EFFECT OF ESTROGENIZATION ON THE DISTRIBUTION OF RADIOMERCURY IN QUAIL

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It is known that mercury derivatives are transported into eggs developing in the reproductive organs of birds (1-12). An egg consists of three components; yolk which develops in the ovary, albumen and shell which are secreted from the site of magnum and the site of uterus of the oviduct, respectively (13). It is known also that alkoxy mercury derivatives (9, 10) and inorganic mercury (10, 12) are transported mostly into egg yolk, and alkyl mercury compounds into egg albumen (1, 3, 5, 6, 10) and shell (1). The difference in the mode of the transportation may be due to the difference in chemical form among these mercurials (9, 12).

On the other hand, the onset of laying in domestic fowls is accompanied by changes in the majority of the plasma components (13-30). Similar changes in the plasma were induced in immature pullets, nonlaying hens, cockerels, and other birds by treatment with estrogens (20, 23, 30-52). Large quantities of phospholipids and phosphoprotein in the serum of the laying hen are synthesized in the liver in response to estrogens (20, 49, 52), and the phospholipids and the phosphoprotein are transported and accumulated into ovarian follicles (20).

Rissanen and Miettinen reported that inorganic mercury or alkoxy mercury derivatives possibly bound to the yolk proteins (9). These forms of mercurials would be transported into ovarian follicles via the circulating blood plasma in a yolk-protein bound form. There is a possibility that retention of mercury in birds may be controlled by treatment with estrogens. In this paper, the effect of estrogenization on distribution of radiomercury is compared between male and immature female quail and laying quail, and interrelationship among all the four entities, i.e., laying plasma, estrogens, plasma radiomercury, and yolk radiomercury, is discussed.

METHODS

Quail (*Coturnix coturnix japonica*) used in this experiment were of the JQ-NIBS closed strain and were supplied by the Nippon Institute for Biological Science Tachikawa. Both immature female and adult male quail of 4 and 6 weeks, respectively, were employed. Weight was approx. 90 g each while the laying quail weighed approx. 130 g. Quail which

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laid more than 5 eggs a week were selected (12).

Comparison of retention of ^{203}Hg in blood plasma between male and laying quail

Radioactive mercuric nitrate ($^{203}\text{Hg}(\text{NO}_3)_2$) was injected into the jugular vein of thirty male quail and the same number of laying ones. The injection was scheduled to take place at the time when laying birds had an egg in the hard shell stage in uterus, as reported previously (12). The dose of $^{203}\text{Hg}(\text{NO}_3)_2$ for a bird was 0.05 mg of mercury (specific activity; 20 $\mu\text{Ci}/\text{mg Hg}$) per 100 g of body weight in a volume of 0.1 ml in 0.01 N HNO_3 solution. This amount of mercury had no depressant effect on egg production (12). The quail to which radiomercury had been administered were divided into five groups of 6 and sacrificed 5 and 30 min and 1, 3, and 6 hr, respectively, after injection. Decapitation was carried out and blood plasma was collected as samples for radio-assay.

Estrogenization on retention of ^{203}Hg in blood plasma, liver, and kidney of quail

Eight male quail 6 weeks old, and 8 female quail 4 weeks old were given i.m. a single dose of 0.2 mg of water suspended estradiol benzoate (1, 3, 5, (10)-estratriene-3, 17 β -diol-3-monobenzoate) (Teikoku Zoki Co., Ltd., Tokyo) per 100 g of body weight. Forty-eight hr after estrogenization, $^{203}\text{Hg}(\text{NO}_3)_2$ was injected as described herein. Decapitation was carried out 3 hr after injection of the radiomercury. Blood, liver, and kidney were removed and blood plasma separated.

Dose of estradiol on retention of ^{203}Hg in blood plasma, liver and kidney

Twenty-four male quail of 6 weeks were divided into 3 groups, and treated with 3 dose levels of 0.02, 0.1, and 0.2 mg of estradiol benzoate, respectively. Radiomercury was injected 48 hr after estrogenization. All birds were sacrificed 3 hr after injection of radiomercury and blood plasma, liver, and kidneys were removed.

Estrogenization on excretion of ^{203}Hg

Eight male quail were treated with estradiol benzoate at a single dose of 0.2 mg per 100 g of body weight. Eight other male quail were given only distilled water serving as control. Forty-eight hr after injection of estradiol benzoate or distilled water, these birds, as well as eight laying quail, were injected with $^{203}\text{Hg}(\text{NO}_3)_2$. Feces and urine were collected 1, 3, 6, 12, 24, 48, 72, and 96 hr after injection of the radiomercury.

Determination of ^{203}Hg

Amount of radiomercury contained in each sample was counted by a well type scintillation detector (Tokyo Atomic Industries Co., Ltd., Tokyo) and results were expressed as a percentage to the dose injected.

The $^{203}\text{Hg}(\text{NO}_3)_2$ (specific activity 7.53 mCi/mg Hg) used was supplied by the New England Nuclear Corporation (U.S.A.).

RESULTS

Comparison of retention of radiomercury in blood plasma between male and laying quail

Fig. 1 shows changes in the amount of radiomercury in blood of male and laying quail up to 6 hr after injection of the radioisotope. Radioactivity in the whole blood of male quail (Fig. 1, left) decreased rapidly during the first 30 min and after that slowly. The

FIG. 1. Comparison of ^{203}Hg content in blood between male and laying quail injected i.v. with $^{203}\text{Hg}(\text{NO}_3)_2$.

The standard error of mean of six experiments is shown by a vertical line, where possible.

amount of ^{203}Hg in blood-corpusele fraction decreased similarly as that in the whole blood. The radioactivity in blood plasma fraction was much less than that in blood-corpusele fraction up to 1 hr, suggesting that the decrease in radioactivity in the whole blood was due to the rapid decrease in the isotope from blood-corpusele fraction in male quail.

In laying quail (Fig. 1, right), the radioactivity in plasma fraction was always much higher than that in blood-corpusele fraction, suggesting that the decrease in radioactivity in the whole blood was mostly due to the decrease of that in plasma fraction. Further, the higher retention of mercury in the whole blood of laying quail during the period of observation was attributed to the maintenance of higher level of mercury in plasma fraction.

Effect of estrogenization on retention of ^{203}Hg in blood plasma, liver, and kidney of quail

In the next experiments, the effect of estrogenization on retention of mercury in blood plasma, liver, and kidney of quail was studied. The radioactivity in blood plasma fraction of unestrogenized male or immature female quail, which was approx. one-tenth of that of laying quail, increased after estrogenization to the level much higher than that of laying quail (Fig. 2). The amount of mercury in blood-corpusele fraction, however, was scarcely affected by the estradiol treatment. On the other hand, the estrogenization decreased the amount of mercury in both liver (Fig. 3) and kidneys (Fig. 4) of male and immature female quail nearly to the level of laying quail.

Effect of the estrogenization on male quail is summarized in Fig. 5. The radioactivity

FIG. 2. Effect of estrogenization on retention of ^{203}Hg in blood plasma 3 hr after injection of $^{203}\text{Hg}(\text{NO}_3)_2$.

The standard error of mean of eight experiments is shown by a horizontal line.

FIG. 3. Effect of estrogenization on retention of ^{203}Hg in liver 3 hr after injection of $^{203}\text{Hg}(\text{NO}_3)_2$.

The standard error of mean of eight experiments is shown by a horizontal line.

in the whole blood and in plasma fraction increased with an increase in the dose of estradiol benzoate, but that in blood-corpuscle fraction remained at a constant level. On the other hand, the amount of mercury in liver or kidney was greatly reduced by the treatment.

Fig. 6 shows excretion of mercury into feces and urine of estrogenized male, untreated male and laying quail. In unestrogenized male quail, the amount of mercury excreted into feces and urine increased rapidly to 83% of the administered dose during the first 24 hr after injection of $^{203}\text{Hg}(\text{NO}_3)_2$, and slowly increased thereafter up to 96% during 96 hr. After estrogenization, the amount of mercury excreted was 58% for the first 24 hr and

FIG. 4. Effect of estrogenization on retention of ^{203}Hg in kidney 3 hr after injection of $^{203}\text{Hg}(\text{NO}_3)_2$.

The standard error of mean of eight experiments is shown by a horizontal line.

FIG. 5. Dose dependency of the effect of estrogenization on retention of ^{203}Hg in blood plasma, liver, and kidney in male quail 3 hr after injection of $^{203}\text{Hg}(\text{NO}_3)_2$.

Each point represents a mean of eight experiments.

FIG. 6. Effect of estrogenization on excretion of ^{203}Hg into feces and urine.

The standard error of mean of eight experiments never exceeded the size of the printed symbol.

FIG. 7. Comparison of the amounts of mercury excreted into feces and urine in unestrogenized and estrogenized male quail and into feces and urine plus yolk in laying quail 96 hr after injection of $^{103}\text{Hg}(\text{NO}_3)_2$.

The standard error of mean of eight experiments is shown by a vertical line.

88% after 96 hr, showing that the excretion of mercury is delayed by treatment with estradiol benzoate. In laying quail, however, excreted mercury was only 33 and 47% for 24 and 96 hr, respectively. During the observation period, each of the laying quail produced four eggs. These eggs contained radiomercury in the yolk except one which was laid for the first time after the injection of $^{203}\text{Hg}(\text{NO}_3)_2$. When the amount of mercury in the yolk was added to that in the excreta of the laying quail, as shown in Fig. 7, the total amount of mercury reached 91% of the administered dose, which was almost the same as that excreted by unestrogenized or estradiol treated male quail.

DISCUSSION

Difference in the amount of mercury in blood plasma between male and laying quail:

Laying quail maintained a high level of mercury in blood plasma for a longer period than male ones. In male quail, mercury in blood rapidly decreased and was excreted into feces and urine. In laying quail, however, the amount of mercury in feces and urine was small, while a considerable amount was found in the yolk. These data suggest the possibility that plasma mercury of laying quail exists as a form which is hardly excreted into feces and urine but transported into yolk.

Effect of estrogenization on retention of mercury: There are many studies on changes in the composition of plasma or sera of birds by the onset of laying (13-30) and by estrogenization (20, 23, 30-52). In many of birds, the onset of laying caused changes in the majority of the constituents in plasma or sera, such as increases in total lipids (15-17) and total calcium (13, 14, 18, 23) and appearances of serum vitellin (15, 16, 18, 22, 23) and a phosphoprotein (15-17). Estrogenization also caused hyperlipemia (31-39), hypercalcemia (23, 31, 32, 41, 44, 46, 48, 50, 51), and appearances of serum vitellin (20, 49, 52) and a phosphoprotein (40, 45-47, 51). The phosphoprotein was proved to be phosvitin (28, 29) which had been found as one of the yolk proteins (53). It is now accepted that serum vitellin and phosvitin exist in serum as a lipovitellin-phosvitin- γ -livetin complex (24). This complex was also found in egg yolk (54). Hosoda et al. (20, 49, 52) found that the protein complex was synthesized in response to the transfer of estrogens to liver and supplied to ovarian follicles for construction of the yolk proteins. On the other hand, serum calcium level was reported to increase during the laying period or the treatment with estrogens in pigeon (33, 34), chicken (35-37), sparrow (38), duck (39), and bobwhite quail (44). It was also made clear that calcium binds to phosvitin of the complex (47, 48, 53) and is transported into ovarian follicles in a phosvitin bound form (55). ^{65}Zn was also reported to bind to a component of the complex, lipovitellin (56). In the present data, high level of radiomercury in plasma of laying quail was hardly excreted into feces and urine. High concentrations of radiomercury were detected in the yolk of eggs laid after injection of $^{203}\text{Hg}(\text{NO}_3)_2$. In the male and immature female quail treated with estradiol benzoate, radiomercury in blood plasma fraction increased. This effect depended on the dose of estradiol benzoate. In the male and immature female quail treated with estradiol, which have no ovarian follicles or on mature ones, respectively, plasma radiomercury

increased more distinctly. From these data, it is assumed that a substantial part of the plasma mercury of laying or estrogenized quail might bind to the lipovitellin-phosvitin- γ -livetin complex. It is obscure, however, whether or not the plasma mercury has a specific relationship with a component of the complex as is the case with calcium or zinc. To settle this, investigation is now under way to separate the mercury-bound protein from sera and yolk of laying and estrogenized quail.

SUMMARY

Effect of estrogenization on the distribution of ^{203}Hg was studied in male and immature female quail, as compared with laying ones.

1. The concentrations of blood mercury were maintained at a higher level in laying quail than in male ones. In the laying quail, high concentrations of mercury in the blood were derived from the plasma mercury.

2. Mercury excreted into feces and urine was less in laying quail than in male ones. However, a considerable amount was found in yolk of eggs laid during the observation period.

3. The estrogenization induced an increase in plasma mercury, a decrease in the amount of mercury in the liver and the kidneys, and a reduction of mercury excreted into feces and urine. The effect of the estrogenization depended on the dose of estradiol benzoate.

4. It is suggested that mercury in the blood plasma of laying quail is closely related to some components appearing in the plasma in response to estradiol benzoate and is transported into ovarian follicles together with those plasma components.

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