milliliter of the mixture was placed in dialysis tubing (7 × 0.6 cm) composed of regenerated cellulose. The tubing had a pore size of 4.8 mμ and, theoretically, retained substances with a mol wt of 12,000 or more. Dialysis was carried out at room temperature (24 ± 2°) for 48 hr *vs.* 1000 vol of distilled water. One-milliliter aliquots of $CH_3{}^{203}HgCl$ standard solution were also subjected to the same dialysis procedure. The radioactivity remaining in the dialysis tubes after 48 hr was determined.

Aliquots of radioactive egg white, diluted 1:4 with water, and of a $CH_3{}^{203}HgCl$ standard solution were subjected to ultrafiltration using a pressurized filtration device equipped with PM-10 filter membranes. The PM-10 filter membrane is designed to retain substances having a mol wt of 10,000 or more. Nitrogen gas was used to pressurize the filtration cell at 2 kg per cm^2. Following filtration, radioactivity of the filtrates and the nonfiltrable fractions was determined.

Additional evidence concerning the association of mercury with the proteins of egg white was obtained using gel filtration. Egg white obtained from hens treated with $CH_3{}^{203}HgCl$ was diluted with distilled water (1:4). One milliliter of aliquots of diluted radioactive egg white and of a $CH_3{}^{203}HgCl$ standard solution were each independently passed through 0.9 × 60 cm columns of Sephadex G-10. Distilled water was the eluent and column flow was 0.5 ml/min. One-milliliter fractions were collected, and the radioactivity in all fractions was determined. The protein content of all fractions was determined by the method of Lowry *et al.* (1951).

In preparation for disc electrophoresis, egg white was mixed with a 30% sucrose solution (1:5) and the mixture centrifuged to remove ovomucin from suspension. Fifty microliters of diluted egg white (devoid of ovomucin) were pipetted onto polyacrylamide resolving gel in a continuous Tris–glycine buffer system (pH 8.3) and the proteins were separated electrophoretically. The gel solution contained 7% acrylamide and 0.1% *N,N,N′,N′*-tetramethylethylenediamine (TMED). An 8.5 × 0.6 cm resolving gel was used for each column. Electrophoresis was performed for 60 min at room temperature (24 ± 2°) with a constant current of 3 mA/column. The proteins in the gel were stained with 0.5% Amido Black in 7% acetic acid for 30 min and were destained with 7% acetic acid. The stained bands were identified by comparing them with those obtained from electrophoresis of the individual pure proteins, ovotransferrin (conalbumin), ovoglobulins, ovalbumin, and ovomucoids. The stained bands of each column were separated following identification and the radioactivity of each band was determined.

Electrophoresis was also performed on mixtures of the pure proteins of egg white as well as the individual proteins after the *in vitro* addition of $CH_3{}^{203}HgCl$ to the media. The concentrations of pure proteins used were equivalent to the theoretical levels of ovotransferrin, ovoglobulin, ovomucoid, and ovalbumin present in 1 ml of egg white. The mixtures of proteins and $CH_3{}^{203}HgCl$ were allowed to stand 30 min at room temperature prior to electrophoresis in one phase of the research, and about 12 hr in another phase. The time of standing did not alter the qualitative distribution of the radioactivity among the protein bands.

RESULTS AND DISCUSSION

Treatment of the hens with 10 ppm of dietary CH_3HgCl for 10 days did not adversely affect rate of egg production or feed consumption. The hens consumed 101 g of feed per day during the treatment period, thereby ingesting a total of 8 mg of mercury per hen by the time CH_3HgCl feeding was terminated.

A portion of the data collected concerning levels and proportions of mercury and radioactivity found in eggs is presented in Table I. These data were selected for presentation since they represented the patterns of responses observed following CH_3HgCl treatment. On day 4 of the trial, mercury was readily detected in the whites and yolks of eggs produced by hens fed 10 ppm of CH_3HgCl. Similarly, considerable radioactivity was present in white, yolk, and shell (including membranes) of eggs produced by hens given $CH_3{}^{203}HgCl$ within 3 days of injection. The largest concentrations of Hg and of ^{203}Hg in egg white occurred on days 12 and 8 after initiation of treatment, re-

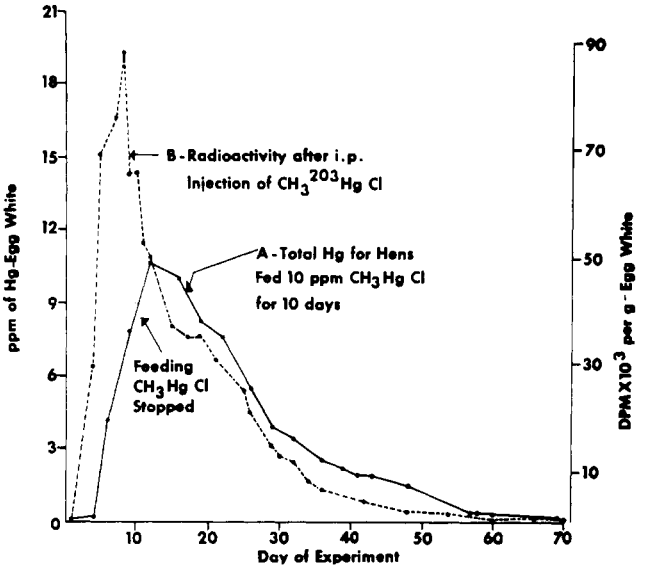

Figure 1. Patterns of change in Hg or ^{203}Hg in egg whites from hens treated with methylmercuric chloride.

spectively (Table I and Figure 1). The highest concentration of Hg in yolk also was observed on day 8 while peak radioactivities in yolks of hens injected with $CH_3{}^{203}HgCl$ occurred on day 10 of the trial. The radioactivity of shell and membranes reached a maximum level relatively soon after treatment (day 4).

The largest proportion of total mercury and of radioactivity was found in egg white, regardless of time after treatment (Table I). As would be expected on the basis of time required for yolk formation, there was a lag period of 10–12 days post-treatment before the proportion of mercury present in the yolk attained its highest level. At that time, the mercury and radioactivity of yolk comprised about 15–20% of the total Hg and total radioactivity, respectively, found in the eggs, and remained near this proportion for the remainder of the 70-day experiment. Campbell *et al.* (1971) found that the concentration of mercury in egg white was much higher than in yolk when hens were fed fish meal contaminated with mercury. Smart and Lloyd (1963), Tejning and Vesterberg (1964), and Kiwimäe *et al.* (1969) reported that the oral administration of alkylmercury compounds to hens resulted in an uneven distribution of mercury in eggs; the white invariably contained more mercury than the yolk. On the other hand, Takabe *et al.* (1972) noted that mercury was most concentrated in yolks rather than whites of eggs produced by hens fed phenylmercuric acetate. Apparently, the chemical form of the mercury administered influences its deposition in and distribution among egg components.

The relatively high levels of mercury in egg white of hens fed 10 ppm of CH_3HgCl were of interest. Although the equivalent of 8 ppm of Hg was fed, levels of 10 or more ppm of Hg in egg white were observed immediately after the end of the 10-day Hg-feeding period. These data indicated that the dietary mercury was readily transferred to egg white and became very concentrated in that component. Consequently, egg production was a major route of elimination of mercury from the body. Based on the analyses and weights of eggs produced, it was calculated that about 55% of the total Hg consumed by hens fed the ration containing 10 ppm of CH_3HgCl ration was deposited in eggs produced during the 70-day experiment. In the case of hens given the $CH_3{}^{203}HgCl$ injection, about 65% of the total radioactivity administered was deposited in eggs produced in 70 days.

The data presented graphically in Figure 1 show that the changes in total mercury and in radioactivity of egg white paralleled one another closely, indicating that Hg

Table II. Dialysis and Ultrafiltration of Standard $CH_3{}^{203}HgCl$ and of Egg White from $CH_3{}^{203}HgCl$-Treated Hens

	Radioact., %			
	48-hr dialysis[a]		Ultrafiltration[b]	
Source of radioact.	Dialyzable	Nondialyzable	Filtrable	Nonfiltrable
$CH_3{}^{203}HgCl$ standard (26,240 dpm)	96.8	3.2	95.7	4.3
Egg white from $CH_3{}^{203}HgCl$-treated hens (24,430 dpm)	2.8	97.2	1.2	98.8

[a] Dialysis was performed utilizing 1 ml of diluted egg white (1:4 distilled water) or 1 ml of standard $CH_3{}^{203}HgCl$ solution *vs.* 1000 vol of water for 48 hr. [b] One milliliter of diluted egg white (1:4 distilled water) and 1 ml of standard $CH_3{}^{203}HgCl$ solution were subjected to ultrafiltration through a membrane which retained substances of a mol wt of 10,000 or more.

Table III. Distribution of Radioactivity among Some Proteins of White of Eggs from Hens Treated with $CH_3{}^{203}HgCl$

Protein fraction	Ranges in % of total radioact., ^{203}Hg[a]
Ovotransferrin	0.7–3.3
Ovoglobulins (two bands)	2.6–6.4
Ovomucoids	1.2–3.5
Ovalbumin (three bands)	78.7–93.0
Not associated with protein bands	2.5–8.1

[a] Ranges represent the low and high values obtained when electrophoresis was performed on the whites of 12 different eggs. Electrophoresis was conducted in duplicate for each egg white.

derived from dietary CH_3HgCl (10 mg) or from a trace dose of $CH_3{}^{203}HgCl$ given i.p. (0.19 mg) was deposited in egg white in a similar fashion.

Since the majority of mercury found in eggs was in the white, further investigation was conducted to characterize the distribution of mercury in this substance. Dialysis conducted on whites of eggs obtained from hens treated with $CH_3{}^{203}HgCl$ showed that less than 3% of the radioactivity passed through the dialysis tubing (Table II) indicating that Hg was bound to an organic constituent of egg white. In contrast, approximately 97% of the radioactivity placed in dialysis tubes as standard $CH_3{}^{203}HgCl$ solution was dialyzable in the identical type of aqueous medium.

More than 95% of the radioactivity of an aliquot of $CH_3{}^{203}HgCl$ standard solution passed through an ultrafilter membrane capable of retaining substances having a molecular weight of 10,000 or more. On the other hand, essentially no radioactivity was removed from ^{203}Hg-containing egg white during ultrafiltration. These results indicated that the Hg isotope deposited in egg white was associated with substances having molecular weights of 10,000 or more. Nearly all the proteins of egg white fit this criterion.

When egg white from hens given $CH_3{}^{203}HgCl$ was subjected to gel filtration, the elution patterns demonstrated that nearly all radioactivity of egg white passed through the Sephadex G-10 column at the same rate as the proteins of egg white. Egg white radioactivity and protein emerged immediately after the void volume, occurring in the 7- to 11-ml fractions of eluent. In contrast, $CH_3{}^{203}HgCl$ standard emerged from the same type of column at a much slower rate (17–30 ml of eluent). Proteins with a molecular weight greater than 700 do not penetrate

Table IV. Distribution of Radioactivity among Proteins of Egg White Following Electrophoresis

Protein fraction	Total Radioact. per mg of protein, %			
	$CH_3{}^{203}HgCl$ added to solutions of individual proteins[a]	$CH_3{}^{203}HgCl$ added to mixture of pure proteins[a]	$CH_3{}^{203}HgCl$ added to egg white *in vitro*	Egg white containing $CH_3{}^{203}HgCl$ deposited *in vivo*
Ovotransferrin	1.41[1,b]	1.10[1]	0.75[1]	1.20[1]
Ovoglobulin	15.97[3]	1.22[1]	1.02[1]	3.94[2]
Ovomucoid	1.61[1]	1.40[1]	1.16[1]	1.38[1]
Ovalbumin	3.49[2]	3.40[2]	3.97[2]	6.22[3]

[a] The quantities of pure proteins in the mixtures corresponded to the theoretical amounts present in 1 ml of egg white based upon egg white containing 11% protein comprised of 12% ovotransferrin, 8% ovoglobulin, 11% ovomucoid, 54% ovalbumin, and 15% other proteins (Gilbert, 1971). [b] Each value is the mean of three determinations and means within a column not followed by the same superscript number are significantly different ($P \leq 0.05$).

Sephadex G-10 beads. Thus, these results together with those obtained from dialysis and ultrafiltration showed that ^{203}Hg in egg white was associated with substances having molecular weights in excess of 12,000, probably proteins.

Subsequent electrophoresis of egg white and determination of the distribution of radioactivity in the protein bands showed that 78–93% of the ^{203}Hg was associated with ovalbumin (Table III). Only small amounts of radioactivity were found in bands corresponding to ovotransferrin, ovoglobulins, and ovomucoids. The protein of egg white contains about 54% ovalbumin, 12% ovotransferrin, 8% globulins, 11% ovomucoids, and 15% other proteins (Gilbert, 1971). Correction for differences in the concentration of these various proteins in egg white change the picture slightly; the concentration of ^{203}Hg per unit of protein was highest for ovalbumin and ovoglobulin and was low for ovotransferrin and ovomucoid. These observations correspond to those of Yagi and White (1958) who found that mercury was associated primarily with the albumin and globulin-like fractions of kidney tissue of rats.

Additional electrophoresis of solutions of pure protein containing $CH_3{}^{203}HgCl$ added *in vitro* showed that, on a per unit protein basis, ovoglobulin as well as ovalbumin had a strong affinity for $CH_3{}^{203}HgCl$ when either was the only protein in the solution (Table IV). Ovalbumin also appeared to bind $CH_3{}^{203}HgCl$ preferentially even when other proteins were present in the mixture, while, in contrast, preferential association of $CH_3{}^{203}HgCl$ with ovoglobulins was not apparent when the solution contained other proteins. The association of radioactivity from $CH_3{}^{203}HgCl$ with the ovalbumin and ovoglobulin produced by hens given $CH_3{}^{203}HgCl$ was much more marked than when this form of mercury was added to egg white *in vitro* (Table IV).

It was anticipated that ovotransferrin, a strong metal-binding protein (Gilbert, 1971), would bind Hg preferentially. However, this was not the case and an explanation for the predominant association of egg white mercury with ovalbumin, and to a lesser extent with ovoglobulin, is not evident. It has been postulated that mercury complexes with a sulfhydryl group such as that found on cystine (Harvey, 1970), but the cystine content of ovalbumin is much less than that of ovotransferrin (Gilbert, 1971). Noteworthy, however, is that ovalbumin contains about 2.5 times as much methionine as ovotransferrin.

The patterns of distribution of mercury among the physical and chemical components of eggs are of interest since they indicate that fairly specific metabolic pathways may exist for the handling of this element. However, the physiological significance, if any, of differential accumulation of mercury in egg components remains to be determined. The accumulation of mercury in eggs has been shown to adversely affect hatchability and embryonic development (Tejning, 1967; Borg *et al.*, 1969; Fimreite, 1971; and Hill and Shaffner, 1973), but to the authors' knowledge, a direct relationship between embryonic death due to mercury and the predominant association of mercury with specific egg components has not been described.

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