The Effect of Age on Egg Production in the Domestic Hen

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The effect of age on egg production was investigated in the domestic fowl. The rate of egg production was reduced with increasing age and the incidence of thin-shelled and cracked eggs was markedly increased. Older hens which remained in lay produced fewer but larger eggs than the younger birds. The ovaries of these two groups of hens were of similar appearance but the largest preovulatory follicle and the oviduct were significantly heavier in the older birds. No differences in the circulating levels of progesterone or estradiol were apparent between the young and old birds but the activity of the renal 25-hydroxycholecalciferol 1α-hydroxylase was found to be significantly reduced with age. Circulating levels of total and ionized calcium were very similar in the young and old laying birds, plasma ionized calcium levels being markedly depressed during egg shell calcification in both groups. The decreased rate of ovulation in the older birds and the increase with age in the size of the follicles ovulated is thought to be associated with a reduced rate of recruitment of follicles for rapid growth followed by a prolonged period of follicular growth and development. The poor calcification of egg shell in old birds would appear to be due to some dysfunction of the shell gland, possibly associated with reduced synthesis of 1,25-dihydroxycholecalciferol by the kidney. The maintenance of plasma ionized calcium concentrations does not appear to be of primary importance in this respect.

Egg production in the hen generally increases for between 6 and 8 weeks after the onset of lay to reach a maximum which is maintained for a few months. Thereafter, the birds lay progressively fewer and larger eggs with thinner shells. As a result of these changes the economic life of a bird is often as short as 2 years, and cracked eggs remain a major problem to the poultry industry. A number of studies have been conducted to investigate the effects of age on follicular development and ovulation and on egg shell calcification but the underlying mechanisms involved in the observed changes are still poorly understood.

It has been suggested that the reduction with age in the rate of ovarian follicular development is associated with a reduced rate of recruitment of follicles into the rapid growth phase (Williams and Sharp, 1978a). In addition, evidence has been produced that the reduced rate of ovulation may result from changes in the sensitivity of the hypothalamus to positive feedback by progesterone (Williams and Sharp, 1978b). This is consistent with the observation made in aging hens by Tanabe et al. (1981) that there is a positive correlation between plasma luteinizing hormone (LH) concentrations and egg production which is not accompanied by a fall in circulating progesterone levels.

A marked decrease in egg shell quality is commonly observed in aging hens. Intestinal calcium absorption has been shown to decrease with age in both the rat and man (Schachater et al., 1960; Bullamore et al., 1970) and experiments in the rat have shown that this is accompanied by parallel changes in 1,25-dihydroxycholecalciferol (1,25-DHCC)-dependent calcium-binding protein (Armbrecht et al., 1979) which could result from decreased circulating levels of 1,25-DHCC. More recent studies...
have shown that renal 25-hydroxycholecalciferol (25-HCC) 1α-hydroxylase activity is reduced with age in both quail (Baksi and Kenny, 1981) and hens (Abe et al., 1982). Abe et al. also noted a reduction with age in the accumulation of radiolabeled 1,25-DHCC in a number of target tissues following the injection of [3H]-25-HCC. As a result of these studies, Abe et al. concluded that the increased incidence of cracked and thin-shelled eggs seen in aged birds is associated with a disorder of vitamin D metabolism. In the present study, renal vitamin D metabolism has been examined in young and old laying hens and in old hens which have ceased egg production. In addition, plasma levels of the reproductive steroids, progesterone and estradiol, have been measured together with plasma ionized and total calcium concentrations. Ovarian follicle and oviduct weights were also recorded. The possible causes for the change with age in the size and number of ovulated follicles and the subsequent calcification of egg shells are discussed.

MATERIALS AND METHODS

Animals and their treatment. Day-old female chicks (Rhode Island Red X Sussex Light) were raised to maturity using commercial feeds. Artificial lighting was provided for 16 hr per day between 0600 and 2200 hr. The birds were used either at 30-40 weeks (young layers group) or at 150-200 weeks of age. The older birds were divided into two groups according to their rates of lay. Birds which were laying sequences of eggs were assigned to the "old layers" group, while others which had ceased egg production were assigned to the "old nonlayers" group. Egg laying records were kept during a 10-day period prior to the start of the experiment.

Anaerobic blood samples were collected from the birds by wing vein puncture at 0700 and 1600 hr. The plasma obtained was assayed for ionized calcium and total calcium concentration. The birds were killed at 0800 hr by cervical dislocation and aortic blood was collected for the estimation, by radioimmunoassay, of plasma progesterone and estradiol concentrations. Samples of kidney were removed and assayed for 25-HCC hydroxylase activities. The weights of the oviduct and of the large ovarian follicles were also recorded.

Ionized calcium assay. Plasma concentrations of Ca2+ were determined using a radiometer calcium electrode (F2112) as described by Luck and Scanes (1979), with minor modifications: syringes used to collect blood were primed with 50 IU lithium heparin; the Analar Tris buffer was 0.2 mM to which 0.1% sodium azide was added. The precision of the assay in our laboratory was 1.05 μmol/liter.

Determination of total plasma calcium. Total plasma calcium measurements were made using an atomic absorption spectrophotometer (Perkin Elmer 280) operating at 423 nm. Analar calcium carbonate was used to provide standards between 0.5 and 4.0 μg. Lanthanum chloride (0.1%) was added to both standards and samples.

Radioimmunoassay. Plasma concentrations of progesterone and 17β-estradiol were measured by specific radioimmunoassays. The methods used have been described in detail by Brain (1982). Briefly, antibodies to progesterone and 17β-estradiol were raised in rabbits against progesterone-11-carboxymethyl-oxime-bovine serum albumin and 17β-estradiol-6-carboxymethyl-oxime-bovine serum albumin, respectively. Estradiol-17α, estrone, and estriol were found to cross-react with the 17β-estradiol antiserum 50.0, 24.9, and 9.1%, respectively, while other steroids tested (testosterone, androstenedione, progesterone, and 11α-progesterone) caused less than 1% displacement. 11α-Progesterone and 17α-progesterone were found to cross-react with the progesterone antiserum 95 and 25%, respectively. All androgens and estrogens tested caused less than 1% displacement. The sensitivities of the two assays were 2.5 x 10^-11 and 2 x 10^-12 mol/tube, respectively.

Measurement of renal 25-HCC hydroxylase activity. The procedure used for the measurement of renal 25-HCC hydroxylase activity was based on the method of Kenny (1976) and has been described in some detail by Williams (1984). Briefly, samples of kidney were homogenized and incubated with [3H]-25-HCC. The metabolites produced were extracted prior to separation by thin-layer chromatography. Nonradioactive 25-HCC, 1,25-DHCC, and 24,25-DHCC were used to identify the corresponding tritiated vitamin D metabolites. Enzyme activities are expressed as picamoles of product produced per gram wet weight of tissue per hour.

Statistical treatment of results. Results are expressed as means ± SEM. Data were analyzed by one- or two-way analysis of variance and the means compared by Duncan's multiple range test. P < 0.05 was considered to be significant.

RESULTS

The rate of lay in the old birds was substantially lower than in the young birds and
EFFECT OF AGE ON EGG PRODUCTION

TABLE 1
THE EFFECT OF AGE ON THE OVARIAN FOLLICULAR HIERARCHY AND OVIDUCT WEIGHT IN THE HEN

<table>
<thead>
<tr>
<th></th>
<th>Mean number of yolky follicles &gt;1 g in weight</th>
<th>Mean weight of the largest yolk filled follicle (g)</th>
<th>Mean oviduct weight (g)</th>
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</thead>
<tbody>
<tr>
<td>Young layers</td>
<td>4.60± ± 0.22 (n = 10)</td>
<td>15.25± ± 0.55 (n = 10)</td>
<td>49.5± ± 3.2 (n = 9)</td>
</tr>
<tr>
<td>Old layers</td>
<td>4.17± ± 0.17 (n = 6)</td>
<td>18.43± ± 0.51 (n = 6)</td>
<td>67.2± ± 3.8 (n = 6)</td>
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<tr>
<td>Old nonlayers</td>
<td>0.83± ± 0.65 (n = 6)</td>
<td>3.27± ± 2.54 (n = 6)</td>
<td>16.4± ± 6.5 (n = 6)</td>
</tr>
</tbody>
</table>

Note. Values are expressed as means ± SEM. For any given variable, means without a common superscript are significantly different.

at the time of the experiment many had ceased egg production completely. In the old birds which were still laying, rates of egg production were in the range from 66 to 77%, compared with over 90% in the young birds. Eggs produced by the old birds were appreciably larger than those of the younger birds and the incidence of cracked shells was much greater.

Table 1 shows the mean number of large yolky follicles present in the ovary, the mean weight of the largest yolk filled follicle (F1), and the mean weight of the oviduct for each of the groups studied. The mean number of large yolky follicles in the ovary was markedly reduced in the old birds which had ceased egg production when compared with the laying birds, but there was no significant difference between the numbers of large yolky follicles present in the young and old laying hens. The mean weight of the largest of the follicles present in the ovary was significantly greater in the old than in the young layers (P < 0.05), while the oviducts of the old birds which had ceased egg production had atrophied.

Table 2 shows the mean plasma levels of progesterone and estradiol at approximately 0800 hr in each of the groups of birds. Neither hormone differed significantly between the young and old laying hens. In the nonlaying birds, however, plasma levels of both steroids were significantly lower than in the laying birds (P < 0.01).

The activities of the 25-HCC 1α- and 24-hydroxylase enzymes were measured in samples of kidney removed from each of the three groups of birds (see Table 3). Assays were performed at approximately 0800 hr at a time when most of the laying birds were judged to be in the final stages of shell calcification. Birds without a calcified egg in the oviduct were excluded from the study. Comparison of the renal hydroxylase enzyme activities in the two groups of laying birds did not show any significant differences for the activity of the 24-hy-
TABLE 3
THE EFFECT OF AGE ON THE RENAL METABOLISM OF VITAMIN D IN HENS

<table>
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<tr>
<th>Age of Hens</th>
<th>Renal 25-HCC 1-hydroxylase activity (pmol/g/hr)</th>
<th>Renal 25-HCC 24-hydroxylase activity (pmol/g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young layers (n = 8)</td>
<td>227.8 ± 18.6</td>
<td>117.5 ± 18.0</td>
</tr>
<tr>
<td>Old layers (n = 6)</td>
<td>146.2 ± 32.7</td>
<td>131.6 ± 40.2</td>
</tr>
<tr>
<td>Old nonlayers (n = 6)</td>
<td>31.2 ± 15.4</td>
<td>332.7 ± 94.0</td>
</tr>
</tbody>
</table>

Note. Enzyme activities were measured at 08.00 hr, when the laying birds were calcifying an egg shell, using an *in vitro* method as described in the text. Values are expressed as means ± SEM. Means without a common superscript differ significantly (P < 0.01).

Comparison of total plasma calcium concentrations at the two sampling times did not indicate any significant diurnal variation for any of the groups of birds.

Plasma ionized calcium concentrations in the old nonlaying birds were not significantly different at the two times of day studied and levels were similar to those observed in both groups of laying birds at 1600 hr, when the birds were not in the process of egg shell calcification. There were no significant differences in the mean plasma ionized calcium concentrations between young and old laying birds at either time of sampling and in both groups of birds ionized calcium levels were significantly lower at 0700 than at 1600 hr (P < 0.05).

DISCUSSION

The often reported observation that aging in hens is associated with reduced egg production and poor egg shell quality has been confirmed in the present study. Egg production in the old hens which remained in lay was lower than in the younger birds and the incidence of thin-shelled and misshaped eggs was much greater, while other old birds ceased egg production completely. The rate of ovulation and the size of the ovulated follicles are, at least in part, related to the fre-

TABLE 4
THE EFFECT OF AGE ON PLASMA IONIZED CALCIUM CONCENTRATION AND TOTAL CALCIUM CONCENTRATION IN THE HEN AT 07.00–07.30 HOURS (DURING EGG SHELL CALCIFICATION IN THE LAYING BIRDS) AND AT 16.00–16.30 HOURS (AFTER OVIPOSITION IN THE LAYING BIRDS, WHEN NO CALCIFICATION WAS OCCURRING

<table>
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<tr>
<th>Time of blood sampling:</th>
<th>Total plasma calcium concentration (mmol/liter)</th>
<th>Plasma ionized calcium concentration (mmol/liter)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>07.00 hr</td>
<td>16.00 hr</td>
</tr>
<tr>
<td>Young layers</td>
<td>5.7 ± 0.3</td>
<td>5.8 ± 0.2</td>
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<tr>
<td>(n = 7)</td>
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<tr>
<td>Old layers</td>
<td>5.1 ± 0.2</td>
<td>5.5 ± 0.2</td>
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<tr>
<td>(n = 5)</td>
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<td></td>
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<tr>
<td>Old nonlayers</td>
<td>4.1 ± 0.4</td>
<td>4.4 ± 0.3</td>
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<tr>
<td>(n = 8)</td>
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<td></td>
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</tbody>
</table>

Note. Values are expressed as means ± SEM. Means without a common superscript differ significantly (P < 0.01 for total calcium concentrations and P < 0.05 for ionized calcium concentrations).
quency with which follicles are recruited for growth and to the rate and duration of that growth.

In the present study, the ovaries of young and old laying birds were of similar appearance. The ovaries of both groups contained equal numbers of large yolky follicles though the largest of these, the Fl follicle, was significantly larger in the older birds. The older laying birds also had larger oviducts and laid larger eggs. The absence of large atretic follicles in the old laying birds would suggest that the reduced rate of ovulation in these birds is associated with an equally reduced rate of recruitment of follicles into the rapid growth phase.

The ovaries of the birds which had ceased egg production were almost completely devoid of large yolky follicles and their oviducts had regressed. Predictably, in the absence of these follicles, plasma levels of progesterone and estradiol were markedly reduced. Total plasma calcium concentrations were also significantly reduced suggesting that, as might be anticipated, the plasma levels of vitellogenin, a yolk protein precursor with a high capacity to bind calcium, were also reduced. Ionized calcium levels in these birds were similar to those in the laying birds at 1600 hr (noncalcifying) and no diurnal variation was apparent. With the loss of lay, the requirement for calcium is reduced and so presumably is the requirement for 1,25-DHCC. A low renal 25-HCC 1-hydroxylase activity accompanied by a high 24-hydroxylase activity is, therefore, appropriate in these birds.

In marked contrast to the old nonlaying birds, the laying birds, both young and old, had high circulating levels of both progesterone and estradiol. Plasma concentrations of progesterone in these birds were very similar and do not point to any major differences in the magnitude of the positive feedback stimulus required for the induction of ovulation. Estradiol levels at the time of blood sampling were also very similar in the young and old laying birds. No indication was obtained that a reduced estrogenic stimulus was responsible for any age related changes in calcium metabolism or yolk protein synthesis. The possibility remains, however, that more frequent blood sampling might detect important differences in the circulating levels of progesterone and estradiol.

Total plasma calcium concentrations were apparently unaffected by increasing age while laying persisted, perhaps suggesting that plasma levels of vitellogenin were also maintained. The levels of plasma ionized calcium in the young and old laying birds were also very similar indicating that the homeostatic mechanisms which regulate the concentration of ionized calcium in the blood are unimpaired in the old birds. It would appear, therefore, that if the shell gland of aging birds is still fully functional, then adequate amounts of calcium could be supplied for complete shell calcification. The production by old birds of thin-shelled eggs thus suggests that the efficiency of the shell gland in removing calcium from the circulation and depositing it in the shell is in some way reduced. This could result from changes in the function of the gland itself or simply from a reduced blood flow restricting the supply of calcium to the gland. The increased surface area of eggs laid by aging hens could also be a contributory factor.

The mechanism by which the calcification of the egg shell in the shell gland is controlled is still poorly understood but 1,25-DHCC receptors and calcium-binding protein have been shown to be present in the shell gland (Takahashi et al., 1980) and 1,25-DHCC may well be required for the uptake of calcium from the blood and its deposition in shell. In the present study, 25-HCC 1α-hydroxylase activity was reduced in the older birds and presumably, as a result, circulating 1,25-DHCC levels were also lower. Reduced plasma levels of 1,25-DHCC could be the cause of the reduced
shell calcification but equally, if calcium uptake by the shell gland was reduced for some other reason, then the decline in the activity of the 1-hydroxylase could simply be a reflection of a reduced requirement for calcium. There is, however, some direct evidence that the reduction with age in shell thickness may be due to reduced production of 1,25-DHCC. In an experiment using hens which had been in lay for 9 months, Morris et al. (1977) demonstrated a significant increase in shell thickness within 48 hr of feeding a diet rich in 1,25-DHCC, derived from the leaves of the plant Solanum malacoxylon. The poor calcification of the egg shell in old birds would appear, therefore, to be due to some dysfunction of the shell gland, possibly as a result of reduced renal synthesis of 1,25-DHCC. Low circulating levels of 1,25-DHCC in aging hens would also result in reduced intestinal calcium uptake. However, the plasma ionized calcium data provide indirect evidence that the rate of uptake of calcium by the intestine and the maintenance of plasma ionized calcium concentrations are not of primary importance in the poor calcification of shell by aging hens.

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REFERENCES


