AN AUTORADIOGRAPHIC STUDY ON THE DISTRIBUTION OF MERCURY AND ITS TRANSFER TO THE EGG IN THE LAYING QUAIL

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It has been reported that mercury was contained in the tissue of, and eggs laid by hens fed experimentally seed grains treated with methyl mercury dicyandiamide (1, 2). In the field, it has also been pointed out the remarkably high mercury contents in seed-eating birds, such as pheasants, and predatory animals living on such birds, suggesting that it may be explained by the existence of alkyl mercury treated seed grains left in the field at the sowing time (3). In 1967, Teining (4) reviewed the pharmacological effect of methyl mercury dicyandiamide on the fowl Gallus gallus L. Some other investigators also studied the retention and movement of organic mercury compounds and their transfer to eggs in birds (5, 6).

The autoradiographic technique is one of the most useful methods to examine the body distribution of radioactive substances (7, 8). This technique has been employed actually to study the distribution of several radioactive compounds in the mouse and other small mammals (9–11), but not in any in the fowls.

The present paper reports a retention and movements of inorganic mercury, in the form of $^{203}$Hg-mercuric nitrate, and its transfer to egg in laying quail using a whole-body autoradiographic and radioisotope tracer techniques.

METHODS

Laying quails (Coturnix coturnix japonica, JQ-NIBS closed colony strain) 8 to 20 weeks old, weighing about 130 g, were supplied by the Nippon Institute for Biological Science. They were selected for the present experiments, as they had a history of having laid more than five eggs a week.

Autoradiography: Twelve laying quails were injected radioactive mercuric nitrate ($^{203}$Hg(NO$_3$)$_2$) into the cervical vein when they had an egg with hard shell in the uterus. The dose of $^{203}$Hg(NO$_3$)$_2$ for a quail employed in the autoradiographic experiment was 0.05 mg (equivalent to 40 μCi) per 100 g of body weight in a volume of 0.1 ml. Its specific activity was 0.8 mCi per mg of mercury. This activity of mercury had no depressant effect on the laying. Each group of two birds of them was sacrificed 1, 24, 48, 96, 192, and 384 hours after injection of $^{203}$(HgNO$_3$)$_2$. Each bird was killed by inhalation with

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chloroform. The carcass was sheared feathers and immersed in a mixture of carbon di-
oxide and acetone at about 68°C. When the whole body was sliced sagittally into sec-
tions about 30 μ thick along the central axis, each section exhibited as many organs as
possible. Fig. 1 shows a schematic arrangement of various organs in the section through
the central axis of quail. After each bird was sliced into sections, autoradiograms were
obtained by allowing the whole-body sections to get into contact with X-ray film (Fuji
Industrial 200) for a week.

Two birds were injected intravenously ³²Hg(NO₃)₂, 0.05 mg of mercury (equivalent
to 40 μCi) per 100 g of body weight. Four eggs were collected from each of two quails,
divided into the order of laying after the administration, and macroautoradiographed by
the method of Heller (12).

![Fig. 1. Schematic arrangement of various organs in the female quail. A median
aspect of the whole body.](image)

![Fig. 2. Schematic arrangement of reproductive organ in the laying quail.
The ova in the ovary were named I, II, III and IV in the decreasing order
of size which was supposed to correspond with the ovulating order of them.](image)
Fig. 3. Autoradiograms of laying quails injected intravenously with $^{203}\text{Hg(NO}_3\text{)}_2$ and taken 1, 24, 48, 96 hours after injection.
Quantitative examination in the body: The amount of radiomercury was examined in blood, liver, kidney, and ova. Six to eight laying quails were employed as a group. Each bird was injected $^{203}\text{Hg(NO}_3\text{)}_2$ into the cervical vein at a dose of 0.05 mg of mercury per 100 g of body weight. This dose varied from 1 to 4 $\mu\text{Ci}$ in radioactivity. The each group was sacrificed 5 and 30 minutes and 1, 3, 6, 12, 24, and 48 hours after the administration. Blood, liver, kidney, and ova were removed from the carcass. When mercury was dosed all the ova in the same bird, except one being in the uterus, were named I, II, III, and IV in the decreasing order of size which was supposed to correspond in the ovulating order of them (Fig. 2).

Determination of radiomercury in eggs: After administration of radiomercury, 8 eggs laid by each of the birds were collected for 9 days. These eggs were divided into 8 groups in the order of laying. Each of them was separated into yolk, albumen, and shell. Radioactivity of gamma emitter was measured in these samples by a scintillation detector (Tokyo Atomic Co., Ltd.).

The $^{203}\text{Hg(NO}_3\text{)}_2$ (3.82 mCi/mg Hg) was supplied by the New England Nuclear Corporation (USA).

RESULTS

Autoradiographic distribution of $^{203}\text{Hg}$ in laying quails

Fig. 3 shows the autoradiograms of sections obtained from laying quails 1, 24, 48, and 96 hours after injection of $^{203}\text{Hg(NO}_3\text{)}_2$. In the autoradiograms, high radioactivity was distributed in the blood, liver, kidney, ovary, ova, bone, lung, pancreas, and intestinal wall of the sample obtained at 1 hour; in the liver, kidney, ovary, ova, and the wall and contents of the intestine at 24 hours; and in the liver, kidney, and ova at 48 hours. At 96 hours, the activity of $^{203}\text{Hg}$ was remained only in the kidney and ova. It was seen that the radioactivity decreased in accordance with the lapse of time after the injection. Especially, radiomercury in the blood, bone, and lung decreased more rapidly than in the other tissues. An extremely low activity was noticed in the central nervous system and

![Autoradiogram of the area of laying ovary of a quail injected intravenously $^{203}\text{Hg(NO}_3\text{)}_2$. It was taken 192 hours after injection.](image)

Kidney, liver, kidney, ova, oviduct, albumen, ovum (yolk), follicle, uterus, shell, ovum (yolk), ova, albumen, yolk.
the muscles even in samples obtained 1 hour after dosing. At the 48th hour, the distribution of radiomercury exhibited a ring-shaped pattern in the yolk of the egg located in the uterus. Fig. 4 shows a high-power magnification of the ovary and egg in the uterus of a laying quail injected radiomercury 192 hours before. The follicular membrane of each ova in the ovary had high activity. In the ova, including one ovum being in the uterus which will be in the ovary at dosing radiomercury, radioactivity localized more highly in the central part of them, and the activity lowered gradually toward the surface. No activity was detectable, however, in the albumen or shell.

Quantitative examination of $^{203}$Hg in blood, liver, kidney, and ova

The radioactivity of $^{203}$Hg in the whole blood, liver, kidney, and four ova (see method) is shown in Table 1 and Fig. 5. Radiomercury in the whole blood decreased rapidly during first 12 hours and slowly thereafter. In the liver, radioactivity reached a maximum value 30 minutes after administration, and decreased rapidly till the 12th hour and slowly thereafter. Radioactivity in the kidney reached a maximum value at the 6th hour, and decreased more rapidly during the 6th to the 12th hour than after the 12th hour. On the contrary, radiomercury in the four ova increased up to the 12th hour and reached a plateau thereafter. At this time the
Radioactivity in the ova was about 42% of the dose administered. The radioactivity in ovum I increased rapidly up to the 6th hour, reaching a plateau thereafter. In ova II, III, and IV, however, the radioactivity reached a plateau after the 12th hour. Over a period from the 12th to the 48th hour, the amount of radiomercury in these ova was in the decreasing order of ovum I, II, III, and IV (Table 2 and Fig. 6).

Radioactivity in eggs laid after administration of $^{203}$Hg(NO$_3$)$_2$

Fig. 7 is an autoradiogram showing the distribution of radioactivity in the four whole eggs (see method). The radiomercury was distributed in the yolk, but not in the albumen or shell. The first egg, which was in the uterus when

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**Table 2. Distribution of $^{203}$Hg in ova I, II, III and IV.**

<table>
<thead>
<tr>
<th>Time after inj.</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min (6)*</td>
<td>0.25±0.03</td>
<td>0.29±0.03</td>
<td>0.15±0.03</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>30 min (6)</td>
<td>1.34±0.10</td>
<td>1.54±0.24</td>
<td>0.83±0.15</td>
<td>0.22±0.03</td>
</tr>
<tr>
<td>1 hr (8)</td>
<td>2.50±0.17</td>
<td>2.31±0.13</td>
<td>1.18±0.07</td>
<td>0.41±0.07</td>
</tr>
<tr>
<td>3 hr (8)</td>
<td>6.22±0.40</td>
<td>5.47±0.28</td>
<td>2.88±0.40</td>
<td>0.88±0.18</td>
</tr>
<tr>
<td>6 hr (8)</td>
<td>13.34±0.82</td>
<td>11.33±0.38</td>
<td>5.90±0.33</td>
<td>1.67±0.09</td>
</tr>
<tr>
<td>12 hr (6)</td>
<td>13.77±0.60</td>
<td>15.78±0.53</td>
<td>9.33±0.63</td>
<td>3.14±0.65</td>
</tr>
<tr>
<td>24 hr (6)</td>
<td>13.88±0.45</td>
<td>16.12±0.82</td>
<td>9.42±0.97</td>
<td>3.21±0.47</td>
</tr>
<tr>
<td>48 hr (6)</td>
<td>13.48±0.71</td>
<td>17.16±0.58</td>
<td>9.59±0.56</td>
<td>3.33±0.41</td>
</tr>
</tbody>
</table>

Each value is expressed as a mean±SE.

* The number of experiments.

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**Fig. 6. Changes in radioactivity of the ova I, II, III, and IV of a laying quail injected intravenously with $^{203}$Hg(NO$_3$)$_2$.**

See also Figs. 2 and 5.

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**Fig. 7. Autoradiogram of four whole eggs laid by a quail after intravenous injection of $^{203}$Hg(NO$_3$)$_2$.**

See also Figs. 2 and 8.
mercury was administered, had no activity of $^{203}\text{Hg}$. The radioactivity in each yolk of the eggs except first one was observed to have a ring-shaped pattern, the ring increased in area as the oviposition went on. In the yolk of the third egg, the edge showed a lower activity than the other part. Radioactivity in the egg was examined quantitatively in the three divisions, yolk, albumen, and shell. It was found that the activity of $^{203}\text{Hg}$ was high in the yolk, especially the highest in that of the third egg (Fig. 8). The activity of $^{211}\text{Hg}$ was undetectable, however, in the albumen or shell of these eggs.

DISCUSSION

Application of the autoradiographic technique to quail

 Autoradiographic technique is a useful one in investigation the distribution of radioactive compounds in the body. Although it has been applied to the mouse and other small mammals for study of the distribution of several radioactive compounds, it has scarcely been applied to the fowls for the same purpose. It was difficult to obtain a whole-body section of the laying hen for the large size of its body. So, in the present experiment, the autoradiographic technique was applied to quail to investigate the distribution and movement of $^{203}\text{Hg(NO}_3\text{)}_2$ and the transfer of this element to the egg in the domestic fowl. The laying Japanese quail, Coturnix coturnix japonica, is about 16 cm long from ehad to cloaca and about 4.5 cm long from back to abdomen. Accordingly, it was possible to obtain a sagittal whole-body section from this bird in the same manner as from small mammals.

Distribution and movement of radiomercury

It was found that the radiomercury was distributed all over the section of the quail soon after administration. The decrease in activity of $^{203}\text{Hg}$ was rapid in blood, lung, and other blood-rich tissues, but slow in the liver and kidney. These findings were almost the same as those reported in mice (9–11), rats (13, 14), and poultry (15, 16). The decreasing phase of the radiomercury in the blood, liver, and kidney was divided into two parts, i.e., a fast and a slow, with the 12th hour after injection as the dividing line. A large concentration of radiomercury was seen in the intestinal contents in the autoradiogram, suggesting that the intestinal tract might have been a route for the excretion of mer-
cury from the body. These findings are consistent with those findings obtained from rats by Pricket et al (17).

Transfer of radiomercury to egg

In the autoradiograms examined, an accumulation of radiomercury in ova developing in the ovary was found. However, there was no activity in the albumen or shell. The time course of accumulation of radiomercury in the ova was also divided into two parts, i.e., a fast and a slow. The fast part of the accumulation course was kept up to the 12th hour after injection. Furthermore, it was revealed that the accumulation of radiomercury in the ova during the fast part corresponded to the decrease of radioactivity in the blood and liver, and that almost the accumulation of radiomercury was completed within first twelve hours after injection. The radioactivity reached nearly a plateau in ovum I at the 6th hour after injection and in ova II, III, and IV at the 12th hour. If the rate of permeation of mercury through the follicular membrane of these ova is constant, the amount of this element accumulated in an ovum will be in the decreasing order of ova I, II, III, and IV. On the contrary to this expectation, the activity of $^{203}$Hg actually evidenced in an ovum was in the decreasing order of ova II, I, III, and IV. It is an explanation for this disorder that the accumulation of the radiomercury to ovum I may have probably been completed because of this ovum would be ovulated about 6 hours after dosing $^{203}$Hg(NO$_3$)$_2$, and that the other ova may have continued to accumulate till the 12th hour. After the 12th hour, the radiomercury in each ovum remained at a plateau. This result may be related to the fact that the decrease in radioactivity in the blood and liver became slow at the same time.

After reaching the plateau in radioactivity of ova, the amount of radiomercury in ovum II was the largest of the four. The yolk of the third egg contained the largest amount of the radiomercury in the yolk of eight eggs laid after injection of $^{203}$Hg(NO$_3$)$_2$. It is thought, therefore, that ovum II will correspond to yolk of the third egg laid after injection of radiomercury. From the above reason, it will be an explanation for the assumption that the decreasing order of size of ova in the ovary will correspond to the ovulating order of them.

Smart and Lloyd (1) and Tejning (4) determined the amount of mercury in the eggs of hens orally given seeds dressed with alkyl mercury compound. They found that the mercury content in the egg was much higher in the albumen than in the yolk. However, the present investigation demonstrated that mercury in the egg was distributed mostly in the yolk and scarcely in the albumen and shell. This inconsistency may be resulted from the difference in chemical form of these mercurials, but not from the difference in mode of administration or species of birds. It was clarified that in eggs obtained from laying quails orally given $^{106}$Hg(NO$_3$)$_2$, the radiomercury was contained only in the yolk and not in the albumen or shell (unpublished data).

SUMMARY

The autoradiographic and tracer techniques were employed to investigate the distribution and movement of radiomercury, in the form of $^{203}$Hg-mercuric nitrate, and its
Hg TRANSFER TO THE QUAIL EGG

transfer to eggs in laying quails.

1. Whole-body sections were prepared from laying quails weighing about 130 g. Autoradiograms were obtained at 1, 24, 48, 96, 192, and 384 hours after an intravenous injection of $^{203}$Hg(NO$_3$)$_2$.

2. One hour after the injection, radioactivity in the body was detected all over the body of the quail. Forty-eight hours after the injection, it remained only in liver, kidney, and ova, and after 96 hours, radioactivity was detected mainly in the kidney and ova.

3. Radioactivity in blood, liver, kidney and ova were counted by the scintillation technique. Soon after the injection, a high radioactivity was detected in the blood and liver, but it decreased rapidly within 12 hours.

4. Radioactivity in the ova in the ovary increased rapidly during first 12 hours after the injection and remained at a plateau thereafter. After the 12th hour of the injection, the largest amount of radiomercury was found in the second-sized ovum in the ovary.

5. Radioactivity in the eggs was examined after laying. It was high in the yolk, but undetectable in the albumen and shell. The largest amount of radiomercury was found in the yolk of the third egg laid after the injection.

6. It is concluded that when mercury, in the form of mercuric nitrate, is administered intravenously in the laying quail, it is distributed mainly in the liver, kidney, and ova. The ova containing mercury constitute the yolk of laid eggs. The total amount of mercury in the yolk is the largest in that of the third egg laid after the administration.

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