Methylmercury Compounds in Eggs from Hens after Oral Administration of Mercury Compounds

Arnold Kiwimäe, Åke Swensson, Ulf Ulfvarson, and Gunnel Westöö

White leghorn hens were fed for 140 days with wheat treated with methylmercury hydroxide, phenylmercury hydroxide, methoxyethylmercury hydroxide, and mercury(II) nitrate. The daily administration was 400 or 1600 μg. of mercury. After administration of phenylmercury hydroxide, methoxyethylmercury hydroxide, or mercury(II) nitrate, a proportion of the mercury was transformed to methylmercury compounds. The organs of the hens and the eggs contained different levels of methylmercury compounds. Since the methylation of mercury in the hen is neither rapid nor complete, the level of mercury concentration in the organism of the hen and in the eggs is mainly determined by the rate of excretion of the actual mercury compound administered.

Small amounts of methylmercury compounds in biological material may be determined quantitatively by gas and thin-layer chromatography according to the methods of Westöö (1966, 1967) and Kitamura *et al.* (1966). In many cases, although an external source of the methylmercury compounds can be excluded, compounds of this type occur in fish (Norén and Westöö, 1967).

Therefore methylation of mercury in other biological systems would seem possible. The sites and mechanism of such methylations are still unknown.

This investigation is part of a greater investigation (Kiwimäe *et al.*, 1969), and its object has been to study the methylmercury content of eggs from hens which were fed different mercury compounds experimentally.

EXPERIMENTAL

The experiment was performed with 72 White Leghorn hens, distributed among nine groups of eight hens each. The hens were kept in separate cages and were fed 50 grams of wheat per day, with free access to additional food and water. The hens were artificially inseminated with mixed semen from eight cocks.

The wheat given to the hens was treated with methylmercury hydroxide, phenylmercury hydroxide, methoxyethylmercury hydroxide, and mercury(II) nitrate in concentrations of 8 and 32 mg. of mercury per kg. The daily administration of mercury to the hens thus was 400 and 1600 μg., respectively. One group of hens was given untreated wheat.

The feeding period lasted for 140 days. Eggs were collected during another 4 weeks after the mercury administration ceased.

All eggs from each hen were collected. Some of the eggs were taken for determination of total mercury by neutron activation analysis according to Sjöstrand (1964). These analyses were made by Isotoptekniska Laboratoriet, Stockholm. Eggs were also analyzed for methylmercury compounds according to Westöö (1966, 1967).

National Institute of Occupational Health, Division of Occupational Medicine and the King Gustaf V Research Institute, Stockholm 60, Sweden.

RESULTS

The results of the determination of total mercury in eggs and in the organs of the hens and the hatched chickens will be reported later (Kiwimäe *et al.*, 1969). Some results are, however, summarized here as a background for the results reported.

The concentration of total mercury in eggs increased rapidly after mercury administration started. After 1 or 2 months the rate of increase was only moderate.

The concentration level in the eggs depended on the level in the food. At the same concentration of mercury in the food, the highest concentration in the eggs was reached with methylmercury hydroxide, then in decreasing order with phenylmercury hydroxide, methoxyethylmercury hydroxide, and mercury(II) nitrate.

The relation between the concentrations in the yolk and the white showed characteristic variations with the type of mercury compound. In the control group and in the group that received methylmercury hydroxide the concentration was higher in the white; in all other groups, it was higher in the yolk.

Characteristic differences between the mercury compounds were also found as regards the effect of the increase in the dose level on the concentrations in the white and the yolk. When the two groups that were given methylmercury hydroxide were compared, a fourfold increase in the dose level resulted in a fourfold increase in the concentration of mercury in the white. With mercury(II) nitrate and phenylmercury hydroxide the dose level affected the concentration in the white only slightly. With methoxyethylmercury hydroxide this concentration was about doubled. In the yolk, on the other hand, the mercury concentration increased fourfold with all mercury compounds when the dose level was increased fourfold.

Table I gives the mercury concentrations in the white and the yolk of eggs laid at different times after feeding started.

Table I. Total Mercury Concentration and Per Cent of Methylmercury Compounds in Eggs from Hens Fed Different Mercury Compound[a]

Time after Feeding Started, Days	Concentration of Mercury, Mg./Kg.											
	Dose Level, 400 μg. per Hen per Day						Dose Level, 1600 μg. per Hen per Day					
	Mercury(II) Nitrate		Phenylmercury Hydroxide		Methoxyethyl-mercury Hydroxide		Mercury(II) Nitrate		Phenylmercury Hydroxide		Methoxyethyl-mercury Hydroxide	
	Total Hg	% MeHg[b]	Total Hg	% MeHg[b]	Total Hg	% MeHg[b]	Total Hg	% MeHg[b]	Total Hg	% MeHg[b]	Total Hg	% MeHg[b]
						Egg Whites						
7–9	0.12	(72)	0.36	84	0.19	89	0.16	94	0.29	(59)	0.36	81
15–17	0.15	(53)	0.29	87	0.24	92	0.18	83	0.33	79	0.46	(98)
30–32	0.16	100	0.38	99	0.33	~100	0.23	(96)	0.53	(66)	0.72	93
53–55, 62–64, 75–77	0.35	93	0.49	75	0.34	84	0.35	(100)	0.63	(63)	0.82	94
111–113, 137–139 (last day of feeding)	0.31	...	0.53	...	0.46	...	0.44	89	0.85	~100	0.88	89
29 days after feeding stopped	0.058	(86)	0.064	...	0.086	(73)	0.064	(81)	0.12	(92)	0.10	(~100)
								Yolk				
137–139							2.12	1.8	4.53	3.8	2.89	3.3

[a] Egg whites from hens fed untreated wheat: Total Hg 0.029 mg./kg., 90% MeHg. Yolk: Total Hg 0.012 mg./kg.
[b] Percentages within brackets means total mercury and methylmercury determination made on different eggs laid at same time.

Table II. Total Mercury Concentration and Per Cent of Methylmercury Compounds in Organs from Hens Fed 1600 μg. of Mercury per Day[a]

Mercury Compound	Concentration of Mercury in Organs, Mg./Kg.							
	Blood		Liver		Kidney		Muscle	
	Total Hg	% MeHg	Total mercury	% MeHg	Total mercury	% MeHg	Total Hg	% Me Hg
Mercury(II) nitrate	0.20	80	2.65	12	3.17	9	0.16	85
Phenylmercury hydroxide	0.58	50	4.80	10	10.0	4.7	0.23	87
Methoxyethyl-mercury hydroxide	0.48	56	...		4.80	10	0.37	59
Methylmercury hydroxide (two hens fed 400 μg. Hg per day)	...		...		...		1.25	94
							3.90	100

[a] Hens killed and dissected immediately after feeding for 139 days stopped. Organs from one hen analyzed in each group.

Total mercury was usually determined on a mixture of white or of yolks from one egg per hen in each group. Methylmercury compounds were sometimes determined on other eggs laid at the same time. Therefore the concentrations of total mercury and of methylmercury compounds are sometimes not exactly comparable. In these cases the percentage of methylmercury compound is given in brackets. When the concentration of methylmercury found exceeded the concentration of total mercury found, the percentage of methylmercury compounds is stated as ~100%.

Table I shows that the proportion of methylmercury compounds was between 81 and 94% in the whites one week after feeding with mercury-treated wheat started (figures in brackets not considered). No tendency to decrease this proportion was found during the feeding period. As the recovery of methylmercury compounds added to egg white was about 90%, this means that practically all the mercury in the whites was methylated. After the feeding ceased, the mercury concentration in the whites decreased rather rapidly.

In the yolks the proportion of methylmercury compounds was much lower than in the whites. At the high dose level the concentrations of total mercury in the yolk were very high, three to 10 times higher than in the whites, and the ratio of methylmercury compounds to total mercury was low. At the low dose level the concentration in the yolk was only slightly higher than in the white, the proportion of methylmercury compounds to total mercury was only a few per cent. Part of the methylmercury found in the yolk may have originated from the white, as it was not always completely separated from yolk.

In the eggs from the control group the total mercury concentration was of course much lower. The average concentration in the white amounted to 29 ng. per gram. The proportion of methylmercury compounds to total mercury was, however, about the same as after the experimental administration of mercury, or about 90%. In the yolk the average concentration of total mercury was 12 ng. per gram.

The mercury concentration in eggs from hens fed wheat containing methylmercury hydroxide was also determined. One month after the mercury administration started the eggs contained about 10 or 400 mg. of mercury per kg. in the white and 2 or 10 mg. of mercury per kg. in the yolk, depending on the dose level. The proportion of methylmercury compounds to total mercury was close to 100%, as expected.

Concentrations of methylmercury compounds and total mercury in the organs of one hen from each of the groups that were given a high dose of mercury are reported in Table II. Only single analyses were performed. The hens were

killed immediately after the mercury administration ceased. Table II also reports analyses of muscles from two hens that were given methylmercury hydroxide at the low dose level.

The hens given mercury(II) nitrate, phenylmercury hydroxide, or methoxyethylmercury hydroxide had a rather high proportion of methylmercury compounds in the blood and the muscles (between about 60 and 90%). In the liver and kidneys the percentage of methylmercury compounds was much lower (up to 12%). The muscles of the hens given methylmercury hydroxide probably contained exclusively methylmercury compounds.

DISCUSSION

After administration of mercury(II) nitrate, phenylmercury hydroxide, or methoxyethylmercury hydroxide to hens a proportion of the mercury compounds was transformed into methylmercury compounds in the hens. Table II indicates that the different compounds are likely to be distributed independently of each other among the organs in a way which would be expected for each compound. The methylmercury compounds were rather evenly distributed among the organs, while the other mercury compounds gave very high concentrations in the liver and kidneys compared to the other organs. This distribution pattern was found by Swensson and Ulfvarson (1968) after a single injection of the mercury compounds here investigated in young White Leghorn cocks.

Interesting differences in the proportion of methylmercury compounds to total mercury were shown in the white and the yolk of the eggs from the hens that were given mercury(II) nitrate, phenylmercury hydroxide, or methoxyethylmercury hydroxide. While the proportion in the white was similar to that in the blood and the muscles, the proportion in the yolk was similar to that in the liver and the kidneys.

The mercury concentration in the whites was less affected by the dose level of mercury in the food than the concentration in the yolks. The white and yolk seem to behave differently as to the partition of mercury between them and the organs in the hen. Furthermore, there seems to be no partition equilibrium between the white and the yolk.

The fact that methylmercury compounds were formed from other mercury compounds in the hen does not mean that the nature of the mercury administered to the hen is unimportant. Since the methylation is neither fast nor complete, the level of mercury concentration in the hen and in the eggs laid will be determined mainly by the rate of excretion of the actual mercury compounds administered to the hen. Although the white of the eggs from hens given mercury(II) nitrate, phenylmercury hydroxide, or methoxyethylmercury hydroxide contained mainly methylmercury compounds, the concentration varied with the compound given to the hens, and was in all cases much lower than when the hens were given the same quantity of methylmercury hydroxide from the beginning.

LITERATURE CITED

Kitamura, S., Tsukamoto, T., Hayakawa, K., Sumino, K., Shibata, T., *Med. Biol. (Tokyo)* **72** (5), 274–81 (1966).

Kiwimäe, A., Swensson, Å., Ulfvarson, U., unpublished data, 1969.

Norén, Koidu, Westöö, Gunnel, *Vår foda* **19** (2), 13–17 (in Swedish) (1967).

Sjöstrand, B., *Anal. Chem.* **36,** 814–19 (1964).

Swensson, Å., Ulfvarson, U., *Acta Pharm. Toxicol.* **26,** 259–74 (1968).

Westöö, Gunnel, *Acta Chem. Scand.* **20,** 2131–7 (1966).

Westöö, Gunnel, *Acta Chem. Scand.* **21,** 1790–800 (1967).

Received for review April 19, 1968. Accepted February 24, 1969.