A Transport Mechanism of Methyl Mercury to Egg Albumen in Laying Japanese Quail

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Abstract. A transport mechanism of methylmercury to egg albumen was investigated in laying Japanese quail which had received a single intravenous injection of ^{203}Hg -methylmercuric chloride.

Whole body distribution of ^{203}Hg was analyzed by means of autoradiography. It was notable that the uptake of ^{203}Hg was higher in the oviduct than in any other tissue checked. Further, the ^{203}Hg was detectable in egg albumen which had been synthesized and secreted in the magnumean portion of the oviduct. From the observations mentioned above, it is suggested that methylmercury in the vascular system was taken up cumulatively by the oviduct and deposited in egg albumen by the secretory way.

On the tissue level of the benzene-extractable form of ^{203}Hg compounds (RHg) quantitative analysis was made in the respective organs. RHg decreased from the blood and liver with the lapse of time. The renal level of RHg showed a gradual increase which was followed by a rapid decrease. In contrast to the tissue tested above, a magnumean uptake of RHg increased gradually and reached the highest level of the four tissue after 24 hours. The RHg content was analyzed in the egg shell, albumen and yolk. It was the largest in the albumen and second largest in the yolk, but negligible in the shell. In 7 eggs laid over a period of 8 days after the injection, the total RHg content was approximately 44% of the dose of the radiocompound administered. The largest amount of RHg was found in the albumen of an egg the third in the order of oviposition after the injection. The fecal and urinary content of ^{203}Hg was approximately 23% of the dose during the same period of time. These quantitative data suggest that the major part of methylmercury in the body is transported into egg albumen via the secretory mechanism of the magnumean portion of the oviduct.

Starch gel electrophoresis of the soluble cytoplasmic fraction of the magnumean homogenate and of egg albumen revealed that ^{203}Hg was associated with protein bands which had migrated near the front. The protein bands were considered to be ovalbumin. It was confirmed from an examination in vitro that ^{203}Hg -methylmercuric chloride bound to a purified hen's ovalbumin preparation.

These results mentioned above suggest that methylmercury is readily bound to ovalbumin which was newly synthesized in large quantity in the magnumean portion of the oviduct.

Mercury residues in hen's egg were reported by many workers [4, 5, 9, 13, 14]. It is well accepted that the intake of eggs contaminated with mercury causes toxiderma in children while there are no knowledge on the mechanism of the toxic

effect [3]. The content of mercury in eggs and the rate of increase in this content have been summarized by Howell [4] to depend on the kind of mercurial compounds, the level of mercury in dose, the length of dosing period, individual varia-

tion within a flock, and strain or species of birds. In many experiments, birds were administered with mercurial compounds in feeding with seeds dressed with the compounds, and the level of mercury in eggs were analyzed [4, 5, 14]. When the mercurial level was examined in the egg components in birds administered with methylmercuric compounds, it was relatively high in the egg albumen. In birds administered with methoxy ethylmercury, phenylmercury, and inorganic mercury derivatives, the content of mercury was relatively high in the yolk [5, 8, 13].

Recently, it has been made possible to determine methylmercury compounds in biological materials, such as fish, egg, meat and liver [14]. The great majority of mercurial compounds in the egg albumen has been clarified to be in a methylmercuric form in birds which had received not only methylmercury derivatives but also methoxy ethyl-, phenyl- or inorganic mercurials [5].

The present investigation was performed to elucidate the transport mechanism of organic mercury compounds into the egg albumen in relation to mercurial metabolism in the reproductive organs in laying Japanese quail which had been injected intravenously with ^{203}Hg -methylmercuric chloride.

Materials and Methods

Laying Japanese quail (*Coturnix coturnix japonica*) used in this experiment were of JQ-NIBS closed colony and supplied by the Nippon Institute for Biological Science in Tachikawa, Tokyo. They were 10 to 32 weeks old and weighed approximately 130 g. These laying birds had a history of having produced more than six eggs a week.

Whole body autoradiography: Ten laying birds received a single intravenous injection of ^{203}Hg -labeled methylmercuric chloride (CH_3HgCl , 3 mCi/mg of Hg, from the New England Nuclear Corporation, U.S.A.) into the jugular vein. The dose

of the compound for a bird was 0.05 mg of Hg (equivalent to 40 μCi) per 100 g of body weight in 0.1 ml of 0.01 N hydrochloric acid solution with carrier. The dose level of the compound had no inhibitory effect on the egg production. Two birds were sacrificed by inhalation with chloroform at various post-injection time intervals and examined by the conventional method of whole body autoradiography [13].

Organic mercury extraction: The organic form of radiomercury in the tissue and eggs were extracted by the procedure recommended by Westöö [15]. The test for the extraction of organic mercury gave a considerable recovery (79–83% in respective tissue). When extracted by this procedure, radiomercury in the tissue, yolk or albumen was expressed as organic mercurial compounds (RHg).

Quantitative experiment on distribution of RHg: Forty laying birds were selected for this experiment and divided into 5 groups of 8 birds each. Each bird received a single intravenous injection of ^{203}Hg -methylmercuric chloride solution into the jugular vein. The dose level of the compound for a bird was 0.05 mg of Hg per 100 g of body weight. Birds in each group were sacrificed at various post-injection time intervals. Various tissue were removed from the carcass and subjected to the RHg extraction procedure.

Fecal and urinary excretion and transport to egg of radiomercury: Twelve birds were given a single intravenous injection of the same dose of ^{203}Hg -methylmercuric chloride as used in the quantitative examination mentioned above. The injection was done when quail had an egg with hard shell in the uterine portion of the oviduct. After that, 7 eggs were laid for 8 days from each of birds. Each egg was separated into a yolk, albumen and shell. Furthermore, the yolk was separated into a yolk granular fraction and a water-soluble one [1]. Mixtures of feces and urine were collected at various post-injection time intervals for 8 days. They were placed in a dish containing a small amount of water, homogenized, and suspended in 100 ml of hot water at about 80 °C. A small aliquot (2 ml) of the resulting suspension was placed into a polyethylene vial for detection of radioactivity. At the end of excretory examination, all the birds were killed by decapitation. Specimens were harvested from their organs. RHg was extracted from them in the manner as described above.

Electrophoretic examination: Starch gel electrophoresis was performed for fractionation of egg albumen proteins. Samples for the electrophoresis were composed of a soluble cytoplasmic fraction of the magnumean homogenate of the oviduct, egg

albumen, and a purified hen's ovalumin preparation (Sigma Chemical Co., St. Louis, Mo., U.S.A.).

The soluble cytoplasmic fraction of the magnumean homogenate was prepared in the following manner. A laying quail was injected intravenously with ^{203}Hg -methylmercuric chloride solution. The dose level of the compound was 0.05 mg of Hg (equivalent to 100 μCi) per 100 g of body weight. After 24 hours, the bird was decapitated and a magnumean portion of the oviduct was removed. Five grams of the magnum was added to 15 ml of 0.25 M sucrose in 0.076 M tris citrate buffer (0.076 M tris and 0.005 M citric acid) at pH 8.65 [6], homogenized with a Potter-Elvehjem homogenizer, and centrifuged at $100,000\times g$ for 30 minutes. The resulting supernatant fraction was separated and subjected to electrophoresis.

A sample of egg albumen was subjected to electrophoresis. A laying quail was injected intravenously with the same dose of ^{203}Hg -methylmercuric chloride solution as used in the analysis of the magnumean homogenate. A thin layer of egg albumen was removed from an egg which had been the third in oviposition after the injection and diluted with an equal volume of 0.076 M tris citrate buffer at pH 8.65.

Purified hen's ovalbumin was dissolved in 0.076 M tris citrate buffer, pH 8.65, to 4% (w/v). An aliquot (0.5 ml) of it was added to 1 μl of ^{203}Hg -methylmercuric chloride solution (1 μg of Hg which was equivalent to 2 μCi), incubated at 4 °C for 60 minutes, and dialyzed with the tris citrate buffer at 4 °C for 24 hours. The dialyzed preparation was centrifuged at $1,200\times g$ for 60 minutes and a very small portion of the resulting white sediments was discarded.

Starch gel electrophoresis: The modified discontinuous buffer system described by Lush [6] was used (buffer for gel: 0.076 M tris and 0.005 M citric acid, pH 8.65; buffer for electrode vessels: 0.3 M boric acid and 0.1 M sodium hydroxide, pH 8.81). The gel was stained with 1% (w/v) amido black 10 B for 10 minutes, and photographed. Then it was divided into segments 2 mm in width to count the radioactivity of ^{203}Hg .

All the samples were placed into polyethylene vials with a total volume adjusted to 2 ml. The radioactivity of gamma emitter was measured in these samples by a well-type scintillation detector (Tokyo Atomic Co., Ltd., Tokyo).

Results

Autoradiographic observation: Fig. 1 shows autoradiograms of laying quail in-

jected intravenously with ^{203}Hg -methylmercuric chloride. All the birds weighed approximately 130 g each and received the same amount of the radiocompound. The autoradiograms reveal a distribution pattern of ^{203}Hg 1, 24, 48, 96 and 192 hours after the injection. All of them were obtained with the same exposure period of time and under the same developing and printing conditions.

One hour after the injection, much ^{203}Hg was readily detectable in the tissue of liver, kidney, lung, oviduct, ovary, small intestine, pancreas, spleen, heart, proventriculus, blood, bone marrow, gallbladder and parotid gland. In the ovary, radioactivity was condensed in the peripheral part of ova. The brain and skeletal muscle retained a poor activity. The skeletal muscle and gizzard increased the radioactivity considerably 24 hours after the injection. On the contrary, the brain and egg shell in the uterus revealed a poor activity. The proventricular ^{203}Hg decreased slightly with the lapse of time, but no ^{203}Hg was detectable in the cavity of this organ and of the gizzard, which was situated on the anal side posterior to the proventriculus.

It was noted that ^{203}Hg was evidently found in egg albumen and in the peripheral part of the yolk of an egg 24 hours after the injection. After 48 hours, ^{203}Hg decreased considerably from the quail body. The residual activity of ^{203}Hg , however, was relatively high in the tissue of liver, oviduct, kidney, lung and ovary. It was very high in the ova and albumen. Similar observations were obtained from an autoradiogram after 96 hours. At this time, considerable activity was visible in the brain. Further, the brain increased ^{203}Hg concentration in the course of time, as is shown in an autoradiogram after 192 hours. At this time, residual ^{203}Hg was

Fig. 2. Quantitative presentation of RHg concentration in percentage of dose in blood, liver, kidney and magnum (I) and in infundibulum, magnum, isthmus and uterus of oviduct (II) in laying quail 1, 3, 6, 12 and 24 hours after an intravenous injection of ^{203}Hg -methyl mercuric chloride

Remarks.

Vertical bar shows SE of the mean of 8 experiments.

detectable considerably in the oviduct, kidney, lung, egg albumen and yolk, slightly in the liver, and not at all in any other organ. Through out the observation period, the bill retained a considerable level of radioactivity.

Quantitative examination of extractable radiomercury: It was clarified from the

extraction of organic radiomercurial compounds that the radiomercury compounds in the tissue tested were mainly organic ones (RHg). Fig. 2-I shows the results of quantitative determination of RHg in four tissue, e.g., blood, liver, kidney and magnumean portion of the oviduct, as adjusted to percentage of the dose. After an hour,

Fig. 3. Cumulative ^{203}Hg or RHg content in percentage of dose in albumen and yolk of eggs laid by quail injected intravenously with ^{203}Hg -methyl mercuric chloride

Remarks.

A solid line: Total ^{203}Hg content. A dotted line: RHg content. RHg: Extractable organic forms of radiomercury. MMC: ^{203}Hg -methyl mercuric chloride. SE of the mean of 12 experiments is shown by a vertical bar, where possible.

Fig. 4. Cumulative ^{203}Hg content in percentage of dose in the yolk granular fraction (YGF*) and water-soluble fraction of eggs laid by quail injected intravenously with ^{203}Hg -methyl mercuric chloride

Remarks.

Vertical bar shows SE of the mean of 12 experiments.

the liver retained the highest concentration of RHg of the four tissue and followed

Fig. 5. Cumulative ^{203}Hg content in percentage of dose in feces and urine for 8 days in laying quail injected intravenously with ^{203}Hg -methyl mercuric chloride

Remarks.

SE of the mean of 12 experiments is shown by a vertical bar, where possible.

Fig. 6. RHg concentration in percentage of dose in some tissue in laying quail 8 days after an intravenous injection of ^{203}Hg -methyl mercuric chloride

Remarks.

*1: Superficial pectoral muscle. *2: Profound pectoral muscle. Horizontal bar shows SE of the mean of 12 experiments, where possible.

by the kidney, blood and magnum in the order listed. For 24 hours after the injection, the liver and blood decreased RHg concentration with the lapse of time. On the other hand, the kidney and magnum increased RHg concentration in the course of time up to 12 hours after. Thereafter, the magnumean RHg concentration increased and reached the highest level of

the four tissue, while the renal RHg concentration decreased rapidly.

As to RHg concentration, the oviduct was divided into four portions; infundibulum, magnum, isthmus and uterus (Fig. 2-II). Every portion retained a similar level of RHg 1 hour after the injection. Thereafter, the magnumean portion showed a remarkable increase in this level up to 24 hours after the injection, while the other three portions remained at an almost constant level.

Radiomercury in egg: In eggs collected for 8 days after the injection, RHg was detectable in the albumen and yolk, but negligible in the shell. The RHg contents in the egg components are expressed cumulatively in Fig. 3. In all the eggs, except one, laid during the observation period, egg albumen contained a much larger amount of RHg than yolk. The exceptional egg was the first in oviposition after the injection and had no RHg detectable in any component. It was situated in the uterine portion of the oviduct at the time of injection of the labeled compound. The albumen fraction of an egg the third in oviposition after the injection contained the largest amount of RHg of all the eggs examined. The total radioactivity and the RHg contents in the albumen increased considerably with the lapse of time. Both of them reached approximately 40% of the administered dose within 8 days. Those in the yolk reached approximately 4.5 and 3.5%, respectively, during the same period of exposure. Yolk preparations were divided into a water-soluble and a yolk granular fraction (Fig. 4). In this figure, total radiomercury contents are shown. The yolk granular fraction contained a larger amount of radioactivity than the water-soluble fraction.

Fecal and urinary excretion of radio-

mercury: Total radiomercury contents in feces and urine are plotted cumulatively in Fig. 5. For 8 days after the injection, approximately 25% of the dose administered was discharged into a mixture of feces and urine. The cumulative curve of radiomercurial excretion was composed of two phases, a fast and a slow. The fast phase was seen for the first 2 days and followed by the slow one in which a gradual increase was noticed.

At the end of the excretory analysis, the laying quail administered with the labeled compound were subjected to tissue level examination. Fig. 6 shows the RHg concentrations in some selected tissue on the 8th day after the injection. Of all the tissue analyzed, the RHg concentration was the highest in the magnumean portion of the oviduct, which was followed by the kidney, pancreas and liver. It was considerably low in the remaining tissue. At that time, the blood plasma fraction retained the lowest level of all the tissue tested.

Fig. 8. Detection of ^{203}Hg in protein bands of electrophoregram of egg albumen of an egg which is the third in oviposition after the bird was injected intravenously with ^{203}Hg -methylmercuric chloride

Remarks.

The arrow indicates sample application. The protein bands sensitive to amido black 10B are divided into 12 parts. Upper: Radioactivity. Lower: Protein bands.

Electrophoretic examination and distribution of radioactivity: Fig. 7 shows a photograph of the electrophoregram of the soluble cytoplasmic fraction of the magnumean homogenate and the results of radioactivity determination. In the electrophoregram, a clear mono-peak of radioactivity is found in conspicuous protein bands which migrated near the front. The other bands retained very poor activity. In the electrophoretic preparation of egg albumen, visible protein bands were separated from one another and served for detection of radioactivity. Fig. 8 illustrates 12 protein bands separable with a razor blade and the distribution pattern of radioactivity. In it the 2nd and the 3rd bands contained the highest activity of ^{203}Hg of all the bands removed. They appeared to be ovalbumin protein according to the diagram summarized by Gilbert [2]. Then an experiment was done to examine binding in vitro between ^{203}Hg -methylmercuric chloride and a purified hen's ovalbumin preparation (Fig. 9). The ovalbumin proteins migrated to the front under this buffer system and electrophoretic conditions used. Therefore, it was confirmed that ovalbumin bound to RHg.

Discussion

Studies have been made on the distribution of mercury in mammals [11] and birds [10, 12] administered with alkylmercury derivatives. They have revealed that the dose of methylmercuric compounds shows a wide distribution of considerably high levels of mercury or its compounds in many tissue, while the distribution of inorganic mercury is restricted mainly in the liver, kidney and pancreas [8, 10]. The present autoradiographic experiment made it possible to confirm that in laying quail injected with ^{203}Hg -methylmercuric chloride,

radioactivity was distributed widely in many tissue, although the differential level of radiomercury depended on the length of exposure period. Radiomercury compounds in the tissue and eggs were proved to maintain its organic form in extraction analysis for at least 8 days after the injection.

The level of extractable radiomercurial compounds (RHg) depended on the course of time in some tissue. The skeletal muscle and gizzard revealed a temporary increase in the RHg level. The brain exhibited a continually increasing uptake of RHg all over the observation period. Most of the other tissue, however, decreased in the concentration of RHg with the lapse of time. Of the tissue tested, the oviduct maintained the highest level of RHg during the observation period. These findings suggest that the wide distribution of a high level of RHg depends on the high solubility of ^{203}Hg -methylmercuric chloride in organic solvents.

When the gastrointestinal tract was examined, no radioactivity was detectable in the cavity of the proventriculus and gizzard in which the RHg level was altered. The intestinal wall and contents showed a poor distribution of radiomercury in later stages, or 48, 96 and 192 hours after the injection. These results of autoradiographic observation suggest that the proventricular, hepatic and intestinal discharge of RHg play little role in the fecal excretion of the compounds.

It was notable that all over the observation period a considerable level of radiomercury was maintained in the calamus and inferior umbilicus of feathers and in the bill which had been well known to be rich in ceratin and thiol groups. Observing in detail the autoradiograms, the radioactivity appeared to be restricted in

newly developed regions of the feathers and bill. The boundary formation of ^{203}Hg uptake contributes to the study of growing process of the feathers and bill.

In the reproductive system, a high level of RHg was noted in the ova, oviduct and albumen and yolk of eggs, except a uterine egg 1 hour later. In laying quail injected with ^{203}Hg -mercuric nitrate, ^{203}Hg was detectable only in the ova in the reproductive system, but neither in the oviduct nor in the albumen, as reported previously [8]. The high oviducal and albumeneous uptake of RHg was specific in laying quail administered with ^{203}Hg -methylmercuric chloride. The albumeneous RHg seemed to have been deposited by oviducal secretion. Inorganic mercury has been reported to be transported in bound form with serum lipovitellin which is a lipoprotein of serum yolk precursors [7]. In this experiment, a minor level of RHg was found in the yolk granular fraction of the yolk which is rich in the lipovitellin. The ovarian uptake of RHg possibly depends on the high lipid solubility of the compounds for rich in lipid in the granular fraction, unlikely that of inorganic mercury. The albumen appeared to take up a major part of RHg, while it had been well known to contain little amount of lipid. The albumeneous uptake of RHg is no explanative to get a concept of the high lipid solubility of the compounds. The lipid solubility of RHg possibly contributes to transport readily across the cell membrane of the oviduct rather than to bind to albumin proteins.

The results of quantitative examination of tissue level of RHg lend to postulate on the oviducal secretion of RHg into the egg albumen. The magnumean portion of the oviduct increased continuously in the RHg level, while the blood, liver and kid-

ney decreased. This result suggests that a partial redistribution of RHg was carried out from some other organs to the magnumean portion by way of the blood circulation. The oviducal RHg level was analyzed separately in the infundibulum, magnum, isthmus and uterus. The magnumean portion continued to increase gradually in the RHg level and the other portions of the oviduct remained at much lower levels than it. The magnumean portion, which is a so-called albumen secreting portion, synthesizes egg albumen proteins under the hormonal control [2]. The accumulation of RHg in the magnum makes it possible to assume that RHg bind to albumen proteins and that the resultant complex forms are secreted in the oviducal cavity and deposited in the egg albumen.

In starch gel electrophoretic analysis of the soluble cytoplasmic fraction of the magnumean homogenate, a single peak of radioactivity was associated with protein bands which had migrated near the front. Very similar bands were found and the location of radiomercury was clarified by the same analysis of the egg albumen proteins. These electrophoregrams suggest that the major protein bands containing radiomercury are probably ovalbumin, judging from their migration. It was confirmed from the reaction in vitro that purified hen's ovalbumin bound to ^{203}Hg -methylmercuric chloride. These data lead to a suggestion about the transport of RHg into the egg albumen; that is, the circulatory translocation of RHg causes an increase in uptake by the magnumean tissue rich in ovalbumin content. In this tissue RHg binds to ovalbumin. Then the bound form of RHg is discharged into the oviducal cavity and deposited finally in egg albumen. As mentioned above, the binding of RHg and ovalbumin is probably

accomplished by the other possible mechanism rather than by its high solubility to lipid. The high lipid solubility of RHg likely makes readily it to penetrate into the magnumean cells. Immediately after the absorption of RHg, that it binds to ovalbumin induces possibly a lowering of the concentration of its free form in the magnumean cells. This might promote the penetration of RHg into the cells. For rich in newly synthesized ovalbumin in the magnum, it is possible the tissue to retain much larger amount of RHg than any other tissue.

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Explanation of Figures

Fig. 1. Whole body autoradiograms showing the distribution of ^{203}Hg in laying quail 1, 24, 48, 96 and 192 hours after an intravenous injection of ^{203}Hg -methyl mercuric chloride.

Fig. 7. Electrophoregram of soluble cytoplasmic fraction of magnumean homogenate and ^{203}Hg distribution in laying quail 24 hours after an intravenous injection of ^{203}Hg -methylmercuric chloride.

Remarks. A dot indicates radioactivity in each

segment dissected from electrophoregram. The arrow indicates sample application. Upper: Radioactivity. Lower: Electrophoregram.

Fig. 9. Distribution of ^{203}Hg and electrophoregram of purified hen's ovalbumin incubated with ^{203}Hg -methylmercuric chloride in vitro.

Remarks. A dot indicates radioactivity in each segment dissected from electrophoregram. The arrow shows sample application. Upper: Radioactivity. Lower: Electrophoregram.

1 hour

24 hours

48 hours

96 hours

192 hours

1